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Herbicide Resistance in Summer Annual Weeds of Australia's Northern Grains Region

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Abstract: For more than two decades, glyphosate has been relied on to control summer annual weeds in fallow systems in Australia's northern grains region. With numerous cases of glyphosate resistance reported in weed species collected from this region, there are concerns about the future viability of this herbicide. A random seed collection survey of summer weeds was conducted between 2016 and 2018 with the aim of determining the frequency and distribution of resistance to glyphosate and other herbicides commonly used for summer weed control. Glyphosate resistance was ubiquitous in fleabane, with all collected populations resistant to this herbicide. Glyphosate resistance was also prevalent in feathertop Rhodes grass, windmill grass, and awnless barnyard grass, with resistance detected in 68%, 58%, and 36% of populations, respectively. Only 14% of sowthistle populations collected between 2013 and 2108 were resistant to glyphosate. Resistance to haloxyfop was detected in feathertop Rhodes grass, albeit at a low frequency (2%). Other herbicides, such as 2,4-D amine, propaquizafop, and clethodim, provided good control of the broadleaf and grass weeds tested. The results from these surveys conducted between 2013 and 2017 provide a first glimpse of the state of herbicide resistance in key crop weeds for Queensland and the northern region of New South Wales. It is clear that farmers and agronomists need to consider incorporating non-chemical weed management tactics to promote the sustainability of current herbicides.

Keywords: glyphosate resistance; sowthistle; fleabane; feathertop Rhodes grass; awnless barnyard grass

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1. Introduction

There is a focus on winter crop production across the large 10 M ha annually cropped area of the northern grains region of Queensland (Qld) and New South Wales (NSW) [1]. The emphasis on winter crop production is driven by dominant market opportunities for winter grain crops rather than growing season patterns. Consequently, this region encompasses a large range of environments in which winter crops are grown. Across the region, annual rainfall patterns transition from winter-dominant in the south to summer-dominant in the north [2]. The environment for winter crop production in the southern portion of the region is characterised by cool, moist winter-spring growing seasons (April–October) interspersed with hot and mostly dry summers (November–March). In the north, winter crop production is practiced in a comparatively shorter season (May to October) of warmer and drier conditions, followed by a hot and humid summer–autumn period (December to April). Consequently, winter crops in the south are supported by in-season rainfall, while in the north, they are reliant on soil moisture stored during the summer fallow phase [3,4]. The region-wide adoption of conservation cropping, based on reduced tillage and residue retention [5], has greatly improved the health of cropping soils, leading to a more sustainable production system [6,7]. Importantly, for the summer-dominant rainfall areas, crop residue retention is critical to the capture and retention of typically high-intensity and sporadic rainfall events [8]. Similarly, reducing the amount of soil disturbance decreases soil moisture losses through evaporation, and the resulting improved soil structure aids rainfall infiltration [9].

Weed control during summer fallow phases is essential for soil moisture storage and nutrient conservation [10]; however, the adoption of conservation cropping has resulted in a reliance on herbicides for weed control and the subsequent risk of resistance evolution. In conservation cropping systems, residue retention and reduced soil tillage remove the use of tillage and burning as valuable weed control tools. Consequently, there is a reliance on herbicides for weed control at all stages throughout the cropping rotation. The risks of herbicide reliance are evident in the high frequencies of evolved resistance in winter annual weed species of the northern cropping region [11–13]. These documented, extensive levels of resistance have resulted from a focus on in-crop herbicides. A similar reliance on herbicides for summer fallow weed control has contributed to the evolution of herbicide resistance in summer annual weed populations. In particular, the focus on glyphosate for summer weed control has led to resistance in the five dominant summer annual weed species from the northern grains region; sowthistle (*Sonchus oleraceus* L.) [14], fleabane (*Conyza* spp.) [15], feathertop Rhodes grass (*Chloris virgata* Sw.) [16], awnless barnyard grass (*Echinochloa colona* (L.) Link) [17,18], and windmill grass (*Chloris truncata* R. Br.) [19]. While glyphosate resistance evolution is the most critical because of the loss of this extremely valuable herbicide, it is not the only herbicide of concern for growers dealing with herbicide resistance. For instance, chlorsulfuron and atrazine resistance in annual weeds from this region was first reported more than two decades ago [20,21]. This herbicide-resistance history, combined with the propensity for weed populations to evolve resistance to ALS-inhibiting herbicides [22] and the increased use of these herbicides, will most likely lead to problematic levels of resistance. Similarly, although not documented, there are now expected to be cases of resistance to ACCase-inhibiting herbicides, which are frequently being used in support of or to replace glyphosate in controlling summer annual grass species.

