Characterisation and Management of Herbicide Resistance in Barley Grass (*Hordeum glaucum* Steud.)

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ABSTRACT

Hordeum glaucum has emerged as a problematic weed in cereal and broadleaf crops of South Australia (SA). Recent reports from growers and agricultural advisors in SA have indicated an increase in the incidence of *H. glaucum* in field crops. The increase in the incidence was suspected due to the evolution of herbicide resistance and an increase in seed dormancy in *H. glaucum* populations. Initially, dose response studies confirmed high levels of resistance to (aryloxyphenoxypropanoate) APP acetyl-coenzyme A carboxylase (ACCase)-inhibiting herbicides in the populations where growers had reported control failures with ACCase-inhibiting herbicides. As a result of previous reports of an increase in seed dormancy and confirmation of herbicide resistance in *H. glaucum*, it was considered important to investigate herbicide resistance status and seedbank behaviour of field populations of this weed species. Therefore, studies were conducted to characterise herbicide resistance, study seedbank behaviour, inheritance of resistance, fitness penalties associated with herbicide resistance and alternative herbicides for the management of ACCase-inhibiting herbicide-resistant *H. glaucum* in field peas. A field survey was undertaken in the Upper North and Eyre Peninsula regions of SA in October 2012. Of the 90 *H. glaucum* populations screened for resistance to quizalofop, 14% exhibited some level of resistance and 86% were susceptible. Resistance to ALS-inhibiting herbicides (imazamox+imazapyr and sulfosulfuron) was low (3% to 12% populations). The majority of *H. glaucum* populations emerged rapidly (median T_{50} = 8d), but some populations displayed an extremely slow emergence pattern with T_{50} >20 d. There was no direct linkage between seed dormancy and herbicide resistance. The majority of *H. glaucum* populations showed a low level or no seedbank persistence but a few populations persisted for one year (up to 20% seedbank persistence). Dose–response studies confirmed that *H. glaucum* populations had variable levels of resistance to both ACCase and ALS-inhibiting herbicides, with greater resistance to ACCase-inhibiting herbicides. Gene sequencing

confirmed the presence of previously known mutations Ile-1781-Leu, Ile-2041-Asn and Gly2096Ala in the *ACCase* gene of some *H. glaucum* populations. No amino acid substitution was found in the *ALS* gene of resistant populations, but the reversal of SU resistance by malathion (a cytochrome P450 inhibitor) and susceptibility to sulfometuron suggest that non-target site mechanisms confer resistance to ALS-inhibitors in this species. The mode of inheritance of resistance to ACCase-inhibiting herbicides was identified as a single gene with a partially-dominant allele. Fitness studies conducted under intraspecific competition and/or interspecific competition in pots and the field with wheat and lentil revealed that the amino acid substitution at 1781 position of the ACCase gene did not impose any fitness costs, but there was some evidence for fitness cost associated with Ile-2041-Asn mutation in *H. glaucum* populations. To identify alternative herbicides to control ACCase-inhibiting herbicide-resistant *H. glaucum*, a range of pre- and post-emergent herbicides were examined in field peas. The results of this investigation suggest that propyzamide or pyroxasulfone applied PP and POST imazamox could be used effectively in the field for the management of ACCase-inhibiting herbicide-resistant *H. glaucum* in South Australia.

PUBLICATIONS ARISING FROM THIS THESIS

- Shergill LS, Malone J, Boutsalis P, Preston C, Gill GS (2015) Target-site point mutations conferring resistance to ACCase-inhibiting herbicides in smooth barley (*Hordeum glaucum*) and hare barley (*Hordeum leporinum*). Weed Science 63(2):408-415

- Shergill LS, Fleet B, Preston C, Gill G (2015) Incidence of herbicide resistance, seedling emergence and seed persistence of smooth barley (*Hordeum glaucum*) in South Australia. Weed Technology 29(4):782-792

- Shergill LS, Malone J, Boutsalis P, Preston C, Gill G (2015) Multiple resistance to ACCase and ALS-inhibitors in *Hordeum glaucum* Steud. Pest Management Science: Submitted

- Shergill LS, Malone J, Boutsalis P, Preston C, Gill G (2015) Inheritance and mechanism of quizalofop resistance in smooth barley (*Hordeum glaucum*) biotype from South Australia. Weed Science: Submitted

- Shergill LS, Boutsalis P, Preston C, Gill G (2015) Fitness costs associated with 1781 and 2041 ACCase–mutant alleles conferring resistance to herbicides in *Hordeum glaucum* Steud. Crop Protection: Submitted

- Shergill LS, Fleet B, Preston C, Gill G (2015) Management of ACCase-inhibiting herbicide-resistant smooth barley (*Hordeum glaucum*) in field pea (*Pisum Sativum*) with alternative herbicides. Weed Technology: In-press

- Shergill LS, Preston C, Boutsalis P, Malone J, Gill G (2014) Amino acid substitutions in *ACCase* gene of barley grass (*Hordeum glaucum* Steud.) associated with resistance to ACCase-inhibiting herbicides. Pages 7-10 In Proceedings of 19th Australasian Weeds Conference Hobart, Tasmania, Australia: Tasmanian Weed Society

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CHAPTER 1

REVIEW OF LITERATURE

1. **Herbicide use in weed control**

The use of herbicides in weed control started around the end of 19th century (Hay 1974). During the 1890s, research on the development of inorganic chemicals for weed control began in Europe. Various inorganic chemicals such as: lime, sodium chloride, copper sulphate, iron sulphate, sulphuric acid, nitric acid, sodium arsenite, carbon bisulfide, sodium chlorate, sodium borate, salt of dinitrophenol, ammonium sulfamate, sodium pentachlorophenate, kerosene, and gasoline were used to control weeds from 19th to mid-20th century (Timmons 1970). Although, copper sulphate was first used for selective weed control in wheat, the arsenicals were the first chemicals to be tested and commercially used as herbicides. The discovery of phenoxyacetic acid herbicides during the 1940s in Britain and USA marked the beginning of herbicide phase of the "Chemical Era of Agriculture" (Timmons 1970). This discovery ushered in a series of discoveries and development of the aliphatic acids, carbanilates and their derivatives (Timmons 1970).

These inventions provided additional herbicides to farmers for the control of weedy grasses. Herbicides were promptly adopted by farmers because of their benefits over other methods of weed control. Due to the increase in demand for herbicides, the herbicide market increased at 6.3% per annum up to 1970s (Cobb and Kirkwood 2000). The success of the phenoxy herbicides created interest among governments and in the industry that provided the stimulus to start weed research as a new science. The number of herbicides in Weed Science list of the Weed Science Society of America of common and chemical names was 15 in 1940 and rose to 25 in 1950 (Timmons 1970). Thousands of new agricultural chemicals (mostly organic compounds) were tested after 1950 and

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approximately 120 herbicides were included in the Weed Science list of common and chemical names by 1969 (Timmons 1970). After another 40 years, the list compiled by the Weed Science Society of America includes 374 herbicides with 16 herbicide modifiers (WSSA 2010). Herbicide modifiers are chemical substances used with herbicides to change their herbicidal properties by a physiological mechanism. They include safeners, synergists, extenders, etc., but do not include compounds such as surfactants that may modify herbicidal activity by chemical or physical mechanisms (WSSA 1994).

Herbicides have helped to increase global food production to feed the ever increasing human population. They have been the most reliable, least expensive methods of weed control, and have helped to harvest the most profitable crops from the field. Although herbicides have made a large contribution to increasing world food production, their ongoing success has been endangered by the evolution of herbicide resistance.

2. Herbicide resistance

Herbicide resistance is a striking example of 'evolution in action'. The heavy reliance on herbicides as the primary method of weed control in cropping systems has resulted in reduction in the use of other methods. Whilst economically this approach has been rewarding to farmers, it has led to herbicide resistance in weeds, just as had happened to pests with fungicides and insecticides. Herbicide resistance, as defined by Powles et al. (1997) is the inherited ability of a weed population to survive a herbicide application that is normally lethal to the vast majority of individuals of that species. In 1970, resistance in *Senecio vulgaris* to simazine and atrazine was reported, which is regarded as beginning of herbicide resistance era (Ryan 1970). Concurrently, various other species of *Amaranthus* and *Chenopodium* were also reported resistant to triazines (Holt 1992). Subsequently, the number of herbicide-resistant weeds has increased at an alarming rate. In the 56 years since Harper predicted the evolution of herbicide resistance (Harper 1956), the world database shows there are 383 resistant biotypes of 250 weed species (145 dicots and 105

monocots) (Heap 2016). In the last few decades, herbicide resistance has been documented across the six continents of the globe.

In Australia, the first case of herbicide resistance was reported in 1980 (Heap and Knight 1982). The numbers of reported herbicide-resistant weeds since then have increased dramatically. According to '*The International Survey of Herbicide-resistant Weeds*', Australia has the second highest number (81) of resistant weed biotypes after US with 151 (Heap 2016). *Lolium rigidum* Gaudin, which is the most widespread weed in southern Australia, has the highest number of resistant biotypes in Australia. *Avena fatua* L.*, A. Sterilis* sp. *ludoviciana,* and *Raphanus raphanistrum* L. are among the other major resistant species in Australia (Preston et al. 1999). Some of the reasons for widespread herbicide resistance in Australia include heavy reliance on ACCase and ALS-inhibiting herbicides in continuous cropping systems and high weed density of weeds like *L. rigidum* (Gill 1996; Preston et al. 1999). Herbicide resistance to all the major groups has been reported in weed species around the world (Figure 1).
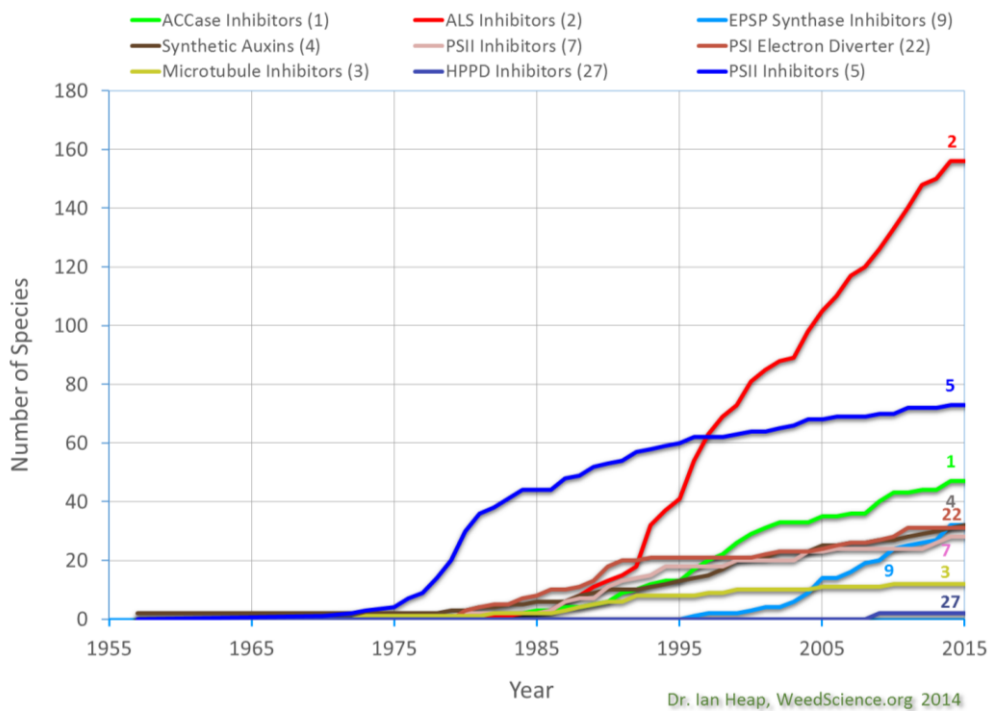
Figure 1: Number of resistant weed species to different herbicide groups in the world (Heap 2015)

3.   Factors contributing to the evolution of herbicide resistance

Herbicide resistance is an evolutionary phenomenon in plant species (Maxwell and Mortimer 1994). In response to repeated treatment with the same group of a herbicide, weed populations change in genetic composition to adapt to intense selection pressure imposed by the herbicide (Jasieniuk et al. 1996). Many factors contribute to the evolution of herbicide resistance. They include the frequency of resistant alleles in natural population, the intensity of selection, the mode of inheritance of resistance, gene flow within and between populations, the nature and extent of herbicide use, and the relative fitness of susceptible and resistant biotypes in the presence and absence of herbicide (Diggle et al. 2003; Jasieniuk et al. 1996; Powles et al. 1997).

3.1 Genetic variation

Genetic variation is a pre-requisite for the evolution of herbicide resistance in a susceptible population. The presence of appropriate variation on which selection can act is the key to the evolution of resistance. Genetic variation could occur by mutation (or recombination) or could be pre-existing (Maxwell and Mortimer 1994). Resistant traits are expressed in a population either as a major gene, or genes, could be present at lower frequencies, or mutate and expressed due to selection from a susceptible population (Maxwell and Mortimer 1994; Preston and Powles 2002). The probability of a resistant mutant arising in a weed population is directly related to the size of the population (Jasieniuk and Maxwell 1994). Some populations may not have sufficient number for the occurrence of a resistant plant. Generally, out-crossing species are likely to be more variable, with large population size, than inbreeding species (Charmet et al. 1996).

3.2 Selection

The evolution of herbicide resistance in weeds is directly related to the intensity of the selection pressure imposed by herbicides. Intense selection pressure coupled with genetic

diversity, provide stimuli for rapid evolution of herbicide resistance (Maxwell and Mortimer 1994). If resistant traits are present in a genetically variable natural population, even at low frequencies, the recurrent selection of only these traits with the repeated herbicide application will increase the frequency of resistant individuals. The use of highly selective herbicides results in rapid evolution of herbicide resistance (Jasieniuk and Maxwell 1994). The selection pressure increases with the application of herbicides that possess a single target site and specific mechanism of action, long term soil residual activity, and frequent and continuous application of the same herbicides (Jasieniuk et al. 1996; LeBaron and McFarland 1990).

3.3 Genetic inheritance

Inheritance is the process of passing of genetic traits from a parent to its offspring. The heritability of traits is governed by nuclear and cytoplasmic inheritance (Rao 2000). Pollen and ovules are the propagules for transmission of nuclear inheritance, whereas, transmission of cytoplasmic inheritance occurs only through ovules (i.e. maternal parent) (Rao 2000). The majority of resistance to various classes of herbicides is associated with nuclear genes (Jasieniuk and Maxwell 1994). Seefeldt et al. (1998) showed that diclofop resistance due to insensitive target-site in *Avena fatua* L. biotypes is controlled by a single nuclear gene, with resistance being dominant at lower herbicide doses and susceptibility being dominant at increased doses. Wang and Darmency (1997) demonstrated that sethoxydim resistance in *Setaria italica* L. was controlled by a single, completely dominant, nuclear gene and later the resistance was confirmed to be due to the 1781 mutation (Délye et al. 2002). Inheritance of resistance to fenoxaprop in a *Alopecurus myosuroides* Huds. population with insensitive ACCase-enzyme and an enhanced herbicide metabolism is conferred by two dominant and independent nuclear genes (Letouzé and Gasquez 2001). However, inheritance of triazine resistance is cytoplasmic in most species, but in *Abutilon theophrasti* it is controlled by nuclear genes (Andersen and

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Gronwald 1987). The chloroplast genome contains the gene for the triazine target site, the D1 protein of Photosystem II, and therefore resistance is passed with the chloroplasts from the maternal parent (Hirschberg and Mcintosh 1983). A notable exception to the partial or complete dominance of herbicide resistance is trifluralin resistance in *Setaria viridis*, which is determined by a single, recessive allele (Jasieniuk et. al. 1994).

3.4 Gene flow

Gene flow is an important phenomenon for herbicide resistance evolution in weeds. It can occur through seed or pollen migration. Gene flow through pollen plays a vital role in genetic variation in natural populations by increasing the frequency of resistant alleles (Campbell and Waser 2001; Hidayat et al. 2006). Pollen migration is usually the mode of transmission of genetic traits in cross-pollinated species, whereas, in self-pollinated species gene flow occurs through seed migration (Darmency 1996). If the rate of gene flow is higher than the rate of mutation this will result in higher frequency of resistant plants in the recipient population (Jasieniuk et al. 1996; Rao 2000). Gene flow helps in the spread of herbicide-resistant alleles in or among populations (Jasieniuk et al. 1996). Gene flow can occur from resistant plants to susceptible plants in the same field or to/from adjacent fields (Hidayat et al. 2006; Preston et al. 1996). The primary source of gene flow for maternally inherited traits are the seeds, whereas, pollen and seed transmit nuclear inherited resistant traits (Darr et al. 1981). The role of pollen in spread of resistance has been calculated to be around 1% in the populations within a few hundred metres vicinity, which is very low (Jasieniuk et al. 1996). Thus, its role in herbicide resistance spread has largely been ignored. However, gene flow with seed dispersal could be important in case of self-pollinated species where pollen flow probability is minimal.

3.5 Fitness

Fitness is the measure of survival and reproduction of a viable offspring under a selection pressure. It is a relative term whereby inter-genotypic comparisons are made relative to the fit populations. If there are no differences in the fitness of two biotypes or resistant/susceptible plants, their relative frequency will not be affected during periods when herbicide is not used. However, if the resistant populations suffer a fitness penalty, their relative frequency will decrease in the absence of herbicide selection pressure (Gill et al. 1996). Whether there will be a decrease in the frequency of resistant traits depends upon the fitness cost; the higher the fitness cost the sooner the replacement of resistance with susceptibility is likely to occur. Thus, herbicide rotations can be used to delay evolution of herbicide resistance, but may lead to multiple resistance evolution (Jasieniuk and Maxwell 1994). Purba et al. (1996) argued that gene modification or association of resistant individuals with deleterious genes could be the possible source of reduced fitness. Herbicide resistance has been associated with a fitness penalty, e.g. in case of triazine resistant broadleaf weeds, it was observed that the number of resistant weeds decreased when triazine herbicides were not sprayed (Gill et al. 1996; Holt and Thill 1994). Surprisingly, many resistance mechanisms do not have measurable associated fitness cost. For example, *H. leporinum* resistant biotypes (paraquat resistant) did not show any fitness penalty relative to susceptible genotype (Purba et al. 1996). Warwick and Black (1994) reported that there were no consistent differences in relative fitness for non-triazine resistant and susceptible biotypes; however, for triazine resistant weed species, resistant plants were generally less fit than susceptible plants, although exceptions do exist. Lower relative fitness of resistant biotypes than susceptible biotypes of *H. glaucum* and *H. leporinum* from the same area has also been reported (reviewed in Powles and Howat 1990).

Theoretically, herbicide-resistant biotypes should have a fitness cost, although it may not be detectable. For the fitness estimates, measures throughout the plant life cycle should be taken in order to encompass the effects of selection on mortality and fecundity (seed set) of survivors (Maxwell and Mortimer 1994). Fitness is a dynamic entity and can change over time with the selection under different environments for more fit individuals (Maxwell and Mortimer 1994).

4. Mechanisms of herbicide resistance

There are number of mechanisms conferring herbicide resistance: modified target site, enhanced detoxification, reduced absorption or translocation, sequestration or compartmentation, and repair of the toxic effects of herbicides. However, these can be grouped under two broad categories, i.e. target site and non-target site resistance.