The extent of herbicide-resistant biotypes in major annual winter weeds of Australian cropping systems has been monitored through the conduct of random routine surveys of cropping regions [12,13,23–25]. These surveys enable the weed control research community to develop better and implement appropriately focused weed research and control programs. Despite several cases of identified herbicide-resistant biotypes in the major summer weed species, there has not been a region-wide survey to identify the extent of resistance to glyphosate and other commonly used herbicides in the northern grain-cropping region. This study aims to determine the frequency and distribution of herbicide resistance in populations of sowthistle, fleabane, feathertop Rhodes grass, barnyard grass, and windmill grass, the dominant summer annual weeds of northern region cropping systems.

2. Materials and Methods

2.1. Weed Seed Collection Survey

To assess the frequency of herbicide resistance in the major summer annual weed species (fleabane, sowthistle, feathertop Rhodes grass, barnyard grass, and windmill grass), a random weed seed collection survey of Queensland and northern NSW was conducted at crop maturity during the 2016/17 and 2017/18 summer growing seasons. Because of the year-round emergence of sowthistle populations, these species were also collected during winter crop surveys of southern NSW conducted between 2013 and 2017 (see Broster et al. [26] for specific details of surveys). Weed seed collection surveys were conducted in stages from north to south through the cropping region to coincide with the time of crop maturity and just prior to crop harvest. Survey sites were identified through a random selection of fields at an approximate distance interval from 5 to 10 km between sites while driving a transect through the cropping region (NSW) or via direct contact with land owners/managers (Qld) for approval to survey a field on the property randomly. The latitude and longitude coordinates of each survey site were recorded with maps created using ArcGIS Desktop (Version 10.6) [27]

Surveys of each cropping field were performed by two people separately walking a V-shaped transect over approximately a 300-metre distance for a 10–15 min period with the encountered weed species identified and recorded. The density of these weeds was visually assessed using the categories adapted from Llewellyn and Powles [28]: very low (occasional plant), low (<1 plant m^{-2}), medium (1–10 plants m^{-2}), high (>10 plants m^{-2}), and very high (>10 plants m^{-2} and dominating crop). Where present, seed heads of the species to be screened for resistance were collected and placed in a separate paper bag for each species.

2.2. Weed Seed Sample Processing and Seedling Establishment

Collected weed seed populations were stored in a temperature-controlled (~20 °C) dry laboratory. Seed heads or florets were hand-threshed with the samples, then aspirated, and the seed was collected.

At the Queensland Department of Agriculture and Fisheries Toowoomba research station (QDAF), weed seedlings were established for herbicide screening during the 2017/2018 and 2018/2019 summer growing seasons. Sowthistle was grown from late March to late November, while fleabane and summer grasses were grown from late November to mid-May. Sowthistle seeds were germinated on 0.6% agar for four to seven days in the glasshouse and transplanted into porous trays (33 cm long × 30 cm wide × 5 cm deep) (50 seedlings/population/tray) filled with potting mix. The trays were then placed into large trays filled with sufficient water to ensure adequate soil moisture during the critical seedling-emergence phase. After seven days, the plants were watered from above. A similar approach was used for fleabane, with the exception that fleabane seeds were sown directly into trays filled with peat:sand (60:40) mix. After seven days, fleabane seedlings were thinned to the required density of 50 seedlings per tray. The seedlings of summer grass species (barnyard grass, feathertop Rhodes grass, and windmill grass) were established by first germinating the seeds on agar and then transplanting them into trays of potting mix. Planted trays were placed in a polyethylene growth house where daily temperature fluctuations were approximately from 15 °C to 35 °C. Seedlings were hand-watered to maintain trays at near field capacity and were fertilised every seven days after emergence, with each pot receiving approximately 50 mL of 1.0 g L^{-1} solution of Thrive® All Purpose Soluble Fertiliser (N 25%, P 5%, K 8.8%, trace elements; Yates Australia, 1 Gow Street, Padstow NSW 2211, Australia).

At Charles Sturt University (CSU), from May to October, the year after each survey sowthistle seeds were placed on top of soil mix (commercial garden loam) in pots (17 cm long × 11 cm wide × 6 cm deep), after which they were gently watered until germination. After germination, they were counted, and where more than 40 plants were present, thinned to between 30 and 40 plants per pot with a minimum of 10 plants required for each of the three replicates. Pots were placed in an air-conditioned glasshouse where daily temperature fluctuations were approximately from 10 °C to 25 °C. Seedlings were hand-watered to maintain trays at near-field capacity and were fertilised every seven days after emergence with the Thrive® All Purpose Soluble Fertiliser.

2.3. Herbicide-Resistance Screening

Herbicide treatments were applied at the upper recommended label rate for each herbicide to the weed species being screened (Table 1) once seedlings had reached the three- to five-leaf growth stage. Fleabane, feathertop Rhodes grass, barnyard grass, windmill grass, and sowthistle collected in the 2016 Queensland and northern NSW survey were screened at QDAF, while the sowthistle collected from the other regions were screened at CSU. At QDAF, herbicides were applied with appropriate adjuvants (if required) using a single nozzle (TeeJet XR110015 flat fan, Springfield, IL, USA) cabinet sprayer calibrated to deliver 114 L of water ha^{-1} at 200 kPa and 4 km h^{-1}. At CSU, herbicides were applied using a twin-nozzle boom (TeeJet XR11001 flat fan) cabinet sprayer calibrated to deliver 85 L of water ha^{-1} at 250 kPa and 5 km h^{-1}.

Table 1. Active ingredient rates and suppliers of herbicides used in screening populations of five weed species randomly collected from the northern grains region cropping fields at the end of the 2016/17 summer growing season.

Active Ingredient and Application Rate	Herbicide Product (Formulation), Company, and Address	Weed Species Screened
Glyphosate (729 g ae ha^{-1}) †	Weedmaster ARGO (540 g/L glyphosate) Nufarm, 103–105 Pipe Road, Laverton North, VIC 3026, Australia	Sowthistle, fleabane, feathertop Rhodes grass, awnless barnyard grass, and windwill grass
2,4-D amine (1050 g ai ha^{-1})	Amicide Advance (700 g/L 2,4-D amine) Nufarm, 103–105 Pipe Road, Laverton North, VIC 3026, Australia	Fleabane and sowthistle
Bromoxynil (210 g ai ha^{-1}) † pyrasulfotole (37.5 g ai ha^{-1})	Velocity (210 g/L bromoxynil and 37.5 g/L pyrasulfotole), Bayer Crop Science Pty Ltd., 8 Redfern Rd, Hawthorn East, VIC 3123, Australia	Sowthistle
Chlorsulfuron (15 g ai ha^{-1}) †	Glean (750 g/kg chlorsulfuron) Corteva Agriscience, Albert Avenue, Chatswood West, NSW 2067, Australia	Sowthistle
Haloxyfop (78 g ai ha^{-1})	Verdict 520 (520 g/L haloxyfop) Corteva Agriscience, Albert Avenue, Chatswood West, NSW 2067, Australia	Feathertop Rhodes grass
Clethodim (48 g ai ha^{-1}) **	Select (240 g/L clethodim) Sumitomo Chemical, Level 5, 51 Rawson St, Epping, NSW 2121, Australia	Feathertop Rhodes grass, awnless barnyard grass, and windmill grass
Paraquat (400 g ai ha^{-1})	Shirquat 250 (250 g/L paraquat) Nufarm, 103–105 Pipe Road, Laverton North, VIC 3026, Australia	Feathertop Rhodes grass
Propaquizafop (60 g ai ha^{-1})	Shogun (100 g/L propaquizafop) Adama, Level 1, Building B/207 Pacific Hwy, St Leonards, NSW 2065, Australia	Awnless barnyard grass and windmill grass
Imazapic (96 g ai ha^{-1}) *	Flame (240 g/L imazapic) BASF, 12/28 Freshwater Pl, Southbank, VIC 3006, Australia	Awnless barnyard grass and windmill grass

† Not registered for control of these weed species; * Used alone as post-emergence herbicide; ** As per APVMA permit PER89322.