4.1 Target site resistance

Herbicides act on proteins by binding or otherwise interacting with them, thereby exhibiting negative effects on plant growth or metabolism. Target site proteins can be altered by mutations, which reduces or eliminates the ability of herbicides to bind or interact with them; alternatively, resistant plants can also over-produce these herbicide-binding proteins (Preston and Mallory-Smith 2001). In this type of resistance, the herbicides reach the site of action at lethal doses but are unable to exhibit the lethal action due to changes at the target site (Powles and Yu 2010).

Photosystem II (PS II) inhibiting herbicides (triazines, ureas and nitriles) block the electron transport chain on the reducing side of PS II leading to production of excess singlet oxygen, resulting in the destruction of lipids and chlorophyll (Preston and Mallory-Smith 2001). In most triazine-resistant plants, the herbicide binding domain on D1 protein of PS II can change. The molecular basis of this change is a single amino acid substitution of Ser 264 to Gly in the D1 protein, which removes a hydrogen bond important for

herbicide binding. Mutations in the D1 protein have been reported in species such as *Potulaca oleraceae* and *Poa annua* with changes of Ser 264 to Thr and Val 219 to Ile, respectively. The Ser 264 to Thr mutation confers resistance to linuron and atrazine, while, the Val 219 to Ile substitution confers resistance to diuron and metribuzin. The Ser 264 to Thr change most likely interferes with the entry of herbicide to Q_B site, which results in herbicide resistance (reviewed in Preston and Mallory-Smith 2001).

Acetolactate synthase (ALS) or acetohydroxyacid synthase (AHAS) inhibiting herbicides include five families i.e. sulfonylureas (SUs), imidazolinone (IMIs), pyridinyloxybenzoate (PTBs), triazolopyrimidine (TPs) and sulfonylamino-carbonyl-triazolinone (SCTs) (Singh and Shaner 1995). AHAS act as a catalyst in the formation of aceto-hydroxybutyrate and acetolactate. These five families of herbicides act on AHAS, thereby stopping synthesis of the branched chain amino acids (valine, leucine and isoleucine) resulting in plant death. In resistant weeds, the amino acid substitutions in the protein sequence at target-sites i.e. Pro 197, Ala 205, Asp 376, Trp 574, Ser 653 and Ala 122 confer resistance to ALS-inhibiting herbicides (Yu et al. 2008).

ACCase-inhibiting herbicides from three different chemical families i.e. aryloxyphenoxypropionate (APPs), cyclohexanedione (CHDs) and phenylpyrazolin (DENs) inhibit acetyl-coenzyme A carboxylase (ACCase) enzyme in graminaceous weeds (Sasaki et al. 1995). This enzyme is responsible for fatty acid synthesis in plants (Burton et al. 1991). ACCase inhibiting herbicides are used for grass control in dicots because grasses contain two different ACCase enzymes with about 80% of the activity associated with the plastid form. The plastid ACCase of dicots is structurally different from that of grasses and is insensitive to ACCase inhibitor herbicides (Preston and Mallory-Smith 2001). This difference in the sensitivity to ACCase-inhibiting herbicides of plastid ACCase of grass weeds and broadleaf crops allows their safe use for the selective control of grasses. Resistance to this herbicide group is caused by amino acid substitution in the

carboxyltransferase (CT) domain of the ACCase gene (Délye 2005) or due to active exclusion or compartmentation of the herbicide from the site of action, reduced herbicide absorption or translocation or both (Deprado et al. 2000). Target-site resistance is most often reported as the mechanism of resistance to ACCase-inhibiting herbicides (Beckie and Tardif 2012; Délye 2005; Yu et al. 2007b). The amino acid substitutions in the protein sequence at target sites i.e. Ile 1781, Trp 1999, Trp 2027, Ile 2041, Asp 2078, Cys 2088 and Gly 2096 in the *ACCase* gene have been reported to confer resistance to ACCase-inhibiting herbicides (Beckie and Tardif 2012; Kaundun 2014). The Ile-1781-Leu mutation is associated with resistance to APP, some CHD and DEN herbicides. The Trp-2027-Cys or Ile-2041-Asn mutations confer resistance to APP and DEN herbicides. The Asp-2078-Gly mutation and Cys-2088-Arg mutation provides high-level resistance to all three classes of herbicides: APP, CHD and DEN. The Gly-2096-Ala mutation confers resistance mainly to APP herbicides and Trp-1999-Cys only to the APP herbicide fenoxaprop (reviewed in Beckie and Tardif 2012).

Changes to micro-tubule assembly in weeds have been shown to confer resistance to dinitroaniline herbicides. The main herbicides belonging to this group include pendimethalin and trifluralin, which target germinating seeds by inhibiting microtubule growth thereby disrupting cell division. Microtubules are formed with the polymerisation of α and β-tubulin. These herbicides bind to tubulin, prevent polymerisation, hence preventing cell division and elongation (Powles and Yu 2010; Preston and Mallory-Smith 2001).

Glyphosate is the broad spectrum herbicide that is widely used in agriculture throughout the world. It inhibits the chloroplast enzyme EPSP synthase, which disrupts the shikimate pathway of amino acid production, resulting in the death of the plants (Powles and Yu 2010). The amino acid substitution of Ser, Thr and Al at Pro 106 within the putative glyphosate binding site confers resistance to glyphosate (Preston and Mallory-

Smith 2001; Sammons and Gaines 2014; Yu et al. 2007a). Similary, a double mutation (TIPS) in the *EPSPS* gene causing high level of glyphosate resistance in *Eleusine indica* has also been recently reported (Yu et al. 2015). The amplification of EPSPS gene in *Dacus carota* resulted in glyphosate resistance in these species. These are examples of over expression of target site or gene amplification causing herbicide resistance (Gaines et al. 2010; Goldsbrough et al. 1990; Shyr et al. 1992).

4.2 Non-target site resistance

In non-target site resistance, the herbicide is unable to reach the target site at a lethal dose, due to modification of mechanisms in plants: e.g. decreased rates of herbicide penetration/translocation and increase in herbicide sequestration/metabolism (Powles and Yu 2010). These mechanisms limit the availability of herbicides at the target-site. Various other plant modifications such as hairy epidermis and waxy cuticles may also limit the availability of herbicide at the target site. Epicuticular waxes have been reported to reduce glyphosate absorption in *Erythroxylum coca* and diclofop penetration in *L. rigidum* (Ferreira and Reddy 2000; Prado et al. 2001). Similarly, glyphosate resistance in *Lolium multiflorum* has also been reported, due to lower spray retention, lower foliar uptake, and altered translocation pattern (Michitte et al. 2007). Selective herbicides are safe to crops generally due to the ability of the crop to rapidly detoxify them. Target weed species often also have the ability to detoxify herbicides to some extent, but cannot detoxify them at a rate required to escape death (Preston and Mallory-Smith 2001). Yuan et al. (2007) explains that detoxification processes can occur in four phases. In Phase I, herbicide molecules are activated, typically with oxidation. Phase II involves conjugation of a bulky hydrophilic molecule to the activated xenobiotic using thiols or sugars, which is then recognised by Phase III. Phase III involves transporting the conjugated molecule into the vacuole or extracellular space by active transport involving ABC transporters. Further degradation of conjugated molecule in the vacuole or extracellular space is done in phase

IV. The major detoxifying proteins involved in these processes include cytochrome P450 in Phase I, Glutathione transferases (GST's) and glycosyltransferases in Phase II, and ABC transporters in Phase III (Yuan et al. 2007). Research has shown that non-target site resistance to ALS-inhibiting herbicides in weeds is usually due to enhanced rates of herbicide metabolism, often involving   cytochrome P450s (reviewed in Yu and Powles 2014). Non-target site resistance to ACCase-inhibiting herbicides is also conferred by the enhanced metabolism of FOP, DIM and DEN compounds mainly by cytochrome P450s and GSTs (reviewed in Kaundun 2014).

5. Barley grass

Hordeum glaucum Steud. (smooth barley) and *Hordeum leporinum* L. (hare barley), collectively known as barley grass, is a prolific seed producer, inbreeding annual weed of Poaceae family, which can germinate under high osmotic pressure (Campbell et al. 1972; Giles and Lefkovitch 1986; Kloot 1987).

H. glaucum and *H. leporinum* belong to the *H. murinum* complex, which consists of three species: *H. murinum* L., *H. leporinum* Link and *H. glaucum* Steud. (Cocks et al. 1976; Covas 1949). Hubbard (1954), Covas (1949) and Morrison (1958) described the complex to be containing three distinct species (*murinum, leporinum* and *glaucum*); however, Humphries (1980) disagreed and gave them a subspecific status (Cited in Giles and Lefkovitch 1986).

5.1 Biology and ecology

Barley grass is a vigorous winter annual, propagating through its seed (Popay and Sanders 1975). It is an obligate self-pollinated plant (Giles and Lefkovitch 1986; Johansen and von Bothmer 1994). *H. murinum* and *H. leporinum* are both cool season annuals, whereas, *H. glaucum* is a warm season annual (DiTomaso and Healy 2007). As reviewed by Giles and Lefkovitch (1986), *H. murinum* tends to occur in the coolest, wettest regions and

leporinum in hotter, drier Mediterranean-type conditions. Furthermore, the *H. glaucum* appears to be confined to the hottest, driest conditions of the Mediterranean climatic zones. Although native to Europe, western Asia and north Africa, this complex (*Hordeum spp.*) is restricted to the Mediterranean region and western Asia, whereas, *H. glaucum* is only found in dry eastern parts of this region (Cocks et al. 1976). They also distinguished its distribution according to the rainfall, and reported *H. glaucum* to occur in areas having rainfall less than 425 mm, whereas, H. *leporinum* was found in areas with rainfall greater than 425 mm. *Hordeum* sp. starts to germinate with late summer rains from late February to early May with autumn rains, when soil temperatures range from 18-24 C (Biddiscombe et al. 1954; Harris 1961; Popay 1981). Popay and Sanders (1975) also reported its germination continuing through winter to spring.

5.2 Introduction and geographical distribution in Australia

Barley grass was introduced into Australia soon after the European settlement (Smith 1968). Its introduction is associated with the import of wool and farm animals in the nineteenth century from England, South Africa, N-W India and Pakistan and also from eastern Mediterranean, with the opening of the Suez canal (Cocks et al. 1976; Davison 1977).

According to Smith (1968), barley grass is a ubiquitous weed in the annual pasture zone of southern Australia with varying population density depending upon the season. It can be found in cropping as well as non-cropping areas such as roadside verges, sheep and cattle enclosures, building sites and waste ground (Davison 1977). *H. glaucum* has naturalised in Australia and can be found in all states of the continent (Figure 2).
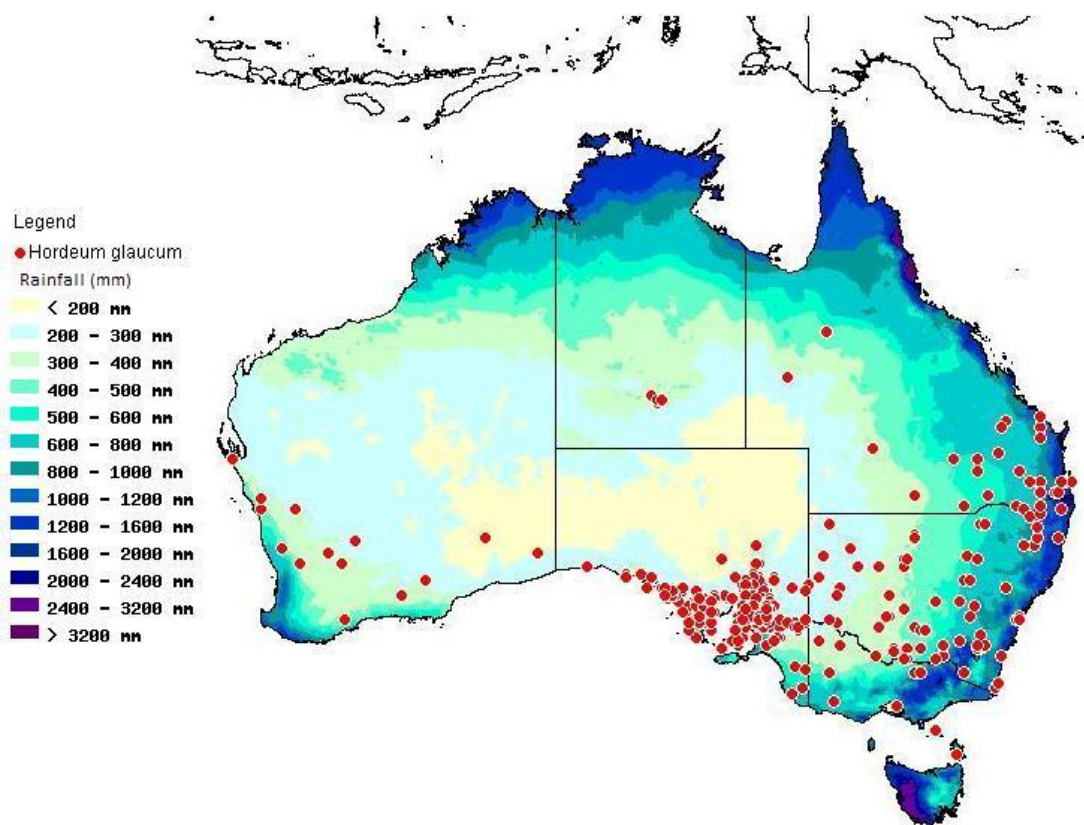
Figure 2: Distribution of *H. glaucum* in Australia (Anonymous 2012)

5.3 Cytology and taxonomy

Jacobsen and Von Bothmer (1995) analysed material from 229 populations and found that *H. glaucum* was a diploid (2n=2x=14), *H. murinum* was a tetraploid (2n=4x=28) and *H. leporinum* was tetraploid (2n=4x=28) as well as hexaploid (2n=6x=42). The results are in agreement with Giles and Lefkovitch (1986). However, Cocks et al. (1976) reported *H. leporinum* only to be a tetraploid.

All three *Hordeum* species are similar in appearance, but Cocks et al. (1976) have illustrated the taxonomy of this complex. According to them, each member of the complex consists of three spikelets at each node of the rachis, in all there may be 20-30 such triads of spikelets. The triad is the seed dispersal unit, which consists of the hermaphroditic central spikelet, while the lateral may be male or sterile or hermaphrodite (Johansen and von Bothmer 1994). According to Cocks et al. (1976), *H. murinum* has sessile (shortly

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pedicellate) central spikelet and lemma awns of the lateral spikelets are shorter than those of the central spikelet, whereas, both *H. leporinum* and *H. glaucum* have pedicellate central spikelets and lemma awns of the lateral spikelets are longer than those of the central spikelet. Further, *H. leporinum* has larger anthers, and a looser spike than *H. glaucum*. These descriptions are in concordance with Covas (1949), who also suggested that *H. glaucum* has no starch grains in the filaments of the anthers.

5.4 Seed germination, phenology and fecundity

The *Hordeum* complex exhibits weak or nil requirement of diurnal temperature fluctuations for maximum germination (Cocks and Donald 1973). Previous studies have shown that the *Hordeum* complex has short-lived innate dormancy; with *H. glaucum* possessing the most and *H. murinum* the least. Furthermore, Popay (1981) has reported 10-22 $^{\circ}$C as the temperature range for maximum germination; with no germination at or above 35 $^{\circ}$C. The overall range of temperature for germination is between 8-30 $^{\circ}$C (Cocks and Donald 1973). On the contrary, Fleet and Gill (2010) recently reported large variation in seed dormancy between *H. glaucum* populations. They found that the populations from cropped fields exhibited high levels of dormancy, while those from fence lines or long-term pastures showed low levels of seed dormancy. In comparison to *Lolium*, *H. leporinum* germinates more rapidly with lesser water content in seed, and germinates from soil surface (Cocks and Donald 1973).

Light is not a pre-requisite for germination of *Hordeum* spp., however burial does not enforce dormancy (Davison 1971; Popay 1981; Popay and Sanders 1975). The minimum and maximum time for flowering, reported by Cocks et al. (1976) for *H. glaucum* was 102 & 144 days from sowing, respectively.

Barley grass is a prolific seed producer. The seed is dispersed as an entire triad by various agents like wind, birds or farm animals (Ridley 1930 cited in Kloot 1985). *H. murinum* produce a seed bank in the range of 708 to 1813 seeds/m^2 (Makarian et al. 2007).

Powles et al. (1992) reported an average population of 987 seeds/m^2 in a field infested with *H. glaucum.* Recent research conducted by Fleet and Gill (2010) reported *H. glaucum* populations of 376 plants/m^2 of with seed production of 8702 seeds/m^2 in the absence of weed control.

5.5 Impact of *Hordeum* spp.

Even though barley grass is readily grazed by animals in pasture in its vegetative stage, it becomes a problem when it matures. The seeds have long barbed awns that irritate the mouths, eyes and noses of the cattle or sheep, or get entangled in wool, resulting in loss of productivity (Campbell et al. 1972; Cocks et al. 1976).

Weeds can be a potential host for various fungi and nematodes. Nematodes are able to multiply or persist in weeds which provide a ready source of inoculum for susceptible crop plants (Belair and Benoit 1996; Townshend and Davidson 1960). The presence of susceptible weeds prior to cropping, or post-harvest in the field enables nematodes with short life cycles such as *Pratylenchus* spp. to produce more generations each year (Vanstone and Russ 2001).

Vanstone and Russ (2001) reported that *H. glaucum, Bromus diandrus and B. rubens* were poor hosts of the root lesion nematode, *Pratylenchus thornei.* However, *Avena sterilis* and *H. leporinum* were good hosts of *P. thornei.* Barley grass also acts a host for *Rhizoctonia solani* Kuhn., which causes patches of poor growth in cereals (Mohammadi et al. 2003; Roget et al. 1987). It also carries soil borne fungus *Gaeumannomyces graminis*, causal organism of Take-all disease in wheat and other cereals (Gutteridge et al. 2005; Kirkegaard et al. 1996; Wong 1975).

5.6 Evolution of herbicide resistance in barley grass

Worldwide adoption of no-till systems began with the commercialisation of paraquat. Studies on paraquat started in the United Kingdom in 1955; however, in Australia, field

experiments with bipyridyl herbicides in no-till started in 1964 (reviewed by Derpsch 1998). With the introduction of herbicides, the use of cultural methods of weed control i.e. tillage, mowing, sheep grazing etc. reduced significantly. No-tillage systems reduce soil erosion, fuel cost, labour requirement, and allowed timely sowing of crops; which were the major reasons for their adoption in Australia (Chauhan et al. 2006; Derpsch 1998; Pratley 1995). Due to short growing seasons and fragile soils, minimum tillage systems have been widely adopted in South Australia (Pratley 1995).

With the adoption of no-till, the weed infestation of some weeds will increase while others may decrease. In a 12 year field investigation to measure the effects of tillage, Legere et al. (2011) discovered that total seedbank density generally increased as tillage was reduced and no-till systems had more weed seeds at or near the soil surface. Their results are in agreement with Conn (2006) and Mohler et al. (2006). In an earlier study, Cocks and Donald (1973) reported that barley grass germinated and established readily on the soil surface. Therefore, it has an advantage in minimum or no-till systems. In a subsequent study in 1987, Medd reported that *H. leporinum* was more prevalent when cultivation intensity was reduced (cited in Pratley 1995).