Plant mortality was assessed 21 days after treatment by determining whether the growing point was chlorotic or if new growth was visible, as well as comparing it with the treated control populations. Known susceptible and resistant biotypes of relevant weed populations were used as controls in all experiments. At QDAF, herbicide screening was repeated during the growing season, and results averaged for each population (always <5% variation between experiments), while at CSU (sowthistle only), three replicate samples of each population were screened. The herbicide-resistance screening was conducted under 'ideal' conditions for plant seedling growth (Section 2.2), herbicide treatment, and treatment effects (Section 2.3); thus, herbicide efficacy in the field may be lower than was observed in this survey.

2.4. Data Treatment

Each weed seed sample collected here is considered a separate population, as each surveyed field has a unique cropping history and soil conditions. Populations were classified based on their percentage survival following herbicide treatment. Susceptible populations were classified as those having 0% plant survival. Populations with at least 20% of plants surviving herbicide treatment were classified as resistant in line with other Australian herbicide-resistance studies [13,23–25].

3. Results

3.1. Summer Annual Weed Species Occurrence

The surveying of 1430 fields at crop maturity resulted in the identification and collection of a large number of sowthistle (384) populations from across the region (Table 2). Less frequently collected were populations of fleabane (109), feathertop Rhodes grass (80), and awnless barnyard grass (67) populations, and only a low number of windmill grass (15) populations were detected (Table 2). Sowthistle populations were collected during both summer and winter cropping surveys, with the majority of populations (94%) collected during the winter crop surveys of the region. Fleabane and barnyard grass were uniformly distributed throughout northern NSW and the southern Qld area that is north of the 32nd parallel (i.e., Dubbo, central NSW), where the rainfall pattern is typically summer-dominant (Figures 1 and 2) [2]. Feathertop Rhodes grass populations were more concentrated in central and south-eastern Qld (Figure 3), while windmill grass was mostly found in northern NSW.

Table 2. Number of populations collected for each weed species and the proportion of these populations in each plant density range recorded during the 2016/17 random survey of summer annual weed species of Australia's northern grains region.

Weed Species	Populations Collected	Proportion of Populations in Each Plant-Density Range				
		Very Low	Low	Medium	High	Very High
				(%)		
Sowthistle	384	52	36	10	1	1
Fleabane	109	56	28	15	1	0
Feathertop Rhodes grass	80	61	35	3	1	0
Awnless barnyard grass	67	37	34	26	3	0
Windmill grass	15	86	7	7	0	0

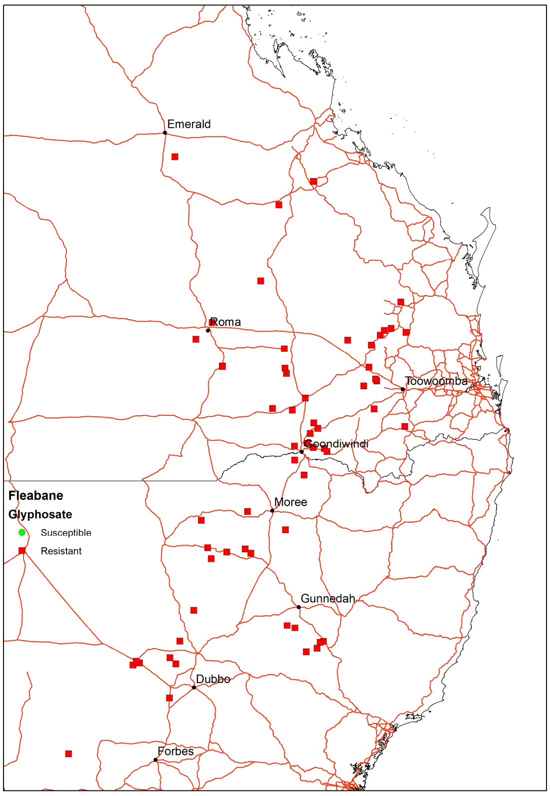

Figure 1. Map of glyphosate-resistant fleabane populations collected during end-of-season random surveys of the northern grains region in 2016 and 2017.

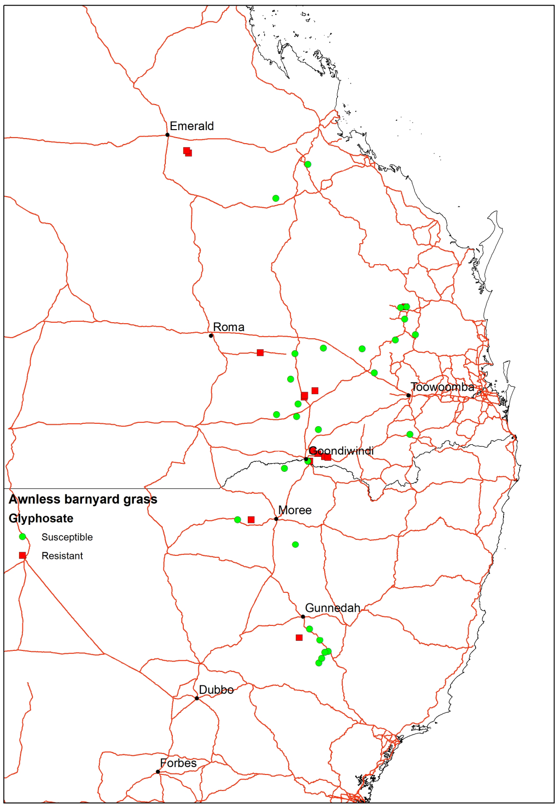

Figure 2. Map of glyphosate-resistant and susceptible awnless barnyard grass populations collected during end-of-season random surveys of the northern grains region in 2016 and 2017.

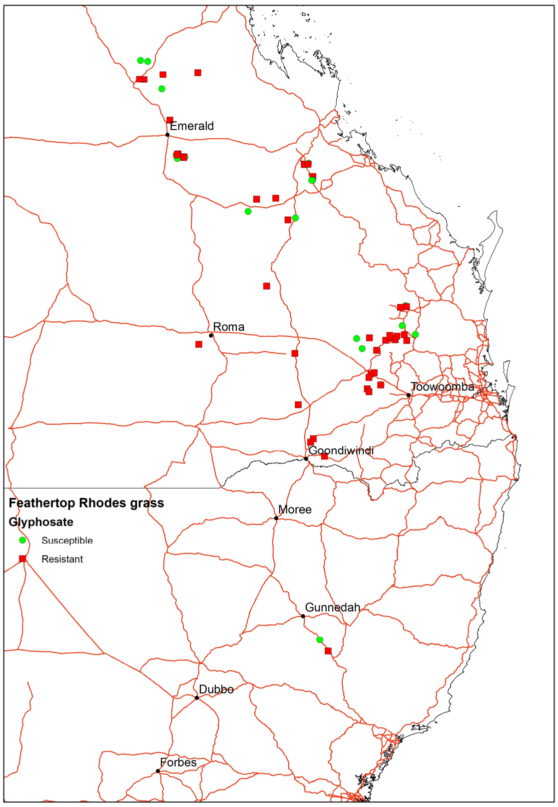

Figure 3. Map of glyphosate-resistant and susceptible feathertop Rhodes grass populations collected during end-of-season random surveys of the northern grains region in 2016 and 2017.

Wherever populations of summer annual weed species were detected in crop and fallow fields at the end of the growing season, they were present at medium to very low densities. The majority of observed plant densities for all collected populations were very low (<1 plant 10 m^{-2}) or low (<1 plant m^{-2}) (Table 2). Sowthistle was the only species where very high densities were reported, with only two populations occurring at >10 plants m^{-2} and dominating crop (Table 2).

3.2. Glyphosate Resistance

Glyphosate resistance was detected in randomly collected populations of all species with particularly high frequencies (>50%) recorded in fleabane, feathertop Rhodes grass, and windmill grass. The highest frequency of glyphosate resistance was found in fleabane, with all collected populations confirmed resistant (Table 3). Similarly, a majority of feathertop Rhodes grass (68%) and windmill grass (67%) populations were resistant to glyphosate. There was a lower but substantial frequency of resistance in awnless barnyard grass (33%). Glyphosate resistance was present but at much lower levels in sowthistle (8%) populations.

Table 3. Proportion of summer annual weed species populations randomly collected from across Australia's northern grains region at the end of 2016/17 and 2017/18 summer growing seasons that are resistant to specific herbicide treatments.