Barley grass (*Hordeum* spp.) is a problematic weed in field crops and pastures of southern Australia (Cocks et al. 1976; Smith 1972). So far it has evolved resistance to three groups of herbicides; namely, ACCase inhibitors, ALS inhibitors, and bipyridiliums (Heap 2015). The first case of paraquat resistance in *H. glaucum* was reported in early 1980s (Powles 1986; Warner and Mackie 1983). *H. glaucum* had evolved herbicide resistance in fields that had been treated with bipyridyl herbicides annually for many years (Alizadeh et al. 1998; Tucker and Powles 1988). For effective control of paraquat resistant biotypes of barley grass, selective and non-selective (knock-down) herbicides, with alternate mechanisms have been successfully used (Powles and Howat 1990). In 2000, Matthews et al. (2000) reported resistance in *H. leporinum* population to ACCase

inhibiting herbicide fluazifop-p-butyl in South Australia. They also reported moderate cross-resistance to other herbicides of ACCase-inhibiting herbicides (i.e. haloxyfop-ethoxyethyl, quizalofop-p-ethyl, sethoxydim and clethodim). This resistance had evolved under the selection pressure of fluazifop-p-butyl. Recently, herbicide resistance in *H. leporinum* to ALS inhibiting herbicides has been reported in Western Australia (Yu et al. 2007c). In a subsequent study, sulfonylurea and imidazolinone cross-resistance has also been confirmed in ALS-inhibiting herbicide-resistant biotypes of *H. leporinum* (Owen et al. 2012).

5.7 Mechanisms of herbicide resistance

As stated earlier, barley grass has been reported to be resistant to three groups of herbicides i.e. bipyridyl, ACCase and ALS-inhibiting herbicides (Matthews et al. 2000; Owen et al. 2012; Preston et al. 1992; Yu et al. 2007c). Preston et al. (1992) reported reduced translocation of paraquat in the resistant plants of *H. glaucum* to be the possible reason for resistance. In *H. glaucum* the cell wall contributes to reduce movement across it and into the chloroplast; however, there is no difference in cell wall function in the case of paraquat resistant *H. leporinum*. Herbicide sequestration in apoplast, i.e. exclusion of herbicide from cytoplasm, has also been reported as the mechanism of paraquat resistance in *H. glaucum* by other workers (Bishop et al. 1987; Powles and Cornic 1987).

Increased herbicide detoxification has been reported as the mechanism for resistance to fluazifop-p-butyl and other aryloxyphenoxypropionate (APP) herbicides, whereas, a modified target enzyme appears to be mechanism involved in sethoxydim resistance in *H. leporinum* (Matthews et al. 2000). In a later study, resistance to ALS inhibiting herbicides in *H. leporinum* was reported to be caused by a mutation resulting from Pro to Ser substitution (Yu et al. 2007c).

5.8 Management of barley grass

Heavy reliance on herbicides, due to their ease of application and effective control, has reduced the use of other methods of weed control. This has led to widespread herbicide resistance in weeds. Moreover, capture of the major portion of the herbicide market by glyphosate and genetically modified (GM) glyphosate resistant crops have diminished herbicide discovery. There has been no major introduction of new site-of-action herbicides in the last two decades (Beckie and Tardif 2012). Therefore, research is needed to develop strategies to preserve current mode of action of herbicides by delaying resistance evolution.

Use of crop and herbicide rotations, knowledge of seed-bank persistence, identification of alternative herbicides, herbicide mixtures and cultural practices may help in achieving this goal. Genetic variation through mutations and the initial frequency of genes conferring resistance cannot be controlled, but the weed densities present in the fields can be managed. In a simulation modelling study, use of herbicide mixtures was found to delay onset of resistance by four years in finite weed populations (Diggle et al. 2003).

Rotating a cereal based cropping system with other competitive crops like canola could reduce the weed seed bank. Diversifying crop rotations would offer opportunities to control herbicide-resistant barley grass with herbicides of different chemistries. It has been observed that a paraquat resistant biotype of *H. glaucum* can be eradicated by preventing seed set for 3 successive years, due to its short residual life in the seed bank (Powles et al. 1992).

H. glaucum has been reported to be increasing in abundance in cropping systems in South Australia (Fleet and Gill 2010). Adoption of the no-till seeding system has also promoted barley grass establishment or infestation. Recent research by Fleet and Gill (2010) has shown that farming practices used in southern Australia have selected barley

grass populations that possess high levels of seed dormancy, which is broken by exposure to cold temperatures in winter. This is an effective escape mechanism that allows these populations to defer establishment until after the crops have been planted. In the past, non-dormant populations of barley grass could be easily controlled with the use of knockdown herbicides applied in late autumn. But the change in weed biology as reported by Fleet and Gill has raised serious concerns about prospects for increasing incidence of *H. glaucum*.

This change in weed biology has increased the selection pressure of post-emergent herbicides, as these are being increasingly relied on for the control of barley grass in broadleaf crops. Recent investigations of a closely related species *H. leporinum* have confirmed resistance to ACCase-inhibiting herbicides in South Australia (Matthews et al. 2000) and to ALS-inhibiting herbicides in Western Australia (Owen et al. 2012; Yu et al. 2007c). Growers are reporting increasing difficulty in managing barley grass with these herbicides.

The combination of increased seed dormancy with herbicide resistance would make it very difficult for Australian farmers to effectively manage this weed species. A new herbicide pyroxasulfone (Sakura) can provide effective control of barley grass in wheat; however, control of ACCase resistant barley grass in pulse crops would be particularly difficult. Therefore, there is an urgent need to undertake a comprehensive study to quantify herbicide resistance status of barley grass populations on South Australian farms, investigate mechanisms, genetics and fitness of resistant biotypes. If resistance is confirmed then field studies would be needed to identify alternative herbicides for the management of herbicide-resistant populations of this weed species.

6. Summary and knowledge gaps

H. glaucum is a problematic weed that has been reported to be increasing in abundance in the cropping systems of South Australia. It has been observed that the management practices used in cropping systems on South Australian farms have selected barley grass

populations that possess high levels of seed dormancy, which is broken by exposure to cold temperatures in winter. This is an effective escape mechanism that allows these populations to defer establishment until after the crops have been planted. Previously, non-dormant populations of *H. glaucum* could be easily controlled with the use of knockdown herbicides applied in late autumn. This change in weed biology has increased the selection pressure of post-emergent herbicides, as these are being increasingly relied on by the growers for the control of *H. glaucum* in broadleaf crops. Resistance to the ACCase and ALS-inhibiting herbicides has already been identified in Australia in a closely related species *H. leporinum*. Growers are reporting increasing difficulty in managing *H. glaucum* with ACCase-inhibiting herbicides. There is a need to better understand the characteristics of resistance to ACCase-inhibiting herbicides in these species and to identify alternative control methods for resistant populations of this species. Therefore, the work presented in this thesis is designed to address the following objectives:

i. To undertake a survey to collect *H. glaucum* populations from South Australian crops to determine their resistance status and seedbank behavior.

ii. To undertake detailed dose response investigations to quantify the level of resistance (LD_{50} and GR_{50}) in different *H. glaucum* populations.

iii. To undertake laboratory investigations to determine the mechanisms of herbicide resistance including identification of mutations conferring resistance.

iv. To undertake crosses between R and S parents and screen the progeny to determine the mode of inheritance of ACCase resistance in *H. glaucum*.

v. To undertake competition experiments to determine the fitness costs of herbicide-resistant biotypes of *H. glaucum*.

vi. To undertake field studies to identify alternatives to ACCase inhibiting herbicides for use in pulse crops.

7. References

Alizadeh HM, Preston C, Powles SB (1998) Paraquat-resistant biotypes of *Hordeum glaucum* from zero-tillage wheat. Weed Research 38:139-142

Andersen RN, Gronwald JW (1987) Noncytoplasmic inheritance of atrazine tolerance in velvetleaf (*Abutilon theophrasti*). Weed Science 35:496-498

Anonymous (2012) Australia's virtual herbarium. http://www.chah.gov.au/avh/index.jsp. Accessed 22 April, 2012

Beckie HJ, Tardif FJ (2012) Herbicide cross resistance in weeds. Crop Protection 35:15-28

Belair G, Benoit DL (1996) Host suitability of 32 common weeds to *Meloidogyne hapla* in organic soils of southwestern Quebec. Journal of Nematology 28:643-647

Biddiscombe E, Cuthbertson E, Hutchings R (1954) Autecology of some natural pasture species at Trangie, NSW. Australian Journal of Botany 2:69-98

Bishop T, Powles SB, Cornic G (1987) Mechanism of paraquat resistance in *Hordeum glaucum*. II. Paraquat uptake and translocation. Australian Journal of Plant Physiology 14:539-47

Burton JD, Gronwald JW, Keith RA, Somers DA, Gengenbach BG, Wyse DL (1991) Kinetics of inhibition of acetyl-coenzyme A carboxylase by sethoxydim and haloxyfop. Pesticide Biochemistry and Physiology 39:100-109

Campbell DR, Waser NM (2001) Genotype-by-environment interaction and the fitness of plant hybrids in the wild. Evolution 55:669-676

Campbell RJ, Robards GE, Saville DG (1972) The effect of grass seed on sheep production. Proc Australian Society of Animal Production 9:225-229

Charmet G, Balfourier F, Chatard V (1996) Taxonomic relationships and interspecific hybridization in the genus *Lolium* (grasses). Genetic Resources and Crop Evolution 43:319-327

Chauhan BS, Gill G, Preston C (2006) Tillage systems affect trifluralin bioavailability in soil. Weed Science 54:941-947

Cobb AH, Kirkwood RC (2000) Challenges for herbicide development. Pages 1-24 *in* Cobb AH, Kirkwood RC, eds. Herbicides and their mechanisms of action Sheffield: Sheffield Academic Press

Cocks PS, Boyce KG, Kloot PM (1976) The *Hordeum murinum* complex in Australia. Australian Journal of Botany 24:651-62

Cocks PS, Donald CM (1973) The germination and establishment of two annual pasture grasses (*Hordeum leporinum* Link and *Lolium rigidum* Guad.). Australian Journal of Agricultural Research 24:1-10

Conn JS (2006) Weed seed bank affected by tillage intensity for barley in Alaska. Soil & Tillage Research 90:156-161

Covas G (1949) Taxonomic observations of the North American species of *Hordeum* Madrono 10:1-21

Darmency H (1996) Movement of resistance genes among plants. Pages 209-220 Molecular Genetics and Evolution of Pesticide Resistance: American Chemical Society

Darr S, Machado VS, Arntzen CJ (1981) Uniparental inheritance of a chloroplast Photosystem II polypeptide controlling herbicide binding. Biochimica et Biophysica Acta (BBA) - Bioenergetics 634:219-228

Davison AW (1971) The ecology of *Hordeum Murinum* L.: II. The ruderal habit. Journal of Ecology 59:493-506

Davison AW (1977) The ecology of *Hordeum murinum* L: III. Some effects of adverse climate. Journal of Ecology 65:523-530

Délye C (2005) Weed resistance to acetyl coenzyme A carboxylase inhibitors: an update. Weed Science 53:728-746

Délye C, Wang T, Darmency H (2002) An isoleucine-leucine substitution in chloroplastic acetyl-CoA carboxylase from green foxtail (*Setaria viridis* L. Beauv.) is responsible for resistance to the cyclohexanedione herbicide sethoxydim. Planta 214: 421-427.

Deprado R, Gonzalez-Gutierrez J, Menéndez J, Gasquez J, Gronwald JW, Gimenez-Espiosa R (2000) Resistance to acetyl CoA carboxylase-inhibiting herbicides in *Lolium multiflorum*. Weed Science 48:311-318

Derpsch R (1998) Historical review of no-tillage cultivation of crops. Pages 205-218 *In* Proceedings of the Workshop on Conservation Tillage for Sustainable Agriculture. Asunción, Paraguay

Diggle AJ, Neve PB, Smith FP (2003) Herbicides used in combination can reduce the probability of herbicide resistance in finite weed populations. Weed Research 43:371-382

DiTomaso JM, Healy EA (2007) Weeds of California and other western states: Agriculture & Natural Resources: University of California

Ferreira JFS, Reddy KN (2000) Absorption and translocation of glyphosate in *Erythroxylum coca* and *E. novogranatense*. Weed Science 48:193-199

Fleet B, Gill G (2010) Barley grass, an emerging weed threat in southern Australian cropping systems. *in* Dove H, Culvenor R, eds. 15th Agronomy Conference. Lincoln, New Zealand: Australian Society of Agronomy

Gaines TA, Zhang W, Wang D, Bukun B, Chisholm ST, Shaner DL, Nissen SJ, Patzoldt WL, Tranel PJ, Culpepper AS, Grey TL, Webster TM, Vencill WK, Sammons RD, Jiang J, Preston C, Leach JE, Westra P (2010) Gene amplification confers glyphosate resistance in *Amaranthus palmeri*. Proceedings of the National Academy of Sciences 107:1029-1034

Giles BE, Lefkovitch LP (1986) A taxonomic investigation of the *Hordeum murinum* complex (Poaceae). Plant Systematics and Evolution 153:181-197

Gill GS (1996) Management of herbicide-resistant ryegrass in Western Australia— research and its adoption. Pages 542-545 *in* Proceedings of the 11th Australian Weeds conference. Melbourne, Victoria: Weed Science Society of Victoria.

Gill GS, Cousens RD, Allan MR (1996) Germination, growth, and development of herbicide resistant and susceptible populations of rigid ryegrass (*Lolium rigidum*). Weed Science 44:252-256

Goldsbrough PB, Hatch EM, Huang B, Kosinski WG, Dyer WE, Herrmann KM, Weller SC (1990) Gene amplification in glyphosate tolerant tobacco cells. Plant Science 72:53-62

Gutteridge RJ, Zhang JP, Jenkyn JF, Bateman GL (2005) Survival and multiplication of *Gaeumannomyces graminis* var. *tritici* (the wheat take-all fungus) and related fungi on different wild and cultivated grasses. Applied Soil Ecology 29:143-154

Harper J (1956) The evolution of weeds in relation to resistance to herbicides. Pages 179-188 *in* Proceedings of 3rd Br. Weed Control Conference.

Harris G (1961) The periodicity of germination in some grass species. New Zealand Journal of Agricultural Research 4:253-260

Hay JR (1974) Gains to the Grower from Weed Science. Weed Science 22:439-442

Heap I (2016) The international survey of herbicide resistant weeds. http://weedscience.org/. Accessed May 05, 2016

Heap J, Knight R (1982) A population of ryegrass tolerant to the herbicide diclofop-methyl. The Journal of the Australian Institute of Agricultural Science 48:156-157

Hidayat I, Baker J, Preston C (2006) Pollen-mediated gene flow between paraquat-resistant and susceptible hare barley (*Hordeum leporinum*). Weed Science 54:685-689

Hirschberg J, Mcintosh L (1983) Molecular basis of herbicide resistance in *Amaranthus hybridus*. Science 222:1346-1349

Holt JS (1992) History of identification of herbicide-resistant weeds. Weed Technology 6:615-620

Holt JS, Thill DC (1994) Growth and productivity of resistant plants. Pages 229-316 *in* Powles SB, Holtum JAM, eds. Herbicide Resistance in Plants: Biology and Biochemistry. Boca Raton, Florida: Lewis Publishers

Hubbard CE (1954) Grasses. A guide to their structure, identification, uses, and distribution in the British Isles: Pelican

Humphries C (1980) *Hordeum* L. Flora Europaea 5: 204-205

Jacobsen N, Von Bothmer R (1995) Taxonomy in the *Hordeum murinum* complex (Poaceae). Nordic Journal of Botany 15:449-458

Jasieniuk M, Brûlé-Babel AL, Morrison IN (1994) Inheritance of trifluralin resistance in green foxtail (*Setaria viridis*). Weed Science 42: 123-127

Jasieniuk M, Brule-Babel AL, Morrison IN (1996) The evolution and genetics of herbicide resistance in weeds. Weed Science 44:176-193

Jasieniuk M, Maxwell BD (1994) Populations genetics and the evolution of herbicide resistance in weeds. Phytoprotection 75:25-35

Johansen B, von Bothmer R (1994) Pollen size in *Hordeum* L.: correlation between size, ploidy level, and breeding system. Sexual Plant Reproduction 7:259-263

Kaundun SS (2014) Resistance to acetyl-CoA carboxylase-inhibiting herbicides. Pest Management Science 70:1405-1417

Kirkegaard J, Wong P, Desmarchelier J (1996) In vitro suppression of fungal root pathogens of cereals by *Brassica* tissues. Plant pathology 45:593-603

Kloot PM (1985) Plant introductions to South Australia prior to 1840. Journal of the Adelaide Botanic Gardens 7:217-231

Kloot PM (1987) Influence of environmental factors on the germination and establishment of barley grass (*Hordeum glaucum*) and annual ryegrass (*Lolium rigidum*). Australian Journal of Experimental Agriculture 27:525-532

LeBaron HM, McFarland J (1990) Herbicide resistance in weeds and crops. Pages 336-352 *in* Green MB, LeBaron HM, Moberg WK, eds. Managing Resistance to Agrochemicals. Washington, DC: American Chemical Society

Legere A, Stevenson FC, Benoit DL (2011) The selective memory of weed seedbanks after 18 years of conservation tillage. Weed Science 59:98-106

Letouzé A, Gasquez J (2001) Inheritance of fenoxaprop-p-ethyl resistance in a blackgrass (*Alopecurus myosuroides* Huds.) population. Theoretical Applied Genetics 103: 288-296.

Makarian H, Rashed Mohassel MH, Bannayan M, Nassiri M (2007) Soil seed bank and seedling populations of *Hordeum murinum* and *Cardaria draba* in saffron fields. Agriculture, ecosystems & environment 120:307-312

Matthews N, Powles SB, Preston C (2000) Mechanisms of resistance to acetyl-coenzyme A carboxylase inhibiting herbicides in a *Hordeum leporinum* population. Pest Management Science 56:441-447

Maxwell BD, Mortimer AM (1994) Selection for herbicide resistance. Pages 1-26 *in* Powles SB, Holtum JAM, eds. Herbicide Resistance in Plants: Biology and Biochemistry. Boca Raton, FL: Lewis Publishers

Michitte P, De Prado R, Espinoza N, Pedro Ruiz-Santaella J, Gauvrit C (2007) Mechanisms of resistance to glyphosate in a ryegrass (*Lolium multiflorum*) biotype from Chile. Weed Science 55:435-440

Mohammadi M, Banihashemi M, Hedjaroude GA, Rahimian H (2003) Genetic diversity among Iranian isolates of *Rhizoctonia solani* Kühn *Anastomosis* Group 1

subgroups based on isozyme analysis and total soluble protein pattern. Journal of Phytopathology 151:162-170

Mohler CL, Frisch JC, McCulloch CE (2006) Vertical movement of weed seed surrogates by tillage implements and natural processes. Soil & Tillage Research 86:110-122

Morrison J (1958) *Hordeum murinum* in Holland. Acta Botanica Neerlandica 7: 654-664.