	Sowthistle	Fleabane	Feathertop Rhodes Grass	Awnless Barnyard Grass	Windmill Grass
Populations tested	337	61	62	42	12
	Proportion of screened populations that were resistant to herbicide treatments (%)				
Glyphosate	8	100	68	33	67
2,4-D amine	0	0	-	-	-
Chlorsulfuron	67	-	-	-	-
Bromoxynil + pyrasulfotole	0	-	-	-	-
Haloxyfop	-	-	2	-	-
Clethodim	-	-	0	0	0
Paraquat	-	-	0	-	-
Propaqizafop	-	-	-	0	0
Imazapic	-	-	-	0	40

Some cropping areas of southeast Qld and northern NSW had high concentrations of glyphosate-resistant populations of summer annual weeds. Fleabane was uniformly resistant wherever collected across northern NSW and southeast Qld (Figure 1). Resistant awnless barnyard grass (Figure 2) and feathertop Rhodes grass (Figure 3) populations were more common in southeast Qld, while the few resistant windmill grass populations were mostly found in northern NSW. Glyphosate-resistant sowthistle populations were more frequent in southeast Qld and northern NSW (Figure 4).

Across the northern grains region, glyphosate resistance was common, occurring in 30% of surveyed fields from which viable populations of sowthistle, fleabane, feathertop Rhodes grass, awnless barnyard grass, or windmill grass had been collected (Figure 5). Concerningly, in 5% of fields, glyphosate resistance was present in at least two of these summer annual weed species.

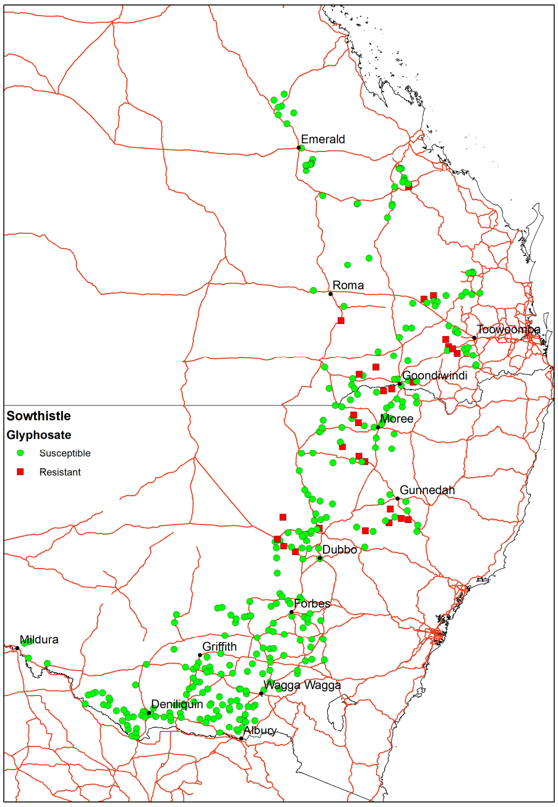

Figure 4. Map of glyphosate-resistant and susceptible sowthistle populations collected during end-of-season random surveys of the northern grains region in 2016 and 2017.

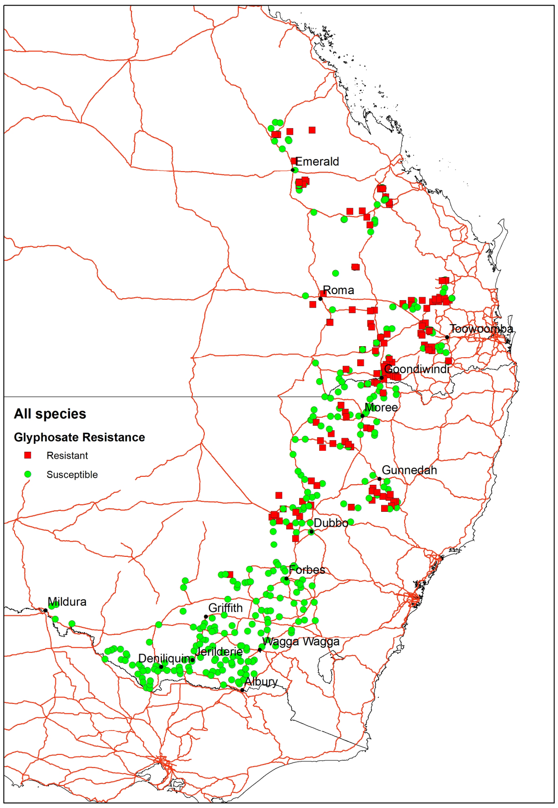

Figure 5. Distribution of glyphosate resistance in randomly collected populations of summer annual weed species occurring in Australia's northern grains region.