Owen MJ, Goggin DE, Powles SB (2012) Identification of resistance to either paraquat or ALS-inhibiting herbicides in two Western Australian *Hordeum leporinum* biotypes. Pest Management Science 68:757-63

Popay A (1981) Germination of seeds of five annual species of barley grass. Journal of Applied Ecology:547-558

Popay A, Sanders P (1975) Effect of depth of burial on seed germination and seedling emergence of barley grass (*Hordeum murinum* L.). New Zealand Journal of Experimental Agriculture 3:77-80

Powles SB (1986) Appearance of a biotype of the weed, *Hordeum glaucum* Steud., resistant to the herbicide paraquat. Weed Research 26:167-172

Powles SB, Cornic G (1987) Mechanism of paraquat resistance in *Hordeum glaucum*. I. Studies with isolated organelles and enzymes. Australian Journal of Plant Physiology 14:81-89

Powles SB, Howat PD (1990) Herbicide-resistant weeds in Australia. Weed Technology:178-185

Powles SB, Preston C, Bryan IB, Jutsum AR (1997) Herbicide resistance: impact and management. Pages 57-93 *in* Donald LS, ed. Advances in Agronomy: Academic Press

Powles SB, Tucker ES, Morgan TR (1992) Eradication of paraquat-resistant *Hordeum glaucum* Steud. by prevention of seed production for 3 years. Weed Research 32:207-211

Powles SB, Yu Q (2010) Evolution in action: Plants resistant to herbicides. Annual Review of Plant Biology 61:317-347

Prado R, Prado JL, Osuna MD, Taberner A, Heredia A (2001) Is diclofop-methyl resistance in *Lolium rigidum* associated with a lack of penetration? Pages 545-550. *in* Proceedings of the British Crop Protection Council Conference: Weeds. Brighton, UK: British Crop Protection Council

Pratley J (1995) Long term investigations of the effect of tillage practices on crop production at Wagga Wagga, New South Wales. Australian Journal of Experimental Agriculture 35:885-892

Preston C, Holtum JAM, Powles SB (1992) On the mechanism of resistance to paraquat in *Hordeum glaucum* and *H. leporinum* - delayed inhibition of photosynthetic O_2 evolution after paraquat application. Plant Physiology 100:630-636

Preston C, Mallory-Smith CA (2001) Biochemical mechanisms, inheritance, and molecular genetics. Pages 23-60 *in* Powles SB, Shaner DL, eds. Herbicide resistance and world grains. Florida: CRC Press

Preston C, Powles SB (2002) Evolution of herbicide resistance in weeds: initial frequency of target site-based resistance to acetolactate synthase-inhibiting herbicides in *Lolium rigidum*. Heredity 88:8-13

Preston C, Roush RT, Powles SB (1999) Herbicide resistance in weeds of southern Australia: why are we the worst in the world? Pages 454-459 *In* Weed Management into the 21st Century: Do we know where we're going? Hobart, Tasmania, Australia: Tasmanian Weed Society

Preston C, Tardif FJ, Christopher JT, Powles SB (1996) Multiple resistance to dissimilar herbicide chemistries in a biotype of *Lolium rigidum* due to enhanced activity of several herbicide degrading enzymes. Pesticide Biochemistry and Physiology 54:123-134

Purba E, Preston C, Powles SB (1996) Growth and competitiveness of paraquat-resistant and susceptible biotypes of *Hordeum leporinum*. Weed Research 36:311-317

Rao VS (2000) Principles of weed science. New Hampshire, USA: Science Publisher. Pp. 557

Roget DK, Venn NR, Rovira AD (1987) Reduction of *Rhizoctonia* root rot of direct-drilled wheat by short-term chemical fallow. Australian journal of experimental agriculture. 27:425-430

Ryan GF (1970) Resistance of common groundsel to simazine and atrazine. Weed Science 18:614-616

Sammons RD, Gaines TA (2014) Glyphosate resistance: state of knowledge. Pest Management Science 70:1367-1377

Sasaki Y, Konishi T, Nagano Y (1995) The compartmentation of acetyl-coenzyme A carboxylase in plants. Plant Physiology 108:445-449

Seefeldt SS, Hoffman DL, Gealy DR, Fuerst EP (1998) Inheritance of diclofop resistance in wild oat (*Avena fatua* L.) biotypes from the Willamette Valley of Oregon. Weed Science 46: 170-175.

Shyr YYJ, Hepburn AG, Widhohn JM (1992) Glyphosate selected amplification of the 5-enolpyruvylshikimate-3-phosphate synthase gene in cultured carrot cells. Molecular and General Genetics MGG 232:377-382

Singh BK, Shaner DL (1995) Biosynthesis of branched chain amino acids: From test tube to field. The Plant Cell 7:935

Smith DF (1968) The growth of barley grass (*Hordeum leporinum*) in annual pasture. 1. Germination and establishment in comparison with other annual pasture species. Australian Journal of Experimental Agriculture and Animal Husbandry 8:478-483

Smith DF (1972) *Hordeum* species in grasslands. Herbage Abstracts 42:213-223

Timmons FL (1970) A history of weed control in the United States and Canada. Weed Science 18:294-307

Townshend J, Davidson T (1960) Some weed hosts of *Pratylenchus penetrans* in premier strawberry plantations. Canadian Journal of Botany 38:267-273

Tucker ES, Powles SB (1988) Occurrence and distribution in south-eastern Australia of barley grass (*Hordeum glaucum* Steud.) resistant to paraquat. Plant Protection Quarterly 3

Vanstone VA, Russ MH (2001) Ability of weeds to host the root lesion nematodes *Pratylenchus neglectus* and *P. thornei.* I. Grass weeds. Australasian Plant Pathology 30:245-250

Wang T, Darmency H (1997) Inheritance of sethoxydim resistance in foxtail millet, Setaria italica (L.) Beauv. Euphytica 94: 69-73.

Warner R, Mackie W (1983) A barley grass *Hordeum leporinum* ssp. *glaucum* Steud. population tolerant to paraquat (Gramoxone). Aust. Weed Res. News 13:16

Warwick SI, Black LD (1994) Relative fitness of herbicide-resistant and susceptible biotypes of weeds. Phytoprotection 75:37-49

Wong PTW (1975) Cross-protection against the wheat and oat take-all fungi by *Gaeumannomyces graminis* var. *graminis*. Soil Biology and Biochemistry 7:189-194

WSSA (2010) Common and chemical names of herbicides approved by the weed science society of America. Weed Science 58:511-518

WSSA (1994) Herbicide Handbook–7th edition: Weed Science Society of America, Lawrence, KS, USA.

Yu Q, Cairns A, Powles SB (2007a) Glyphosate, paraquat and ACCase multiple herbicide resistance evolved in a *Lolium rigidum* biotype. Planta 225:499-513

Yu Q, Collavo A, Zheng MQ, Owen M, Sattin M, Powles SB (2007b) Diversity of acetyl-coenzyme A carboxylase mutations in resistant *Lolium populations*: Evaluation using clethodim. Plant Physiology 145:547-558

Yu Q, Han H, Powles SB (2008) Mutations of the ALS gene endowing resistance to ALS-inhibiting herbicides in *Lolium rigidum* populations. Pest Management Science 64:1229-1236

Yu Q, Jalaludin A, Han H, Chen M, Sammons RD, Powles SB (2015) Evolution of a double amino acid substitution in the 5-enolpyruvylshikimate-3-phosphate synthase in *Eleusine indica* conferring high-level glyphosate resistance. Plant Physiology 167:1440-1447

Yu Q, Nelson JK, Zheng MQ, Jackson M, Powles SB (2007c) Molecular characterisation of resistance to ALS-inhibiting herbicides in *Hordeum leporinum* biotypes. Pest Management Science 63:918-27

Yu Q, Powles SB (2014) Resistance to AHAS inhibitor herbicides: current understanding. Pest Management Science 70:1340-1350

Yuan JS, Tranel PJ, Stewart Jr CN (2007) Non-target-site herbicide resistance: a family business. Trends in Plant Science 12:6-13

CHAPTER 2

TARGET-SITE POINT MUTATIONS CONFERRING RESISTANCE TO ACCASE-INHIBITING HERBICIDES IN SMOOTH BARLEY (*HORDEUM GLAUCUM*) AND HARE BARLEY (*HORDEUM LEPORINUM*)

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Target-Site Point Mutations Conferring Resistance to ACCase-Inhibiting Herbicides in Smooth Barley (*Hordeum glaucum*) and Hare Barley (*Hordeum leporinum*)

Lovreet S. Shergill, Jenna Malone, Peter Boutsalis, Christopher Preston, and Gurjeet Gill*

Acetyl coenzyme A carboxylase (ACCase)-inhibiting herbicides affect fatty acid biosynthesis in plants and are widely used to control smooth and hare barley in dicot crops in Australia. Recently, growers have experienced difficulty in controlling smooth and hare barley with herbicides from this mode of action. Dose–response experiments conducted on five suspected resistant populations confirmed varying levels of resistance to quizalofop and haloxyfop. The level of resistance in these populations was greater than 27-fold to quizalofop and greater than 15-fold to haloxyfop. The quizalofop dose required to reduce shoot biomass by 50% (GR_{50}) for the resistant populations varied from 52.6 to 111.9 g ha^{-1}, and for haloxyfop from 26.5 to 71.3 g ha^{-1}. Sequencing the CT domain of the ACCase gene from resistant plants of different populations confirmed the presence of previously known mutations Ile1781Leu and Gly2096Ala. Amino acid substitution at the 2096 position conferred a greater level of resistance to haloxyfop than the substitution at the 1781 position. This study documents the first known case of field-evolved target-site resistance to ACCase-inhibiting herbicides in Australian populations of smooth barley.

Nomenclature: Quizalofop; haloxyfop; smooth barley, *Hordeum glaucum* (Steud.) Tzvelev; hare barley, *Hordeum leporinum* (Link) Arcang.

Key words: Herbicide resistance, ACCase-inhibiting herbicides, ACCase mutation, mechanism, target site.

Smooth barley and hare barley were introduced into Australia soon after the European settlement (Cocks et al. 1976; Covas 1949; Smith 1968). Introduction of these grass species was associated with the import of wool and farm animals in the 19th century (Cocks et al. 1976; Davison 1977). Since then these grass species have naturalized and become ubiquitous weeds in the annual pasture zone of South Australia (SA) (Smith 1968). Even though hare and smooth barley are readily grazed by animals in the vegetative stage, their seeds can cause physical injury to animals at maturity. Seeds of these species have long barbed awns that can enter eyes and skin of the cattle or sheep, or get entangled in wool, resulting in loss of productivity (Campbell et al. 1972; Cocks et al. 1976). Both of these species are also known to act as a host for various pathogenic fungi and nematodes in cereal growing areas (Belair and Benoit 1996; Townshend and Davidson 1960; Vanstone and Russ 2001). Recently, smooth barley was reported to be increasing in abundance in cropping systems in SA. Fleet and Gill (2010) reported large variation in seed dormancy between smooth barley populations. They found that the populations from cropped fields exhibited high levels of dormancy, whereas those from fence lines or long-term pastures showed low levels of seed dormancy. Since 2010, local growers have been reporting some failures to control smooth and hare barley populations with acetyl–coenzyme A carboxylase (ACCase)-inhibiting herbicides. Therefore, seed dormancy and suspected herbicide resistance maybe associated with the increase in abundance of hare and smooth barley in cropping systems in SA.

ACCase-inhibiting herbicides from subgroups aryloxyphenoxypropionate (APP) and cyclohexanedione (CHD) are commonly used to control grass weeds in legumes and oilseed crops (Preston 2009). Because of their reliably high efficacy, APP herbicides are generally preferred by growers for the control of smooth and hare barley in broadleaf crops. These herbicides inhibit ACCase, the enzyme responsible for fatty acid biosynthesis in grass weeds (Burton et al. 1991). Grasses contain two different ACCase enzymes, but about 80% of the enzyme activity is associated with the plastid form, which is sensitive to ACCase-inhibiting herbicides. The plastid ACCase of dicots is structurally different from that of grasses and is insensitive to ACCase-

Table 1. Geographical locations of the tested smooth and hare barley populations from the southern Australian region.

Population	Species	Resistance status[a]	Location	Coordinates
A743.1	Smooth barley	R	Port Germein, South Australia	32.95°S, 137.99°E
A743.2	Smooth barley	R	Port Germein, South Australia	32.94°S, 137.96°E
A1312	Smooth barley	R	Cummins, South Australia	34.18°S, 135.72°E
A1186	Hare barley	R	Beulah, Victoria	35.88°S, 142.42°E
A777	Hare barley	R	Yeelanna, South Australia	34.09°S, 135.72°E
S81	Hare barley	S	Karoonda, South Australia	35.10°S, 139.86°E
Yaninee	Smooth barley	S	Yaninee, South Australia	32.93°S, 135.24°E

[a] R, resistant; S, susceptible.

inhibiting herbicides (Preston and Mallory-Smith 2001). This difference in the sensitivity to ACCase-inhibiting herbicides of plastid ACCase of grass weeds and broadleaf crops allows their safe use for the selective control of grasses.

Heavy reliance on herbicides, due to their ease of application and high efficacy, has led to widespread evolution of herbicide resistance in weeds. Resistance to ACCase-inhibiting herbicides has been reported in 46 grass weed species throughout the world, either due to insensitive ACCase enzyme or through detoxifying the herbicide rapidly through enhanced rates of metabolism (Heap 2014). Matthews et al. (2000) reported resistance to ACCase-inhibiting herbicides in one hare barley population from SA. Increased herbicide detoxification was identified as the mechanism for resistance to APP herbicides, whereas a modified target enzyme was reported as the mechanism for resistance to CHD herbicides (Matthews et al. 2000). So far there have been no reports of resistance to ACCase-inhibiting herbicides in smooth barley.

Growers in SA are now reporting difficulty in controlling smooth and hare barley populations with ACCase-inhibiting herbicides. Therefore, there is a need to understand resistance to ACCase-inhibiting herbicides and to develop management strategies for the control of resistant populations of these weed species. This study documents the first known case of field-evolved target-site resistance to ACCase-inhibiting herbicides in Australian populations of smooth barley.

Materials and Methods

Plant Material. Seeds of five barley grass populations used in this study were collected from farms in southern Australia, where farmers had reported control failure with ACCase-inhibiting herbicides (Table 1). Seed from numerous plants that had survived herbicide application in the field was collected in 2009 and 2010. According to taxonomic classification given by Cocks et al. (1976), three of these populations (A743.1, A743.2, and A1312) were classified as smooth barley, and two (A1186 and A777) as hare barley. Two known susceptible populations, Yaninee (smooth barley) and S81 (hare barley) with no history of herbicide use were used as susceptible control for these experiments.

Seed Germination and Plant Growth. Seeds for all experiments were germinated in plastic trays (33 by 28 by 5 cm) containing standard cocoa peat potting mix (Boutsalis et al. 2012). For dose–response experiments, germinated seedlings at Z11 stage (Zadoks et al. 1974) were transplanted into 9.5 by 8.5 by 9.5 cm punnet pots (Masrac Plastics, South Australia) containing standard potting mix. Depending upon the level of seed germination, three replicates of seven to nine seedlings per pot for each herbicide dose were transplanted. The pots were maintained outdoors during the normal growing season (May to October) in 2012 and 2013. Pots were watered as needed to maintain them near field capacity.

Dose Response to ACCase-Inhibiting Herbicides. Commercial formulations of the two most commonly used APP herbicides: quizalofop (Targa®, 99.5 g L^{-1}, Sipcam Pacific Australia Pty. Ltd., Victoria, Australia) and haloxyfop (Verdict®, 520 g L^{-1}, Dow AgroSciences Australia Ltd., New South Wales, Australia), were applied with a moving-boom laboratory twin nozzle cabinet sprayer (TeeJet 110° flat fan spraying systems, Wheaton, IL) delivering herbicide in 103 L ha^{-1} water at a pressure of 250 kPa and a speed of 1 m s^{-1}. All the populations were sprayed at Z20 stage with quizalofop at 0, 6.2, 12.4, 24.9, 49.8, and 99.5 g ha^{-1}, and haloxyfop at 0, 9.75, 19.5, 39, 78, and 156 g ha^{-1}. The recommended field rate for quizalofop and haloxyfop in Australia is 24.9

Table 2. Primer sequences used for amplification of ACCase gene in smooth and hare barley from genomic DNA.

Primer	Sequence 5'-3'
Acclr9	ATGGTAGCCTGGATCTTGGACATG
Acclr6	GGAAGTGTCATGCAATTCAGCAA
AccCT 2F	CCACTCCTGAATTTCCCAGTGG
AccCT 2R	CGCGATTTGAGTGTACAAAGGCTG
AccCT MidF	CCTGAGAATACATGTGATCCTCGTG
AccCT MidR	CCATTTCCCTTGGCTGTCATCAATGCC

and 39 g ha^{-1}, respectively. However, an additional low rate of quizalofop (3.1 g ha^{-1}) and haloxyfop (4.9 g ha^{-1}) were applied to the standard susceptible populations. Herbicides were applied as commercial formulation plus adjuvant; Hasten^{TM} adjuvant (Victorian Chemical Co. Pty. Ltd., Victoria, Australia) was added to haloxyfop spray solution at 1% v/v; and BS1000 adjuvant (Crop Care Australasia Pty. Ltd., Queensland, Australia) was added to quizalofop spray solution at 0.2% v/v. Plants were returned and maintained outdoors after herbicide application. At 28 d after treatment (DAT), visual survival assessments were made and aboveground plant biomass was harvested. Plants with new green leaf tissue were recorded as resistant, whereas those that displayed severe chlorosis or no new growth were recorded as susceptible. The harvested plants were dried in an oven at 60 C for 72 h, and weighed. The mean dry weight of all plants was calculated for each population and expressed as a percentage of the untreated controls for that population. LD$_{50}$ (dose of herbicide required to kill 50% of the plants) and GR$_{50}$ (dose of herbicide required to reduce shoot biomass by 50%) were obtained by log logistic analysis (GraphPad Prism v6, GraphPad software, San Diego, CA). Resistance indices (RI) were calculated as the ratio between the LD$_{50}$ (or GR$_{50}$) of each population and the LD$_{50}$ (or GR$_{50}$) of the susceptible control.

Sequencing of ACCase Gene. At 28 DAT, plant material (100 mg) from the new or youngest green leaf tissue of resistant and susceptible plants (five individuals per population) was collected from the dose–response experiment. Fresh leaf tissue thus obtained was snap frozen in liquid nitrogen and stored at −20 C. DNeasy Plant Mini Kit (Qiagen, Australia) was used to extract DNA from 50 to 100 mg plant tissue under liquid nitrogen as per manufacturer's instructions.

An approximately 1,600-bp fragment covering nearly the entire CT domain of the ACCase gene,

where known mutations conferring resistance to ACCase inhibitors had already been reported, was amplified in standard polymerase chain reaction (PCR) conditions with the use of primers (Table 2) designed against blackgrass (*Alopecurus myosuroides* Huds.) (accession number AJ310767) ACCase gene sequence (Délye 2005). A nested PCR approach was employed with oligo set Acclr9 and Acclr6 (Zhang and Powles 2006b) followed by oligo set AccCT 2F and AccCT 2R (Malone et al. 2014). MyFi DNA polymerase kit (Bioline Australia Pty. Ltd., Alexandria, New South Wales, Australia 1435) was used to run a PCR reaction of 25 μl, which contained 1× MyFi reaction buffer, 80 to 100 ng DNA template, 0.8 μM primers each and 2 units of DNA polymerase. An automated DNA thermal cycler (Eppendorf Master Cycler® Gradient, Germany) was used for DNA amplification with PCR conditions as follows: 3 min denaturing at 94 C, 40 cycles of 30 s denaturation at 94 C, 30 s annealing at 56 C, 2 min elongation at 68 C, and a final extension for 7 min at 68 C.

PCR products were visualized on 1× SYBR® Safe DNA stained 1.5% agarose gels. Samples were electrophoresed in 1× TAE Buffer (40 mM Trizma base, 1 mM Na$_2$EDTA, pH to 8 with glacial acetic acid) at 100 volts and photographed under UV light (λ302 nm). DNA fragment sizes were estimated by comparing their mobility to bands of known sizes in a low-mass molecular weight marker (Invitrogen, Australia). PCR products were sequenced at Australian Genome Research Facility Ltd., Adelaide, Australia, with primers CT Mid F and CT Mid R (Malone et al. 2014) to obtain sequence data covering the full CT domain fragment.

Nucleotide sequences were analyzed with the use of the VectorNTi, ContigExpress, and Align X software programs (Invitrogen), and all sequences were visually rechecked with the chromatogram files. All the sequences thus obtained were then aligned to each other and with the blackgrass sequence. The presence of nucleotide substitutions in seven previously characterized positions in the CT domain that cause resistance were analyzed (Preston 2009). According to the ACCase sequence of blackgrass, these amino acid positions are 1781, 1999, 2027, 2041, 2078, 2088, and 2096 (Délye 2005).

Results and Discussion

Dose Response to ACCase-Inhibiting Herbicides. Dose–response studies confirmed resistance in three

Table 3. Estimated LD_{50}, GR_{50}, and resistance index[a] (RI) values for smooth barley and hare barley populations treated with quizalofop. Values in parentheses are 90% confidence intervals. Data are means of two experiments.

| Population | Quizalofop | | | |
	LD_{50}	RI	GR_{50}	RI
	g ha^{-1}		g ha^{-1}	
Smooth barley				
A743.1	> 99.5	> 35.5	107.4 (91.1, 126.7)	39.8
A743.2	> 99.5	> 35.5	111.9 (87.2, 143.7)	41.4
A1312	91.7 (80.4, 104.6)	32.8	58.4 (49.6, 68.9)	21.6
Yaninee	2.8 (2.4, 3.2)		2.7 (2.5, 2.9)	
Hare barley				
A1186	114.4 (93.9, 139.5)	35.8	74.8 (62.9, 89.1)	29.9
A777	86.1 (78.0, 95.0)	26.9	52.6 (44.0, 62.8)	21.0
S81	3.2 (3.0, 3.4)		2.5 (2.3, 2.8)	

[a] Resistance indices (RI) were calculated as the ratio between the LD_{50} (or GR_{50}) of each population and the LD_{50} (or GR_{50}) of the susceptible control. The recommended field rate for quizalofop is 24.9 g ha^{-1}.

smooth barley (A743.1, A743.2, and A1312) and two hare barley (A1186 and A777) populations to the ACCase-inhibiting herbicides quizalofop (Table 3) and haloxyfop (Table 4). At 28 DAT, the susceptible populations, Yaninee and S81, were controlled by quizalofop and haloxyfop at the recommended field rates of 24.9 and 39 g ha^{-1}, respectively (Figures 1 and 2). In contrast, the suspected resistant populations survived (80 to 100% survival) at the recommended field rates of both the herbicides.

For the quizalofop treatment, many resistant plants survived and grew normally even at two to four times the recommended herbicide rate. The LD_{50} for quizalofop for the susceptible smooth barley population (Yaninee) was 2.8 g ha^{-1}, which is approximately 10% of the recommended rate (Table 3). Two smooth barley populations, A743.1 and A743.2, had low or no mortality even at the

maximum rate of quizalofop used (Figure 1A); therefore, LD_{50} could not be predicted but was greater than the maximum dose (> 99.5 g ha^{-1}) of the herbicide used (Table 3). Among both smooth and hare barley populations, the LD_{50} for quizalofop ranged from 86.1 to 114.4 g ha^{-1}, which was 26.9 to 35.8–fold greater than the susceptible populations. Similarly, the doses required to reduce 50% shoot biomass (GR_{50}) for the resistant and susceptible smooth barley populations were also calculated. The GR_{50} for the resistant smooth barley populations ranged from 58.4 to 111.9 g ha^{-1}, which were 21.6 to 41.4–fold greater than the susceptible population (Table 3). For hare barley, the GR_{50} for quizalofop was 52.6 and 74.8 g ha^{-1}, which was 21 and 29.9-fold greater than that of the susceptible population.

Similar to quizalofop, haloxyfop did not substantially reduce plant survival or biomass of the

Table 4. Estimated LD_{50}, GR_{50}, and resistance index[a] (RI) values for smooth barley and hare barley populations treated with haloxyfop. Values in parentheses are 90% confidence intervals. Data are means of two experiments.

| Population | Haloxyfop | | | |
	LD_{50}	RI	GR_{50}	RI
	g ha^{-1}		g ha^{-1}	
Smooth barley				
A743.1	55.1 (52.0, 58.4)	25.6	37.6 (31.8, 44.4)	17.1
A743.2	51.9 (38.9, 69.2)	24.1	33.7 (24.7, 46.1)	15.3
A1312	126.7 (108.7, 147.7)	58.8	71.3 (61.5, 82.8)	32.4
Yaninee	2.2 (2.1, 2.2)		2.2 (2.1, 2.2)	
Hare barley				
A1186	31.3 (28.5, 34.4)	14.6	26.5 (20.8, 33.7)	12.6
A777	82.6 (77.3, 88.2)	38.5	64.5 (56.1, 74.2)	30.7
S81	2.1 (2.1, 2.2)		2.1 (2.1, 2.2)	

[a] Resistance indices (RI) were calculated as the ratio between the LD_{50} (or GR_{50}) of each population and the LD_{50} (or GR_{50}) of the susceptible control. The recommended field rate for haloxyfop is 39 g ha^{-1}.
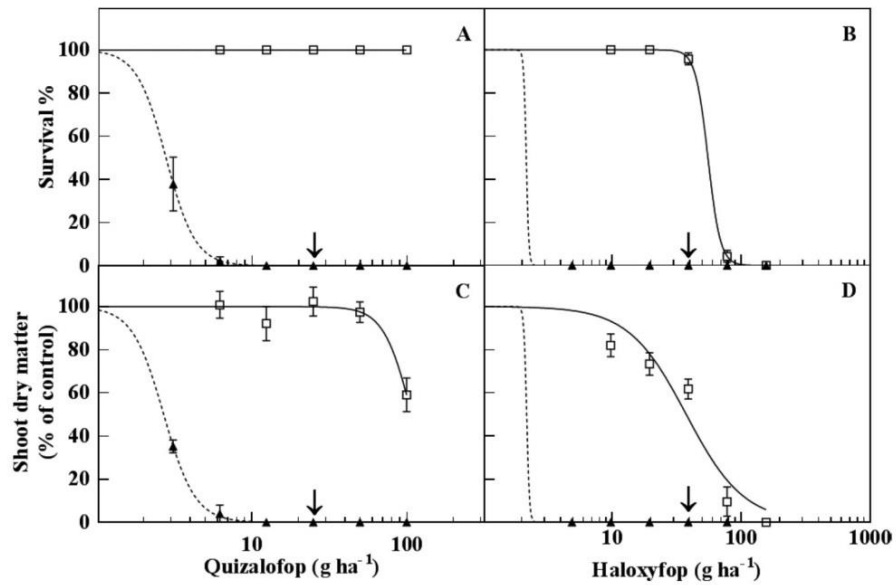
Figure 1. Dose response for survival (A and B) and shoot dry matter (C and D) of one herbicide-resistant (A743.1, squares) and -susceptible (Yaninee, triangles) population of smooth barley treated with quizalofop (A and C) and haloxyfop (B and D). Plants with new green leaf tissue were recorded as resistant, whereas those that displayed severe chlorosis or no new growth were recorded as susceptible. Each data point represents the mean percentage survival $\pm$ SE or mean shoot dry weight expressed as a percentage of the untreated controls $\pm$ SE of the pooled data for both experiments. Arrows indicate the recommended field rate of the herbicide used.
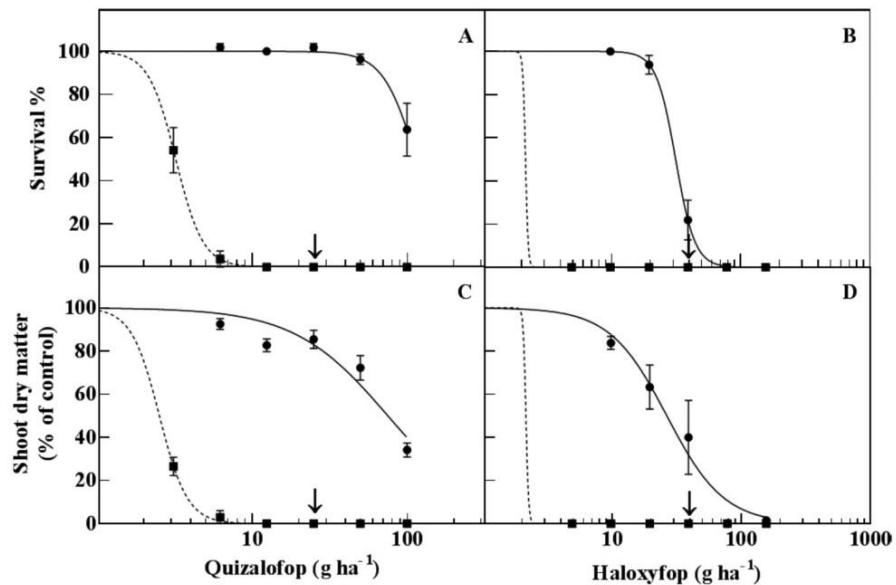
Figure 2. Dose response for survival (A and B) and shoot dry matter (C and D) of one herbicide-resistant population (A1186, circles) and -susceptible population (S81, squares) of hare barley treated with quizalofop (A and C) and haloxyfop (B and D). Plants with new green leaf tissue were recorded as resistant, whereas those that displayed severe chlorosis or no new growth were recorded as susceptible. Each data point represents the mean percentage survival $\pm$ SE or mean shoot dry weight expressed as a percentage of the untreated controls $\pm$ SE of the pooled data for both experiments. Arrows indicate the recommended field rate of the herbicide used.

Table 5. Comparison of nucleotide sequence and derived amino acid sequence of a highly conserved region of the ACCase enzyme from susceptible and resistant populations of smooth barley and hare barley.[a]

Amino acid number	1781	1999	2027	2041	2078	2088	2096
Amino acid	Ile	Trp	Trp	Ile	Asp	Phe	Gly
Consensus sequence	ATA	TGG	TGG	ATT	GAT	TTC	GGC
Smooth barley							
A743.1	CTA	–	–	–	–	–	–
A743.2	CTA	–	–	–	–	–	–
A1312	–	–	–	–	–	–	GCC
Yaninee (S)	–	–	–	–	–	–	–
Hare barley							
A1186	TTA	–	–	–	–	–	–
A777	–	–	–	–	–	–	GCC
S81 (S)	–	–	–	–	–	–	–

[a] The same mutation was identified in all five individuals tested for each population.

suspected resistant populations at the field rate (39 g ha^{-1}) but it was more active than quizalofop on two of the smooth barley (A743.1, A743.2) and one of the hare barley (A1186) populations (Table 4). Haloxyfop controlled the susceptible smooth and hare barley population at low rates with an LD$_{50}$ of 2.2 and 2.1 g ha^{-1}, respectively. A much greater dose was required to control the resistant smooth barley with LD$_{50}$ ranging from 51.9 to 126.7 g ha^{-1}. The resistant smooth barley populations were 24.1 to 58.8-fold resistant to haloxyfop compared with the susceptible population. For smooth barley, the GR$_{50}$ for haloxyfop was 2.2 g ha^{-1} for the susceptible population and ranged from 33.7 to 71.3 g ha^{-1} for the three resistant populations, which were 15.3 to 32.4-fold greater than the susceptible population. Similarly, the GR$_{50}$ for haloxyfop of the susceptible hare barley population was 2.1 g ha^{-1}. The GR$_{50}$ for haloxyfop of the two resistant populations of hare barley were 26.5 and 64.5 g ha^{-1}, which were 12.6 and 30.7-fold greater than the susceptible population (Table 4).

High levels of quizalofop resistance have been reported in other grass species, such as wild oat (*Avena fatua* L.) (Uludag et al. 2008), barnyard grass [*Echinochloa crus-galli* (L.) Beauv.] (Huan et al. 2011, 2013) and giant foxtail (*Setaria faberi* Herrm.) (Stoltenberg and Wiederholt 1995). Similarly, haloxyfop resistance has also been observed in Japanese foxtail (*Alopecurus japonicus* Steud.), where the resistant population was 12-fold resistant to the herbicide (Yang et al. 2007).

The exposure of smooth and hare barley populations to ACCase-inhibiting herbicides in SA has provided selection pressure required for the evolution of resistance. This study has identified the first known case of resistance to the ACCase-inhibiting herbicides in smooth barley. However, ACCase herbicide resistance has been previously reported in hare barley from SA (Matthews et al. 2000). Resistance to ACCase-inhibiting herbicides has been reported in 46 grass weed species around the world (Heap 2014) and resistance to this group in rigid ryegrass (*Lolium rigidum* Gaudin) (Boutsalis et al. 2012; Broster et al. 2012; Zhang and Powles 2006b), ripgut brome (*Bromus dindarus* Roth) (Boutsalis and Preston 2006), wild oat and sterile oat (*Avena sterilis* L.) (Ahmad-Hamdani et al. 2012; Maneechote et al. 1994; Owen and Powles 2009) has been reported previously in Australian cropping systems.

Sequencing of ACCase Gene. The CT domain of the plastidic ACCase gene was sequenced for five individuals from each resistant and susceptible population. Total sequence alignment was 1,600 bp or 533 amino acids long. The nucleotide sequences for the suspected resistant populations differed from that of the susceptible population (Yaninee, accession number KP267754; S81, accession number KP267755) by a single nucleotide, predicting a single amino acid modification. In populations A1312 (smooth barley, accession number KP267751) and A777 (hare barley, accession number KP267753), a codon change from GGC to GCC resulted in a predicted amino acid substitution of Gly2096Ala, whereas a codon change from ATA to CTA/TTA resulted in a predicted amino acid substitution of Ile1781Leu in two smooth barley (A743.1, accession number KP267749; A743.2, accession number KP267750) populations and one hare barley (A1186, accession number KP267752) population (Table 5).

Although the presence of other mechanisms cannot be excluded, the glycine to alanine at 2096 position and isoleucine to leucine amino acid

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substitution at 1781 position in the target enzyme are likely to be the major mechanisms of resistance to ACCase-inhibiting herbicides in smooth barley and hare barley populations. However, a non–target-site mechanism of resistance to APP herbicides and a target-site mechanism of resistance to CHD herbicides has been previously reported in hare barley (Matthews et al. 2000). Previously, Gly2096Ala mutations have been reported to be the basis of resistance to ACCase-inhibiting herbicides in wild oat (Beckie et al. 2012), blackgrass (Délye 2005; Petit et al. 2010) and hood canarygrass (*Phalaris paradoxa* L.) (Cruz-Hipolito et al. 2012). In this study, amino acid substitution at 2096 position in smooth barley (A1312) and hare barley (A777) confers a greater level of resistance to haloxyfop than amino acid substitution at 1781 position (Table 3 and 4). However, amino acid substitutions at both these sites conferred a similar level of resistance to quizalofop. The Ile1781Leu substitution is the most common substitution known to cause high levels of resistance to most ACCase-inhibiting herbicides in several other weed species such as wild oat (Beckie et al. 2012; Christoffers et al. 2002), blackgrass (Délye 2005) and rigid ryegrass (Zhang and Powles 2006a).

This study is the first documented case of field-evolved target-site resistance to the ACCase-inhibiting herbicides in Australian populations of smooth barley. Resistance was associated with a point mutation (Ile1781Leu or Gly2096Ala) at the CT domain of the ACCase gene. As a consequence of the presence of these target-site mutations in field populations of smooth and hare barley, this herbicide group is becoming ineffective against these weeds. Simply increasing the herbicide dose is unlikely to improve weed control in such populations because of the high levels of resistance. Herbicides with different sites of action are needed to provide effective control of these ACCase-resistant populations. Growers should also consider integration of a pasture phase, which allows use of grazing and nonselective herbicides for preventing weed seed production. Greater diversity in weed management tactics is required for the long-term effective management of herbicide-resistant populations.