3.3. Resistance to Chlorsulfuron, Haloxyfop, and Imazapic

The collected summer weed populations were screened with eight herbicides that, because of glyphosate resistance, are increasingly being used for their selective control. There was a very high frequency of chlorsulfuron resistance in sowthistle populations (67%) (Table 3), which was found to be uniformly distributed throughout the NSW and Qld cropping regions (Figure 6). The majority of chlorsulfuron-susceptible sowthistle populations occurred in the southern NSW cropping region. A similarly high frequency of imazapic resistance was identified in windmill grass populations (40%); however, there were only a limited number of populations (12) available for herbicide screening (Table 3). Given the low number of populations available for screening, there is only limited confidence that this result is representative of imazapic resistance frequency in this species for the northern grains region. Resistance to haloxyfop was identified in just one feathertop Rhodes grass population.

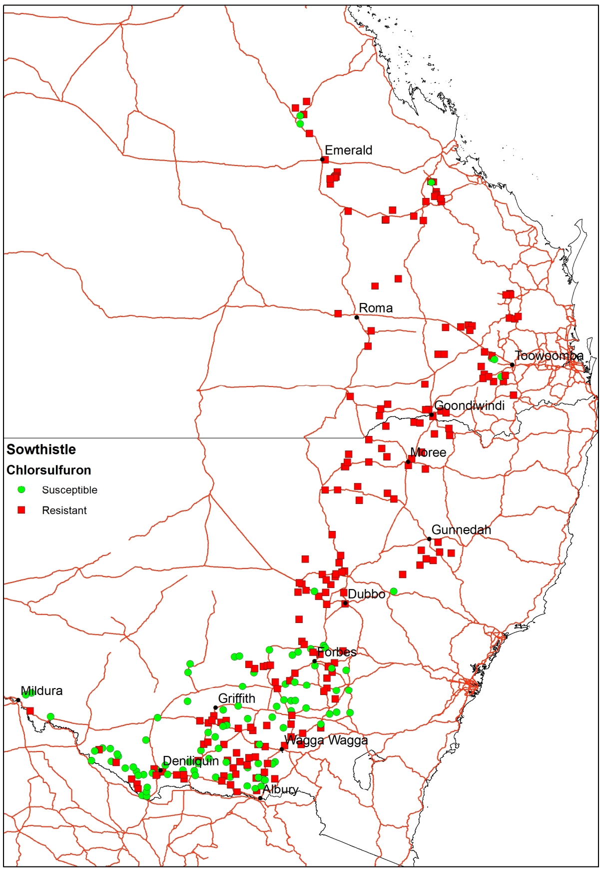

Figure 6. Map of chlorsulfuron-resistant and susceptible populations of sowthistle collected during end-of-season random surveys of the northern grains region in 2016 and 2017.

3.4. Other Herbicides

The comprehensive screening of randomly collected summer weed populations with the range of treatments used to control summer annual weeds confirmed susceptibility to several important herbicides. Pyrasulfotole plus bromoxynil and 2,4-D amine were effective on all sowthistle populations, with the 2,4-D amine also uniformly effective on all tested fleabane populations (Table 3). Clethodim controlled all populations of the three summer annual grass species, while propaquizafop also controlled awnless barnyard grass and windmill grass. These results indicate that several acetyl-CoA carboxylase-inhibiting herbicides are, at this time, highly effective on the summer annual grass species.

4. Discussion

The widespread occurrence of glyphosate resistance in five problematic summer annual weed species is a significant threat to crop production in Australia's northern grains region. The identification of glyphosate resistance in sowthistle, fleabane, feathertop Rhodes grass, windmill grass, and barnyard grass is not surprising, as the occurrence of resistance in these species has previously been reported [14–16,18,19]. This study, however, confirmed that at the time of these surveys, glyphosate resistance was already occurring at high frequencies in randomly collected populations of these weed species. The uniformly glyphosate-resistant fleabane populations identified in this study are particularly concerning but not unexpected. A high frequency of glyphosate resistance (73%) was detected in populations of this weed collected from the region's cotton production fields in 2014 and 2015 [29].