Acknowledgments

Literature Cited

Ahmad-Hamdani MS, Owen MJ, Yu Q, Powles SB (2012) ACCase-inhibiting herbicide-resistant *Avena* spp. populations from the western Australian grain belt. Weed Technol 26: 130–136

Beckie HJ, Warwick SI, Sauder CA (2012) Basis for herbicide resistance in Canadian populations of wild oat (*Avena fatua*). Weed Sci 60:10–18

Belair G, Benoit DL (1996) Host suitability of 32 common weeds to *Meloidogyne hapla* in organic soils of southwestern Quebec. J Nematol 28:643

Boutsalis P, Gill GS, Preston C (2012) Incidence of herbicide resistance in rigid ryegrass (*Lolium rigidum*) across southeastern Australia. Weed Technol 26:391–398

Boutsalis P, Preston C (2006) Resistance to acetyl-coenzyme A carboxylase (ACCase)-inhibiting herbicides in *Bromus* spp. in Australia. Pages 538–540 *in* Proceedings of the 15th Australian Weeds Conference. Adelaide, South Australia: Weed Management Society of South Australia

Broster J, Koetz E, Wu H (2012) Herbicide resistance frequencies in ryegrass ('*Lolium*' spp.) and other grass species in Tasmania. Plant Prot Q 27:36

Burton JD, Gronwald JW, Keith RA, Somers DA, Gengenbach BG, Wyse DL (1991) Kinetics of inhibition of acetyl-coenzyme A carboxylase by sethoxydim and haloxyfop. Pest Biochem Physiol 39:100–109

Campbell RJ, Robards GE, Saville DG (1972) The effect of grass seed on sheep production. Proc Aust Soc Anim Prod 9:225–229

Christoffers MJ, Berg ML, Messersmith CG (2002) An isoleucine to leucine mutation in acetyl-CoA carboxylase confers herbicide resistance in wild oat. Genome 45:1049–1056

Cocks PS, Boyce KG, Kloot PM (1976) The *Hordeum murinum* complex in Australia. Aust J Bot 24:651–662

Covas G (1949) Taxonomic observations of the North American species of *Hordeum*. Madrono 10:1–21

Cruz-Hipolito H, Domínguez-Valenzuela J, Osuna M, Prado R (2012) Resistance mechanism to acetyl coenzyme A carboxylase inhibiting herbicides in *Phalaris paradoxa* collected in Mexican wheat fields. Plant Soil 355:121–130

Davison AW (1977) The ecology of *Hordeum murinum* L.: III. Some effects of adverse climate. J Ecol 65:523–530

Délye C (2005) Weed resistance to acetyl coenzyme A carboxylase inhibitors: an update. Weed Sci 53:728–746

Fleet B, Gill G (2010) Barley grass, an emerging weed threat in southern Australian cropping systems. *In* Proceedings of the 15th Australian Agronomy Conference. Lincoln, New Zealand: Australian Society of Agronomy

Heap I (2014) The International Survey of Herbicide Resistant Weeds. www.weedscience.org. Accessed June 19, 2014

Huan Z, Xu Z, Lv D, Wang J (2013) Determination of ACCase sensitivity and gene expression in quizalofop-ethyl-resistant and -susceptible barnyardgrass (*Echinochloa crus-galli*) biotypes. Weed Sci 61:537–542

Huan Z-B, Zhang H-J, Hou Z, Zhang S-Y, Zhang Y, Liu W-T, Bi Y-L, Wang J-X (2011) Resistance level and metabolism of barnyard-grass (*Echinochloa crus-galli* (L.) Beauv.) populations

to quizalofop-p-ethyl in Heilongjiang Province, China. Agric Sci China 10:1914–1922

Malone JM, Boutsalis P, Baker J, Preston C (2014) Distribution of herbicide–resistant acetyl–coenzyme A carboxylase alleles in *Lolium rigidum* across grain cropping areas of South Australia. Weed Res 54:78–86

Maneechote C, Holtum JA, Preston C, Powles SB (1994) Resistant acetyl-CoA carboxylase is a mechanism of herbicide resistance in a biotype of *Avena sterilis* ssp. ludoviciana. Plant Cell Physiol 35:627–635

Matthews N, Powles SB, Preston C (2000) Mechanisms of resistance to acetyl-coenzyme A carboxylase inhibiting herbicides in a *Hordeum leporinum* population. Pest Manag Sci 56:441–447

Owen MJ, Powles SB (2009) Distribution and frequency of herbicide-resistant wild oat (*Avena* spp.) across the Western Australian grain belt. Crop Pasture Sci 60:25–31

Petit C, Bay G, Pernin F, Délye C (2010) Prevalence of cross-or multiple resistance to the acetyl-coenzyme A carboxylase inhibitors fenoxaprop, clodinafop and pinoxaden in black-grass (*Alopecurus myosuroides* Huds.) in France. Pest Manag Sci 66:168–177

Preston C (2009) Herbicide resistance: target site mutations. Pages 127–148 *in* Stewart CN, ed. Weedy and Invasive Plant Genomics. Chichester, United Kingdom: Wiley-Blackwell

Preston C, Mallory-Smith CA (2001) Biochemical mechanisms, inheritance, and molecular genetics. Pages 23–60 *in* Powles SB, Shaner DL, eds. Herbicide Resistance and World Grains. Boca Raton, FL: CRC Press

Smith DF (1968) The growth of barley grass (*Hordeum leporinum*) in annual pasture. 1. Germination and establish-ment in comparison with other annual pasture species. Aust J Exp Agric Anim Husb 8:478–483

Stoltenberg DE, Wiederholt RJ (1995) Giant foxtail (*Setaria faberi*) resistance to aryloxyphenoxypropionate and cyclohex-anedione herbicides. Weed Sci 43:527–535

Townshend J, Davidson T (1960) Some weed hosts of *Pratylenchus penetrans* in premier strawberry plantations. Can J Bot 38:267–273

Uludag A, Park KW, Cannon J, Mallory–Smith CA (2008) Cross resistance of acetyl-coa carboxylase (ACCase) inhibitor-resistant wild oat (*Avena fatua*) biotypes in the Pacific Northwest. Weed Technol 22:142–145

Vanstone VA, Russ MH (2001) Ability of weeds to host the root lesion nematodes *Pratylenchus neglectus* and *P. thornei*. I. Grass weeds. Australas Plant Pathol 30:245–250

Yang C, Dong L, Li J, Moss SR (2007) Identification of Japanese foxtail (*Alopecurus japonicus*) resistant to haloxyfop using three different assay techniques. Weed Sci 55:537–540

Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. Weed Res 14:415–421

Zhang X-Q, Powles SB (2006a) The molecular bases for resistance to acetyl co-enzyme A carboxylase (ACCase) inhibiting herbicides in two target-based resistant biotypes of annual ryegrass (*Lolium rigidum*). Planta 223:550–557

Zhang X-Q, Powles SB (2006b) Six amino acid substitutions in the carboxyl-transferase domain of the plastidic acetyl-CoA carboxylase gene are linked with resistance to herbicides in a *Lolium rigidum* population. New Phytol 172:636–645

CHAPTER 3

INCIDENCE OF HERBICIDE RESISTANCE, SEEDLING EMERGENCE AND SEED PERSISTENCE OF SMOOTH BARLEY (*HORDEUM GLAUCUM*) IN SOUTH AUSTRALIA

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Overall percentage (%)	85%
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Incidence of Herbicide Resistance, Seedling Emergence, and Seed Persistence of Smooth Barley (*Hordeum glaucum*) in South Australia

Lovreet S. Shergill, Benjamin Fleet, Christopher Preston, and Gurjeet Gill*

Smooth barley has emerged as a problematic weed in cereal crops of South Australia. After the recent reports of herbicide resistance and increase in seed dormancy in smooth barley, it was considered important to determine the herbicide resistance status and seedbank behavior of field populations of this weed species. A field survey was undertaken in the Upper North and Eyre Peninsula regions of South Australia in October 2012. Of the 90 smooth barley populations screened for resistance to quizalofop, 15% exhibited some level of resistance and 85% were susceptible. Resistance to acetolactate synthase (ALS)-inhibiting herbicides was low, with only 3 and 12% of populations classified as developing resistance to imazamox + imazapyr and sulfosulfuron, respectively. No multiple resistance patterns were observed; however, two ALS-inhibiting herbicide-resistant populations had sulfonylurea-to-imidazolinone cross-resistance. At the start of the growing season, the majority of smooth barley populations emerged rapidly (median 50% time to emergence [T_{50}] = 8 d). In contrast, some populations of smooth barley displayed an extremely slow emergence pattern, with T_{50} of > 20 d. No direct linkage between seed dormancy and herbicide resistance was observed. However, two acetyl coenzyme A carboxylase-inhibiting herbicide-resistant populations were highly dormant and exhibited delayed emergence. The majority of smooth barley populations showed low-level or no seedbank persistence, but a few populations persisted for 1 yr. However, some weed populations had up to 20% seedbank persistence from 1 yr to the next. Overall there was a strong negative relationship between smooth barley seedling emergence and the level of seed persistence (R^2 = 0.84, P < 0.05). This association indicated that greater seed dormancy could be responsible for extended persistence of the seedbank of this weed species. The study provides valuable insights into the general pattern of herbicide resistance and the behavior of the seedbank of smooth barley populations on South Australian farms.

Nomenclature: Imazamox + imazapyr; quizalofop; sulfosulfuron; smooth barley, *Hordeum glaucum* (Steud.) Tzvelev.

Key words: ACCase-inhibiting herbicide, ALS-inhibiting herbicide, herbicide resistance, seed dormancy, seedbank persistence, seedling emergence.

Hordeum glaucum ha emergido como una maleza problemática en los cultivos de cereales en el Sur de Australia. Después de reportes recientes de resistencia a herbicidas y el incremento en la dormancia de la semilla en *H. glaucum*, se consideró importante determinar el estatus de la resistencia a herbicidas y el comportamiento del banco de semillas de poblaciones de campo de esta especie. Se realizó un estudio observacional de campo en las regiones Alta Norte y de la península Eyre en el Sur de Australia, en Octubre 2012. De las 90 poblaciones de *H. glaucum* evaluadas por resistencia a quizalofop, 14% exhibieron algún nivel de resistencia y 86% fueron susceptibles. La resistencia a herbicidas inhibidores de acetolactate synthase (ALS) fue baja, ya que solamente 3 y 12% de las poblaciones fueron clasificadas como desarrollando resistencia a imazamox + imazapyr y sulfosulfuron, respectivamente. No se observó ningún patrón de resistencia múltiple. Sin embargo, dos poblaciones resistentes a herbicidas inhibidores de ALS tuvieron resistencia cruzada de sulfonylurea a imidazolinone. Al inicio de la temporada de crecimiento, la mayoría de las poblaciones de *H. glaucum* emergieron rápidamente (mediana del tiempo de 50% de emergencia [T_{50}] = 8 d). En contraste, algunas poblaciones de *H. glaucum* mostraron un patrón de emergencia extremadamente tardío, con T_{50} de > 20 d. No se observó ninguna relación directa entre la dormancia de la semilla y la resistencia a herbicidas. Sin embargo, dos poblaciones resistentes a herbicidas inhibidores de acetyl coenzyme A carboxylase tuvieron una alta dormancia y exhibieron un retraso en la emergencia. La mayoría de las poblaciones de *H. glaucum* mostraron de bajo a ninguna persistencia del banco de semillas, pero algunas poblaciones persistieron por 1 año. Sin embargo, algunas poblaciones tuvieron hasta 20% de persistencia del banco de semillas de un año al otro. En general, hubo una fuerte relación negativa entre la emergencia de plántulas de *H. glaucum* y el nivel de persistencia de la semillas (R^2

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= 0.84, P < 0.05). Esta asociación indicó que una mayor dormancia de la semilla podría ser responsable por la persistencia extendida del banco de semillas de esta especie de maleza. Este estudio brinda una observación valiosa sobre el patrón general de resistencia a herbicida y el comportamiento del banco de semillas de poblaciones de *H. glaucum* en fincas del Sur de Australia.

Herbicide resistance and seed dormancy in weeds are striking examples of evolution in agricultural systems. Intense selection pressure imposed on genetically diverse weed populations provides stimuli for rapid evolution of herbicide resistance (Maxwell and Mortimer 1994). If traits such as herbicide resistance are present in a genetically variable natural population, even at low frequencies, the recurrent selection of these traits with the repeated herbicide application will increase the frequency of resistant individuals (Jasieniuk and Maxwell 1994; Owen et al. 2014). The same analogy can be used to explain the selection of highly dormant individuals in a cropping system in which early-emerging individuals are effectively killed by tillage or non-selective herbicides.

Herbicide-resistant weeds are a major problem in the cropping regions of Australia. Currently, there are over 75 weed species that have evolved herbicide resistance in Australia (Heap 2015). Widespread adoption of minimum tillage, because of its benefits such as reduced soil erosion, reduced fuel and labor cost, and timely sowing, and heavy reliance on herbicides has significantly contributed to the rapid appearance of herbicide resistance (Pratley 1995; Walsh and Powles 2007)

Smooth barley is an ubiquitous weed in the annual pasture zone of southern Australia (Cocks et al. 1976; Smith 1968). Previous studies have shown that this species has short-lived innate dormancy, and was unlikely to be problematic in crops as the majority of the seeds germinated with early autumn rains (Davison 1971; Harris 1961; Smith 1968) and the seedbank did not persist from one year to the next (Popay 1981). In a later study, Fleet and Gill (2010) showed that farming practices used in southern Australia have selected smooth barley populations that possess high levels of seed dormancy, which is broken by the exposure to cold temperatures in winter. This is an effective escape mechanism that allows some plants in these populations to avoid pre-sowing non-selective herbicides and establish after the crops have been planted. In the past, non-dormant populations of

smooth barley could be easily controlled with the use of burndown herbicides applied in late autumn. But this change in weed biology has increased the selection pressure on POST herbicides, as these are being increasingly relied on for the control of smooth barley in crops. Furthermore, investigations of populations of smooth barley and a closely related species hare barley (*Hordeum leporinum* L.) have confirmed resistance to acetyl coenzyme A carboxylase (ACCase)-inhibiting herbicides in South Australia and Tasmania (Broster et al. 2012; Matthews et al. 2000; Shergill et al. 2015), to acetolactate synthase (ALS)-inhibiting herbicides in Western Australia (Owen et al. 2012; Yu et al. 2007), and to bipyridyliums across southern Australia (Hidayat 2004; Owen et al. 2012; Powles 1986; Preston et al. 1992). The combination of increased seed dormancy with herbicide resistance would make it very difficult for Australian farmers to effectively manage this weed species in cropping systems.

At this stage it is unclear whether there is any link between increased seed dormancy and increased herbicide resistance in smooth barley. A link between herbicide resistance and seed dormancy in other species has been reported occasionally (Ghersa et al. 1994; Gill et al. 1996; Owen et al. 2014; Recasens et al. 2007; Tranel and Dekker 2002; Vila-Aiub et al. 2005). Therefore, detailed knowledge of seed biology, particularly timing of seedling emergence and seedbank persistence, is required for the development of integrated weed management practices. It is also important to quantify the occurrence of herbicide resistance, which should also aid in the development of effective weed management systems.

In this paper we report the findings of a random survey of cropping fields across the grain-cropping regions of South Australia. The objectives of the studies reported here were to (1) quantify the occurrence of herbicide resistance in smooth barley populations across South Australia, (2) determine the level of variation in seed dormancy and persistence in smooth barley populations across

Figure 1. Map of surveyed agronomic regions (names in capital letters) across South Australia, showing the location (•) of surveyed fields where smooth barley populations were collected for herbicide resistance screening and weed biology studies.

South Australia, and (3) examine the relationship between seed dormancy and herbicide resistance in populations of smooth barley. This study also documents the first known case of resistance to ALS-inhibiting herbicides in Australian populations of smooth barley.

Materials and Methods

Collection of Plant Material. Smooth barley generally starts flowering in August–September and produces mature seed by October, well before grain harvest in November–December. Therefore, a 3-wk field survey before grain harvest was conducted in October 2012. The survey focused on cropping fields in the upper north (UN) and Eyre Peninsula (EP) regions of South Australia (Figure 1). Fields were selected randomly and without any prior knowledge of herbicide history by traveling a predetermined distance of 5 km (UN region) or 10 km (EP region) along major and minor roads and then surveying the nearest crop or pasture field. At each stop a single field was surveyed. Fields were surveyed by two people moving in different directions; each followed an inverted W pattern through at least 1 ha of the field, beginning at least 20 m from the edge of the crop. For fields with patchy distribution of smooth barley, a representative sample was obtained by collecting approximately equal amounts of seed from most of the patches in the field. Sampling was discontinued once three-quarters of a 20-L bucket was full of seeds or panicles or after 30 min, whichever

occurred first. After collection, seeds or panicles obtained were bulked to obtain a single sample and designated as a single population. The sample thus obtained was placed in a labeled paper bag and global positioning system (GPS) coordinates were recorded from a handheld GPS unit (Garmin eTrex Vista®, Garmin Australasia, Eastern Creek, New South Wales). Additional information of the crop being cultivated and visual smooth barley severity/distribution were also recorded and a weed distribution score was given to each sampled field. Immediately after collection, the seed samples were stored in the laboratory under dry conditions at room temperature at The University of Adelaide, Roseworthy Campus (34.52°S, 138.68°E), until February 2013 when they were threshed and cleaned manually.

Herbicide Resistance Screening. Resistance screening was conducted twice for ACCase-inhibiting and ALS-inhibiting herbicides in the normal growing season from May to August 2013. Only the populations that survived in the first screening were included in the second screening. Seeds of each of the 90 populations were germinated in plastic trays (33 cm by 28 cm by 5 cm) containing standard University of California (UC) potting mix (pasteurized potting soil based on a 60 : 40 mix of sand and peat moss). Germinated seedlings (10 per pot with three replicates) were transplanted into 9.5-cm by 8.5-cm by 9.5-cm punnet pots (Masrac Plastics, South Australia) containing the potting mix. A standard susceptible smooth barley population, Yaninee (from EP, South Australia) (Shergill et al. 2014) and a previously confirmed ACCase-inhibiting herbicide-resistant smooth barley population, F.P (from Baroota, South Australia), were used as susceptible and resistant controls. These populations were screened with ACCase and ALS-inhibiting herbicides.

ACCase-Inhibiting Herbicides. A commercial formulation of the most commonly used aryloxyphenoxypropionate (APP) ACCase-inhibiting herbicide quizalofop (Targa®, 99.5 g L^{-1}, Sipcam Pacific Australia Pty Ltd., Geelong, Victoria) at 24.9 g ai ha^{-1} was sprayed using a moving-boom laboratory twin-nozzle cabinet sprayer (Tee-jet 110° flat-fan spraying systems, Wheaton, IL) delivering herbicide in 121 L ha^{-1} water at a pressure of 250 kPa and a speed of 1 m s^{-1}. All populations were sprayed with

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commercial formulation plus 0.2% v/v BS1000 adjuvant (Crop Care Australasia Pty Ltd., Queensland) at the Z12–Z13 stage (Zadoks et al. 1974). Plants were returned and maintained outdoors after herbicide application and assessed for survival at 28 d after treatment. The plants with new green leaf tissue were recorded as resistant, whereas plants that displayed severe chlorosis or no new growth were recorded as susceptible. The populations were classified as resistant if 20% or more of the individuals in the population survived herbicide application. The populations with 1 to 19% survival were classified as developing resistance. Where there was less than 1% survival, the populations were classified as susceptible.

ALS-Inhibiting Herbicides. Because of poor germination of some populations, 74 populations were screened with the ALS-inhibiting herbicides. The populations were screened with sulfonylurea herbicide sulfosulfuron (Monza®, 750 g kg^{-1}, Nufarm Australia Ltd., Victoria) at 18.7 g ai ha^{-1} and the imidazolinone herbicide mixture imazamox plus imazapyr (Intervix®, 33 g L^{-1} and 15 g L^{-1}, Crop Care Australasia) at 24.8 plus 11.3 g ai ha^{-1}. Herbicides were applied as commercial formulation plus adjuvant; Hasten™ adjuvant (Victorian Chemical Co. Pty. Ltd., Victoria) was added to sulfosulfuron spray solution at 1% v/v; and BS1000 adjuvant was added to imazamox plus imazapyr spray solution at 0.2% v/v. All herbicides were applied at Z11–Z12 stage (Zadoks et al. 1974) with the same laboratory herbicide sprayer described above. The plants were also assessed and classified as described above.

Seedling Recruitment. In the next autumn (April 2013) after the seed collection, seeds (1 g each with three replicates) of 63 smooth barley populations with Yaninee as the standard control (susceptible and non-dormant) were sown in plastic trays (33 cm by 28 cm by 5 cm) containing standard potting UC mix and were maintained outside during the normal growing season at The University of Adelaide, Roseworthy Campus. To estimate weed seedling establishment, emergence counts (at first leaf appearance) were recorded from April until the end of October (7 mo or 190 d). Initially, emergence was recorded at weekly intervals but after 2 mo, because of the decline in emergence, it was recorded at 2-wk intervals. Seedlings were

counted and removed until no further emergence was recorded in three consecutive measurements. The counts thus obtained were expressed as cumulative seedling emergence percentage, i.e., percentage of the total emergence. According to the resistance status confirmed in herbicide resistance screening, the populations were grouped under three resistance classes, i.e., ACCase-inhibiting herbicide resistant (ACC-R), ALS-inhibiting herbicide resistant (ALS-R), and susceptible. Cumulative seedling emergence in these different groups at each sampling time (days after sowing [DAS]) was compared using ANOVA. Cumulative seedling emergence values were fitted to a functional three-parameter sigmoid model with the use of SigmaPlot version 12.5. The model fitted was

$$E(\%) = E_{max}/\{1 + exp - [(x - T_{50})/E_{rate}]\} \quad [1]$$

where E is the cumulative seedling emergence (%) at time x, E_{max} is the maximum seedling emergence (%), T_{50} is the time (d) to reach 50% of maximum seedling emergence, and E_{rate} indicates the slope around T_{50}.

Seed-Bank Persistence. To estimate initial seed viability, 20 seeds were randomly selected from each population and tested for viability. For residual seedbank viability, the smooth barley seeds that failed to germinate during the 2013 winter growing season were recovered from the soil (by sieving) in summer and tested for viability. Seed viability was tested with tetrazolium chloride solution (1% w/v). Sterile florets were removed and seeds were soaked in water for 24 h before slicing them longitudinally to expose the embryo and incubating them in 1% w/v tetrazolium chloride solution for another 24 h in the dark at 30 C (Chauhan et al. 2006b). The extent of pink staining observed under a microscope (Stemi 2000®, Carl Ziess, Sydney, Australia) was used as the indicator of viability or nonviability. Seeds with completely stained (pink) embryo were scored as viable, whereas seeds that lacked integrity of embryo and endosperm were considered nonviable or decayed.

To determine seedbank persistence, three independent samples (1 g each) for each population were drawn and total seeds per replicate were counted. The average number of seeds per population thus obtained and percentage of initial seedbank viability were used to calculate the total number of viable seeds sown, which was thus used

Table 1. Herbicide resistance classification of smooth barley populations randomly collected from Upper North (UN) and Eyre Peninsula (EP) regions of South Australia.

| | Herbicide resistance classification[a] | | |
Herbicide	Resistant (> 20% survival)	Developing resistance (1 to 20% survival)	Susceptible (0% survival)
		% of populations	
Quizalofop			
Total	7 (6)	8 (7)	85 (77)
UN	17 (4)	22 (5)	61 (14)
EP	3 (2)	3 (2)	94 (63)
Imazamox + Imazapyr			
Total	0	3 (2)	97 (72)
UN	0	0	100 (23)
EP	0	4 (2)	96 (49)
Sulfosulfuron			
Total	0	12 (9)	88 (65)
UN	0	13 (3)	87 (20)
EP	0	12 (6)	88 (45)

[a] Values in parentheses are the number of populations classified in each class.

to calculate seedbank persistence. The formula used was

$$\text{Seedbank persistence}(\%) = (\text{Total number of viable seeds recovered} / \text{Total number of viable seeds sown}) \times 100 \quad [2]$$

To determine a relationship between seedbank persistence (%) and emergence (%), the data were fitted to a functional two-parameter logarithmic model with the use of SigmaPlot version 12.5. The model fitted was

$$y = y_0 + a \times \ln(x) \quad [3]$$

where y is the seedbank persistence (%) at emergence (%) x, and y_0 and a are constants.

Results and Discussion

Smooth barley was found to infest many, but not all cropping fields across the survey area in the UN and EP regions of South Australia. In total, 111 fields were surveyed throughout UN and EP regions, of which 78% were infested with smooth barley and 90 samples had sufficient quantity of seed to allow herbicide resistance screening. Such a

high level of occurrence of smooth barley is consistent with the findings of the previous grower survey in which this weed species was rated in the top five most problematic weeds in this region (Fleet and Gill 2010). The majority of surveyed fields had been used to grow wheat (*Triticum aestivum* L.) (62%), barley (*Hordeum vulgare* L.) (12%), peas (*Pisum sativum* L.) (2%), lupins (*Lupinus angustifolius* L.) (2%), canola (*Brassica napus* L.) (1%), and oats (*Avena sativa* L.) (1%). Dominance of cereal crops is consistent with their ability to grow well in low-rainfall environments. Overall, 80% of the collected smooth barley populations came from crop and 20% from the pastures.

Resistance to ACCase-Inhibiting Herbicides. Data were pooled over the two screening experiments and are presented in Table 1. Screening of smooth barley populations randomly collected in the UN and EP regions revealed that the greatest incidence of resistance was to APP herbicide quizalofop, although the overall resistance was low in these regions. Of the 90 smooth barley populations tested for resistance to quizalofop, 13 (15%) populations exhibited some level of resistance and 77 (85%) populations were susceptible. Considerable variation in resistance to ACCase-inhibiting herbicides was identified between the regions. The greatest frequency of quizalofop-resistant populations was observed in the UN region (39%), whereas the frequency of resistance observed in EP was much lower (6%). Overall, 6 (7%) of the tested populations were classified as resistant (> 20% survival), 7 (8%) as developing resistance (1 to 20% survival), and 77 (86%) were susceptible. All resistant populations had greater than 90% survival, whereas plant survival in the populations classified as developing resistance ranged from 2 to 13% at the recommended field rate of quizalofop. This level of survival in the "developing resistance" category is a concern, as anything less than 90% mortality after herbicide treatment is usually regarded as a commercial failure by the growers (Llewellyn and Powles 2001). The majority of the quizalofop-resistant smooth barley populations were collected from wheat fields ($n = 7$, 54%), but resistance was also detected in samples collected from peas ($n = 2$, 15%), barley ($n = 1$, 8%), and pasture ($n = 3$, 23%). Greater frequency of resistance detected in wheat crops appears to be

simply related to the dominance of wheat in these cropping regions. Such high levels of survival (> 90%) in resistant populations indicates repeated use of quizalofop and other ACCase-inhibiting herbicides over many years for the control of grass weeds in the UN and EP regions of South Australia. A survey of the cropping region of Tasmania in 2010 identified just one hare barley population with resistance to APP herbicide haloxyfop (Broster et al. 2012). In contrast, surveys conducted in southern New South Wales in 2007 and Western Australia in 2005 found no resistance to ACCase-inhibiting herbicides in smooth and hare barley populations (Broster et al. 2010; Owen et al. 2012).

Resistance to ACCase-inhibiting herbicides has been reported in 46 grass weed species around the world (Heap 2015). In the majority of cases, target-site point mutations have been reported to confer resistance to ACCase-inhibiting herbicides, with metabolism-based resistance less common (Beckie et al. 2012; Délye 2005; Malone et al. 2014). In the case of smooth barley, ACCase-inhibiting herbicide resistance due to target-site point mutations has been recently reported in three populations from South Australia (Shergill et al. 2015). Therefore, the mechanism(s) responsible for ACCase-inhibiting herbicide resistance in smooth barley populations most likely involves an altered target site(s). However, increased detoxification of APP herbicides has also been previously reported as a mechanism of herbicide resistance in hare barley from South Australia (Matthews et al. 2000).

Resistance to ALS-Inhibiting Herbicides. The survey revealed that resistance to ALS-inhibiting herbicides is still rare. Of the 74 smooth barley populations tested for resistance to imidazolinone herbicide mixture imazamox + imazapyr, only 2 (3%) populations were classified as developing resistance, and none of the populations were classified as resistant (Table 1). Both of these two populations were collected from the EP region; none of the populations from the UN had detectable level of resistance to ALS-inhibiting herbicides. Overall, 97% of the populations were susceptible to imazamox + imazapyr, with 100% susceptibility in the UN region and 94% in the EP. The populations classified as developing resistance to imazamox + imazapyr herbicide were collected from wheat fields and had plant survival ranging from 4 to 7% at the recommended field rate. In

contrast, imazamox-resistant hare barley from Western Australia showed no plant mortality at the field rate of this herbicide (Owen et al. 2012).

The sulfonylurea herbicide sulfosulfuron was less effective in controlling smooth barley populations as compared with imazamox + imazapyr, but none of the populations were highly resistant (> 20% survival). However, nine populations (12%) were classified as developing resistance, with three (13%) from the UN region and six (12%) from EP. All the populations that survived sulfosulfuron application had a low level of survival ranging from 4 to 11% at the recommended field rate. Of the nine smooth barley populations classified as developing resistance to sulfosulfuron, seven populations were collected from wheat fields and one each from barley and pasture fields. In these low-rainfall cropping districts, because of lack of suitable alternative crop species, cereal-based crop rotations are most common, but they are prone to grass weed infestation (Fleet and Gill 2010). Although resistance to ALS-inhibiting herbicides is the most common form of resistance in weed populations (both monocots and dicots) across the globe (Heap 2015), it appears to be still relatively uncommon in Australia in hare barley, with only two cases reported so far (Owen et al. 2012; Yu et al. 2007).

Multiple and Cross-Resistance. Of the total populations screened with ACCase- and ALS-inhibiting herbicides, none of the populations exhibited multiple resistance. However, two ALS-inhibiting herbicide-resistant populations were found to be resistant to both imazamox + imazapyr and sulfosulfuron herbicides. Sulfonylurea-to-imidazolinone cross-resistance has previously been documented in hare barley populations from western Australia (Owen et al. 2012) and in rigid ryegrass populations from southern Australia (Preston and Powles 2002).

Herbicide resistance screening of smooth barley populations showed a greater incidence of resistance to ACCase-inhibiting herbicides compared with ALS-inhibiting herbicides. Higher levels of resistance to ACCase-inhibiting herbicides compared with ALS-inhibiting herbicides has also been reported in a survey of Italian ryegrass (*Lolium multiflorum* Lam.) in northern Idaho and eastern Washington, as a result of the greater use of ACCase-inhibiting herbicides in those regions (Rauch et al. 2010). Information from the current
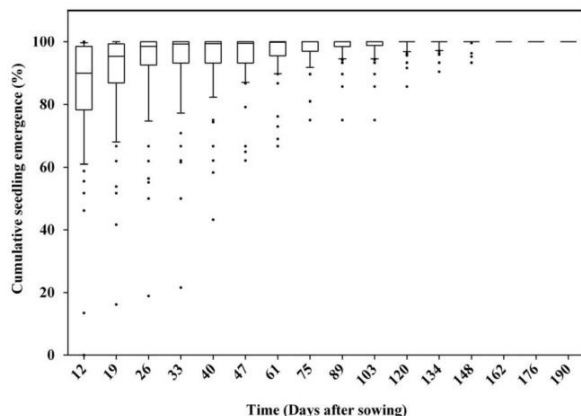
Figure 2. Box and whiskers plot of cumulative seedling emergence percentage of randomly collected smooth barley populations ($n = 63$) measured at different times. Lower and upper boxes represent the second and third quartiles, respectively. Line in the box represents median value. Lower and upper whiskers extend to the 10th and 90th percentiles of the data, respectively.

survey and weed surveys in Tasmania, southern New South Wales (Broster et al. 2012; 2010), and Western Australia (Owen et al. 2012) suggests that herbicide resistance in barley grass is still in early stages of development and weed control with these two modes of action is likely to be effective in the majority of the populations. However, the UN region appears to be a "hot spot" for resistance to ACCase-inhibiting herbicides, with 39% of the populations showing resistance. Growers in those regions need to reconsider their heavy reliance on these herbicides as well as develop strategies to minimize the risk of spread of resistant seeds to the fields that are still susceptible.

Seedling Recruitment. The distribution of cumulative seedling emergence percentage of 63 smooth barley populations measured at different times (DAS) is shown in box and whiskers plot in Figure 2. The majority (75%) of smooth barley populations emerged rapidly and reached cumulative seedling emergence of 87 to 100% within 19 DAS. Even at the first count (12 DAS), nearly half of the populations had reached 78 to 99% cumulative seedling emergence. The susceptible and non-dormant check population, Yaninee, also exhibited rapid emergence ($T_{50} = 8d$); short-lived innate dormancy and rapid emergence after autumn rains is a typical behavior of smooth and hare barley

reported in the Australian literature (Cocks and Donald 1973; Peltzer and Matson 2002; Smith 1968). However, several populations continued to exhibit some seedling emergence even as late as 148 DAS. It is likely that the populations that exhibited delayed emergence possessed greater dormancy compared with the populations with rapid emergence. Similar large variation in seedling emergence between smooth barley populations has been recently reported by Fleet and Gill (2012). They reported that some populations from cropping fields had developed dormancy mechanisms to delay emergence that would allow them to evade pre-sowing weed control in the field. Selection pressure from weed-control treatments in cropping fields appears to have selected physiological mechanisms that increase expression of seed dormancy in smooth barley (Fleet and Gill 2012).

The T_{50} values obtained by fitting a three-parameter sigmoid model (Equation 1) to the cumulative seedling emergence of different smooth barley populations varied from 6 to 44 d, with a range of 6 to 26 d in the populations from the UN region and 6 to 44 d in the EP populations (Figure 3). The median T_{50} value for seedling emergence (8 d) was very similar for the UN and EP populations (unpaired t test, P = 0.66). These results indicate that the majority of smooth barley populations have low seed dormancy and germinate rapidly, which is consistent with the findings of previous research on this species (Cocks and Donald 1973; Peltzer and Matson 2002). However, some smooth barley populations had seven fold greater T_{50} values than others. Higher T_{50} values is an indication of delayed emergence, which is likely to be associated with greater seed dormancy as previously reported in smooth barley by Fleet and Gill (2012). According to Buhler et al. (1997), the knowledge of emergence patterns of weeds could be used to determine optimum timing of cultivation and POST herbicide application. Delaying sowing to allow high weed seed germination and using herbicides to control weed populations has been advocated as an effective weed management tool (Owen et al. 2014). Fleet and Gill (2010) also reported that delaying sowing by 3 wk resulted in 75% reduction in smooth barley infestation in wheat. They further reported that delayed sowing helped dormant smooth barley population to satisfy the cold stratification requirement for germination so they could then be easily
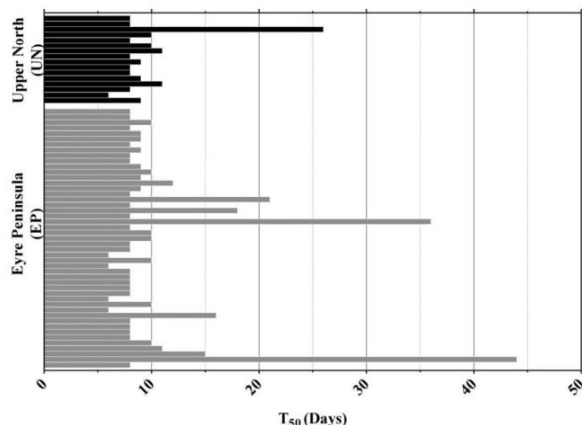
Figure 3. Time (d) taken to reach 50% of maximum seedling emergence (T_{50}) of smooth barley populations randomly collected from the Upper North (UN) and Eyre Peninsula (EP) regions of South Australia. T_{50} values were calculated by fitting cumulative seedling emergence percent data to a functional three-parameter sigmoid model (Equation 1). Each horizontal bar represents T_{50} value for each population ($n = 63$).

Figure 4. Cumulative seedling emergence pattern of three resistance groups; susceptible (solid line, •, $n = 44$), ACC-R (dotted line, ○, $n = 11$), and ALS-R (dashed line, ▼, $n = 8$) of smooth barley populations measured at different times. Vertical bars represent LSD (P > 0.05) according to ANOVA. Lines represent a functional three-parameter sigmoid model (Equation 1) fitted to the mean of cumulative seedling emergence percent data for each at different times.

controlled by PRE herbicides. Similar findings were also reported by Buhler and Gunsolus (1996) in soybean (*Glycine max* L.), where delayed planting reduced weed infestation and improved weed control with rotary hoeing and cultivation. However, delayed sowing can incur a large yield penalty and is generally not preferred by the growers in Australian rain-fed cropping systems (reviewed in Roper et al. 2012). Despite this, delayed sowing could be used occasionally in fields infested with highly dormant weed populations, especially if they are known to be resistant to selective herbicides.

Relationship between Seedling Emergence and Herbicide Resistance. The seedling recruitment data showed that the majority of the herbicide-resistant smooth barley populations were non-dormant and germinated rapidly ($T_{50} < 11$ d). However, there were two ACCase-inhibiting herbicide-resistant populations that had not completely emerged after 47 and 120 DAS. The T_{50} of these two biotypes estimated from the model (Equation 1) were 18 and 26 d, which is much greater than the T_{50} for the non-dormant resistant biotypes (6 to 11 d). The combination of herbicide resistance with high seed dormancy would make it quite difficult to effectively control such weed populations.

The cumulative seedling emergence data for smooth barley populations were grouped under three resistance classes, i.e., ACC-R, ALS-R, and susceptible. There were no significant differences (P > 0.05) in the T_{50} for seedling emergence of smooth barley populations of the three resistance classes (Figure 4). These results suggest that there is no linkage between seed dormancy and resistance status in smooth barley. Gill et al. (1996) also reported no major differences in seedling emergence among the ACC-R and ALS-R and susceptible populations of rigid ryegrass. But in later studies, Vila-Aiub et al. (2005) and Owen et al. (2010) showed some differences in germination and emergence responses between ACC-R or ALS-R and susceptible populations.

In the current study, the greater expression of seed dormancy in two ACCase-resistant populations is unlikely to be directly related to resistance alleles, because many other resistant populations had a much lower T_{50} for seedling emergence (Gundel et al. 2008). The co-occurrence of dormancy and herbicide resistance has been attributed to the impact of selection pressure imposed by management practices associated with decades of intensive cropping rather than herbicide resistance *per se* (Owen et al. 2010). Management practices used in crop production (including cultivation and selective

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Table 2. Seedbank persistence variability among the populations of smooth barley collected from the Upper North (UN) and Eyre Peninsula (EP) regions of South Australia.

Seedbank persistence interval	Total	UN	EP
%		—% of populations[a]—	
0–5	49 (31)	44 (7)	51 (24)
5–10	25 (16)	31 (5)	23 (11)
10–15	21 (13)	25 (4)	19 (9)
15–20	5 (3)	0 (0)	6 (3)

[a] Values in parentheses are the number of populations.

and non-selective herbicide use) are likely to favor survival of late-germinating individuals in a weed population and over time the dormant individuals may become the dominant part of the seedbank (Fleet and Gill 2012; Owen et al. 2014). There is little doubt that highly dormant herbicide-resistant smooth barley populations will be difficult to control in crop fields, especially in cereals, where herbicide options are limited.