Glyphosate resistance was found at consistently higher frequencies in cropping fields of northern NSW and southeast QLD areas where there is a reliance on the storage of soil moisture from summer rainfall for winter crop production. Across these areas, the reliance on glyphosate for weed-free summer fallows has resulted in an intense selection for resistance evolution. Each summer rainfall event frequently leads to the emergence and subsequent control of a weed cohort, the consequence of which is multiple applications of glyphosate during a single fallow period. In a fallow system, there is an absence of crop competition effects on weed plants, which has been shown to support herbicide activity even on resistant plants [30]; therefore, in the absence of crop competition, weeds surviving herbicide treatments will thrive to produce more seed and subsequently larger seedbanks [31].

The very high frequencies of chlorsulfuron resistance in sowthistle populations indicate the potential evolutionary risk of resistance of this weed to additional herbicides, including glyphosate, which currently remains mostly effective on this species. Chlorsulfuron resistance was first reported in a sowthistle population from the northern grains region nearly three decades ago [21] but now occurs at very high frequencies across the region. As this species is ubiquitous throughout the northern grains region, occurring in both summer and winter production systems, the high potential for resistance evolution poses a significant threat to grain production.

Herbicide resistance in the northern grains region is characterised by high frequencies of glyphosate resistance and only low frequencies of resistance to the selective herbicides (Table 3). This pattern of resistance types is especially evident in the summer-rainfall-dominant northern NSW and QLD regions of this survey (Figure 5). In contrast, the winter-rainfall-dominant regions of this and other surveys of Australian cropping regions have commonly identified high frequencies of selective herbicide resistance and only low levels of glyphosate resistance in the endemic winter annual weed species. For example, no sowthistle population samples from the winter-rainfall-dominant region were found to be glyphosate-resistant; however, high frequencies of resistance were detected in populations collected from northern NSW and QLD (Figure 4).

Resistance surveys across south-eastern Australia, including the same paddocks in southern NSW as this research, targeting annual ryegrass (*Lolium rigidum* Gaudin), found only 4% of populations to be resistant to glyphosate compared with 64% and 63% of the selective herbicides diclofop-methyl and sulfometuron, respectively [13]. Similar results have been reported in the most recently published surveys in Western Australia, with only 1% of populations resistant to glyphosate compared with 72% and 89% for resistance recorded in diclofop-methyl and sulfometuron, respectively [24]. In these surveys, many other selective herbicides (e.g., clethodim, trifluralin) also had higher resistance frequencies than glyphosate.

Other species (wild oats, wild radish) have recorded similar findings and higher resistance to selective herbicides compared with glyphosate after collection from the same fields during the same surveys [32,33]. For these species, glyphosate is usually applied not for summer fallow weed control but as a pre-sowing weed control and often followed by a selective pre-emergent herbicide and the planting of the subsequent crop. The differences in the frequency of resistance for the knockdown fallow herbicide glyphosate and the selective in-crop herbicides in winter annual species compared with the summer annual species reported in this paper further emphasize the increased risk of resistance developing when herbicides are applied without a subsequent alternative control and a crop planted soon after herbicide application.

Despite high frequencies of glyphosate resistance in the major summer annual weed species, there are herbicide options that, at this time, provide effective control of resistant populations. All fleabane and sowthistle populations were controlled by 2,4-D amine, while haloxyfop, propaquizafop, and clethodim, the herbicides commonly used to control these summer annual grass species in summer crops, controlled all but one population of feathertop Rhodes grass. However, as indicated by the single haloxyfop-resistant population, these alternate herbicide treatments must be used sparingly to avoid continuing resistance evolution.

5. Conclusions

The high frequencies of glyphosate resistance in summer annual weed populations are a major threat to winter and summer annual crops of Australia's northern grains region. The widespread evolution of glyphosate resistance in fleabane, feathertop Rhodes grass, and awnless barnyard grass has greatly restricted the ability to control these species in summer fallows. Given that, as identified here, there is already resistance to alternative herbicide options, there are doubts about the long-term capability of maintaining weed-free fallow phases for productive winter crops. As these selective herbicides are commonly used to control summer annual weeds in summer crops selectively, the ability to grow these crops successfully is also threatened by resistance. As herbicide resistance will continue to evolve in summer annual weeds of the northern grains region, there is an urgent need for effective, alternative weed-control technologies to be included in weed-management programs.

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