Seedbank Persistence. Seeds of smooth barley populations that failed to germinate during the 2013 winter growing season were recovered from the soil in summer and tested for viability to determine whether they had decayed or were still viable. The results revealed that the majority of the smooth barley populations had a low level or no seedbank persistence. Of the 63 randomly collected smooth barley populations tested for seedbank persistence, 47 (75%) had a low level of persistence (< 10%) (Table 2). Overall seedbank persistence was not different between the two regions (unpaired t test, P = 0.86), with a maximum of 11% in UN and 20% in EP. There were no differences (unpaired t test, P = 0.23) between seedbank persistence of resistant and susceptible populations. Sosnoskie et al. (2013) also reported no detectable differences in seedbank persistence between glyphosate-resistant and glyphosate-susceptible Palmer amaranth (*Amaranthus palmeri* S. Wats) seeds, tested between 0 to 36 mo after burial. All the populations ($n = 17$) with high level of persistence (> 10%) were collected from crop fields, which suggests that the selection pressure imposed by weed-control tactics in crops may have selected for greater seedbank persistence in smooth barley. Previous studies have shown that seeds of smooth barley have a short-lived seedbank and very few
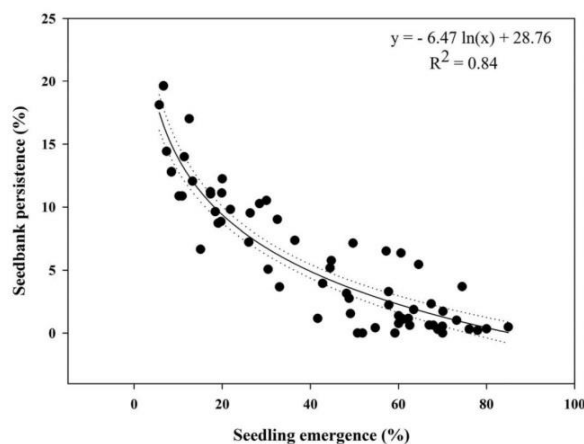
Figure 5. Relationship between seedling emergence (%) and seedbank persistence (%) of smooth barley populations randomly collected from the Upper North (UN) and Eyre Peninsula (EP) regions of South Australia. Lines represent a functional two-parameter logarithmic model fitted (Equation 3) to the seedling emergence (%) and seedbank persistence (%) data for each population ($n = 63$). The band with dotted lines represents the 95% confidence interval.

seeds are likely to be present after 1 yr (Peltzer and Matson 2002; Popay 1981; Powles et al. 1992). In contrast, the results of the present study clearly indicate that some smooth barley populations have adequate seedbank persistence to reinfest crops in the next season.

There was a strong negative relationship between seedling emergence and the level of persistent seedbank of smooth barley populations (Figure 5). Populations that exhibited low seedling recruitment are likely to have a higher level of seed dormancy, which may have enabled greater seedbank persistence. High level of seed decay (50 to 80%) observed in some smooth barley populations was also associated with high seed dormancy. Similar levels of seed decay has been previously reported in other weed species from South Australia. For example, Chauhan et al. (2006a) reported that annual seed decay of rigid ryegrass was > 50% in South Australia cropping systems. Similarly, Kleemann and Gill (2013) reported ~45% seed decay in a population of ripgut brome (*Bromus diandrus* Roth) in South Australia.

In summary, the current study provides valuable insights into the general pattern of herbicide resistance and seedbank behavior of on-farm populations of smooth barley randomly collected

from the UN and EP regions of South Australia. It also reports the first known instances of resistance to ALS-inhibiting herbicides in smooth barley. Although the overall occurrence of resistance on farms was low, 39% of the fields in the UN region had detectable level of resistance to the ACCase-inhibiting herbicide quizalofop. Evidence presented suggests that crop management practices used by the growers in the cropping fields has selected for greater seed dormancy and a persistent weed seedbank. The study also reveals that a large proportion of cropping land still contains herbicide-susceptible smooth barley populations, where rotations including ACCase- and ALS-inhibiting herbicides will still provide effective weed control. Additionally, this study found some smooth barley populations that possess ACCase resistance and high seed dormancy. Such populations will be extremely difficult to manage and growers will need to integrate other non-chemical strategies for long-term weed management.

Acknowledgments

Literature Cited

Beckie HJ, Warwick SI, Sauder CA (2012) Basis for herbicide resistance in Canadian populations of wild oat (*Avena fatua*). Weed Sci 60:10–18

Broster J, Koetz E, Wu H (2012) Herbicide resistance frequencies in ryegrass (*Lolium* spp.) and other grass species in Tasmania. Plant Prot Q 27:36

Broster JC, Koetz EA, Wu H (2010) A survey of southern New South Wales to determine the level of herbicide resistance in brome grass and barley grass populations. Pages 274–277 *in* Proceedings of the 17th Australasian Weeds Conference. Christchurch, New Zealand: New Zealand Plant Protection Society

Buhler DD, Gunsolus JL (1996) Effect of date of preplant tillage and planting on weed populations and mechanical weed control in soybean (*Glycine max*). Weed Sci 44:373–379

Buhler DD, Hartzler RG, Forcella F (1997) Implications of weed seedbank dynamics to weed management. Weed Sci 45:329–336

Chauhan BS, Gill G, Preston C (2006a) Influence of environmental factors on seed germination and seedling emergence of rigid ryegrass (*Lolium rigidum*). Weed Sci 54:1004–1012

Chauhan BS, Gill G, Preston C (2006b) Influence of tillage systems on vertical distribution, seedling recruitment and persistence of rigid ryegrass (*Lolium rigidum*) seed bank. Weed Sci 54:669–676

Cocks P, Donald C (1973) The germination and establishment of two annual pasture grasses (*Hordeum leporinum* Link and *Lolium rigidum* Gaud.). Aust J Agr Res 24:1–10

Cocks PS, Boyce KG, Kloot PM (1976) The *Hordeum murinum* complex in Australia. Aust J Bot 24:651–62

Davison AW (1971) The ecology of *Hordeum Murinum* L.: II. The Ruderal habit. J Ecol 59:493–506

Délye C (2005) Weed resistance to acetyl coenzyme A carboxylase inhibitors: an update. Weed Sci 53:728–746

Fleet B, Gill G (2010) Barley grass, an emerging weed threat in southern Australian cropping systems. *In* Dove H, Culvenor R, eds. 15th Agronomy Conference. Lincoln, New Zealand: Australian Society of Agronomy

Fleet B, Gill G (2012) Seed dormancy and seedling recruitment in smooth barley (*Hordeum murinum* ssp. *glaucum*) populations in southern Australia. Weed Sci 60:394–400

Ghersa CM, Martínez-Ghersa MA, Brewer TG, Roush ML (1994) Selection pressures for diclofop-methyl resistance and germination time of Italian ryegrass. Agron J 86:823–828

Gill GS, Cousens RD, Allan MR (1996) Germination, growth, and development of herbicide resistant and susceptible populations of rigid ryegrass (*Lolium rigidum*). Weed Sci 44:252–256

Gundel PE, Martínez-Ghersa MA, Ghersa CM (2008) Dormancy, germination and ageing of *Lolium multiflorum* seeds following contrasting herbicide selection regimes. Eur J Agron 28:606–613

Harris G (1961) The periodicity of germination in some grass species. N Z J Agr Res 4:253–260

Heap I (2015) The international survey of herbicide resistant weeds. www.weedscience.org. Accessed April 8, 2015

Hidayat I (2004) Evolution and Spread of Paraquat-Resistant Barley Grasses *Hordeum glaucum* Steud. and *H. leporinum* Link. Ph.D dissertation: Adelaide, Australia: The University of Adelaide. 176 p

Jasieniuk M, Maxwell BD (1994) Populations genetics and the evolution of herbicide resistance in weeds. Phytoprotection 75:25–35

Kleemann SGL, Gill GS (2013) Seed dormancy and seedling emergence in ripgut brome (*Bromus diandrus*) populations in southern Australia. Weed Sci 61:222–229

Llewellyn RS, Powles SB (2001) High levels of herbicide resistance in rigid ryegrass (*Lolium rigidum*) in the wheat belt of Western Australia. Weed Technol 15:242–248

Malone JM, Boutsalis P, Baker J, Preston C (2014) Distribution of herbicide-resistant acetyl-coenzyme A carboxylase alleles in

Lolium rigidum across grain cropping areas of South Australia. Weed Res 54:78–86

Matthews N, Powles SB, Preston C (2000) Mechanisms of resistance to acetyl-coenzyme A carboxylase inhibiting herbicides in a *Hordeum leporinum* population. Pest Manag Sci 56:441–447

Maxwell BD, Mortimer AM (1994) Selection for herbicide resistance. Pages 1–26 *in* Powles SB, Holtum JAM, eds. Herbicide Resistance in Plants: Biology and Biochemistry. Boca Raton, FL: Lewis Publishers

Owen MJ, Goggin DE, Powles SB (2012) Identification of resistance to either paraquat or ALS-inhibiting herbicides in two Western Australian *Hordeum leporinum* biotypes. Pest Manag Sci 68:757–763

Owen MJ, Goggin DE, Powles SB (2015) Intensive cropping systems select for greater seed dormancy and increased herbicide resistance levels in *Lolium rigidum* (annual ryegrass). Pest Manag Sci 71:966–971

Owen MJ, Michael PJ, Renton M, Steadman KJ, Powles SB (2010) Towards large-scale prediction of *Lolium rigidum* emergence. II. Correlation between dormancy and herbicide resistance levels suggests an impact of cropping systems. Weed Res 51:133–141

Peltzer SC, Matson PT (2002) How fast do the seedbanks of five annual cropping weeds deplete in the absence of weed seed input? Pages 553–555 *in* Proceedings of the 13th Australian Weeds Conference. Perth, Western Australia: Plant Protection Society of WA Inc

Popay A (1981) Germination of seeds of five annual species of barley grass. J Appl Ecol:547–558

Powles SB (1986) Appearance of a biotype of the weed, *Hordeum glaucum* Steud., resistant to the herbicide paraquat. Weed Res 26:167–172

Powles SB, Tucker ES, Morgan TR (1992) Eradication of paraquat-resistant *Hordeum glaucum* Steud. by prevention of seed production for 3 years. Weed Res 32:207–211

Pratley J (1995) Long-term investigations of the effect of tillage practices on crop production at Wagga Wagga, New South Wales. Aust J Exp Agric 35:885–892

Preston C, Holtum JAM, Powles SB (1992) On the mechanism of resistance to paraquat in *Hordeum glaucum* and *H. leporinum*—delayed inhibition of photosynthetic O_2 evolution after paraquat application. Plant Physiol 100:630–636

Preston C, Powles SB (2002) Evolution of herbicide resistance in weeds: initial frequency of target site-based resistance to acetolactate synthase-inhibiting herbicides in *Lolium rigidum*. Heredity 88:8–13

Rauch TA, Thill DC, Gersdorf SA, Price WJ (2010) Widespread occurrence of herbicide-resistant Italian ryegrass (*Lolium multiflorum*) in Northern Idaho and Eastern Washington. Weed Technol 24:281–288

Recasens J, Caimons O, Torra J, Taberner A (2007) Variation in seed germination and early growth between and within acetolactate synthase herbicide resistant and susceptible (*Lolium rigidum*) accessions. Seed Sci Technol 35:32–47

Roper MM, Milroy SP, Poole ML (2012) Green and brown manures in dryland wheat production systems in Mediterranean-type environments. Pages 275–313 (Chapter 5) *in* Donald LS, ed. Advances in Agronomy. San Diego, USA: Academic Press

Shergill LS, Malone J, Boutsalis P, Preston C, Gill GS (2015) Target-site point mutations conferring resistance to ACCase-inhibiting herbicides in smooth barley (*Hordeum glaucum*) and hare barley (*Hordeum leporinum*). Weed Sci 63:408–415

Shergill LS, Preston C, Boutsalis P, Malone J, Gill G (2014) Amino acid substitutions in ACCase gene of barley grass (*Hordeum glaucum* Steud.) associated with resistance to ACCase-inhibiting herbicides. Pages 7–10 *in* Proceedings of 19th Australasian Weeds Conference. Hobart, Tasmania, Australia: Tasmanian Weed Society

Smith DF (1968) The growth of barley grass (*Hordeum leporinum*) in annual pasture. 1. Germination and establishment in comparison with other annual pasture species. Aust J Exp Agric Anim Husb 8:478–483

Sosnoskie LM, Webster TM, Culpepper AS (2013) Glyphosate resistance does not affect Palmer amaranth (*Amaranthus palmeri*) seedbank longevity. Weed Sci 61:283–288

Tranel D, Dekker J (2002) Differential seed germinability in triazine-resistant and -susceptible giant foxtail (*Setaria faberii*). Asian J Plant Sci 1:334–336

Vila-Aiub MM, Neve P, Steadman KJ, Powles SB (2005) Ecological fitness of a multiple herbicide-resistant *Lolium rigidum* population: dynamics of seed germination and seedling emergence of resistant and susceptible phenotypes. J Appl Ecol 42:288–298

Walsh MJ, Powles SB (2007) Management strategies for herbicide-resistant weed populations in Australian dryland crop production systems. Weed Technol 21:332–338

Yu Q, Nelson JK, Zheng MQ, Jackson M, Powles SB (2007) Molecular characterisation of resistance to ALS-inhibiting herbicides in *Hordeum leporinum* biotypes. Pest Manag Sci 63:918–27

Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. Weed Res 14:415–421

CHAPTER 4

MULTIPLE RESISTANCE TO ACCASE AND ALS-INHIBITORS IN *HORDEUM GLAUCUM* STEUD.

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Name of Principal Author (Candidate)	Lovreet Singh Shergill
Contribution to the Paper	Collected plant material, planned and conducted the studies, data collection, data analysis, data interpretation and wrote manuscript.
Overall percentage (%)	85%
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date 29.1.2016

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Multiple resistance to ACCase and ALS-inhibitors in *Hordeum glaucum* Steud.

Running title: Target and Non-target site resistance in *Hordeum glaucum*

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Abstract

BACKGROUND: Previously, ACCase and/or ALS-inhibitor resistance was identified by herbicide resistance screening in eight populations obtained from cropping regions of South Australia. This study aimed to quantify the level of resistance and characterize the molecular basis of resistance to ACCase and ALS-inhibitors in these *H. glaucum* populations.

RESULTS: *H. glaucum* populations from the upper-north region were highly resistant (RI > 12) to the APP herbicides quizalofop and haloxyfop and less resistant (RI = 2 to 12) to CHD herbicide clethodim. Some mid-north populations had low level of resistance (RI = 2 to 6) to the SU herbicide mesosulfuron and one population had multiple resistance to both ACCase and ALS-inhibitors. Gene sequencing confirmed the presence of Ile-1781-Leu, Ile-2041-Asn and Gly-2096-Ala mutations in the *ACCase* gene, with no mutation found in the *ALS* gene. The use of malathion in combination with mesosulfuron enhanced the activity of herbicide. These populations were also susceptible to SU herbicide sulfometuron.

CONCLUSION: This study has documented herbicide cross-resistance and multiple-resistance to ACCase and ALS-inhibitors in *H. glaucum*. Resistance to ACCase-inhibitors is due to a target site mutation. The reversal of SU resistance by malathion and

susceptibility to sulfometuron suggests that non-target site mechanisms confer resistance to ALS-inhibitors.

Keywords: Smooth barley; *ACCase* gene; *ALS* gene; ACCase mutation; target-site; non-target site

1. INTRODUCTION

Acetyl coenzyme-A carboxylase (ACCase)-inhibiting herbicides (hereafter called ACCase-inhibitors) and acetolactate synthase (ALS) [also called acetohydroxyacid synthase (AHAS)]-inhibiting herbicides (hereafter called ALS-inhibitors) are important herbicide groups commonly used to selectively control grass weeds in a variety of crops in Australia[1-3]. ACCase-inhibitors inhibit ACCase enzyme, responsible for fatty acid biosynthesis in grass weeds[4]. Two different types of ACCase enzymes have been identified in plants i.e. cytoplasmic and plastidic. The plastidic form is sensitive to ACCase-inhibitors and contributes up to 80% of the enzyme activity in grasses[5]. ACCase-inhibitors from three different chemical classes i.e. sulphonylureas (SU), aryloxyphenoxypropionate (APP), cyclohexanedione (CHD) and phenylpyrazolin (DEN) specifically target the carboxylase-transferase (CT) domain of the plastidic ACCase, inhibiting fatty acid biosynthesis and ultimately causing plant death[6]. Similarly, ALS-inhibitors from four different chemical families i.e. imidazolinones (IMI), triazolopyrimidines (TP), pyrimidinyl-thiobenzoates (PTB) and sulfonylamino-carbonyl-triazolinones (SCT) inhibit the AHAS enzyme that catalyzes the first reaction in the biosynthesis of branched-chain amino acids valine, leucine and isoleucine[7, 8]. The reduced synthesis of branched-chain amino acids, which is crucial to the growth of young tissues, leads to starvation of the plant for these amino acids and eventually causes plant death[9, 10].

Widespread use of ACCase-inhibitors has led to the evolution of herbicide resistance in 12 grass weed species throughout Australia[11]. ALS-inhibitors due to their

broad spectrum weed control have also been widely used by Australian growers for weed control in fields. Currently, there are 8 grass weed species resistant to ALS-inhibitors in Australia[11]. An altered target-site has been most often reported as the mechanism of resistance to ACCase[12] and ALS-inhibitors[9, 10]. However, non-target site resistance to ACCase-inhibitors is also increasingly being recognised as a mechanism of resistance[12, 13]. Non-target site based resistance to ALS inhibitors is relatively rare but has been reported in several weed species (reviewed in Corbett and Tardif[9]).

H. glaucum is one of the most problematic annual weeds in the grain cropping regions of South Australia (SA)[14, 15]. ACCase-inhibitors from APP and CHD classes and ALS-inhibitors from SU and IMI classes are commonly used to control *H. glaucum* in Australia. Currently, this species has evolved resistance to three different groups of herbicides: ACCase-inhibitors, ALS-inhibitors and bipyridiliums[16-18]. Previously, 1781-Leu and 2096-Ala mutant alleles in the CT domain of *ACCase* gene have been reported to confer resistance to APP herbicides in populations of *H. glaucum*[16]. So far the mechanism of resistance to ALS-inhibitors has not been reported, however, 197-Thr/Ser mutant alleles in the ALS gene of a closely related species *H. leporinum* have been previously reported to confer resistance to ALS-inhibitors[2, 19].

Recent reports from growers and agricultural advisors in SA indicated an increase in the incidence of *H. glaucum* in field crops. Research by Fleet and Gill[20] has shown that weed management practices used in cropping systems of SA have increased seed dormancy in *H. glaucum*, which may have contributed to its increased abundance in field crops. Incidence of resistance to APP ACCase-inhibitors and ALS-inhibitors in *H. glaucum* populations has already been reported[16, 17]. The combination of herbicide resistance and high seed dormancy would make it difficult for Australian farmers to effectively control such weed populations in their fields. Surveys of crop fields identified resistance to ACCase and ALS-inhibiting herbicides in field populations of this weed

species[17]. In this study we have quantified the level of resistance to ACCase and ALS-inhibitors in *H. glaucum* populations collected from SA and also characterised the molecular basis of resistance to both groups of herbicides.

2. MATERIALS AND METHODS

2.1 Plant material

The eight herbicide-resistant (ACCase or ALS-inhibitor or both) *H. glaucum* populations used in this study were collected from different parts of SA (Table 1). Five populations were obtained in a survey from Upper-North (UN) region of SA in 2012[17] and three other populations were obtained from Mid-North (MN) region in 2013, where ACCase or ALS-inhibitor or both herbicides had provided inadequate control of *H. glaucum* in the field. Previously, herbicide resistance screening of these populations had confirmed that populations collected from UN were resistant to ACCase-inhibitor quizalofop[17] and populations collected from MN were resistant to ALS-inhibitor mesosulfuron (data not shown). Moreover, one population collected from MN region was resistant to both ACCase and ALS-inhibitors tested. Therefore, detailed dose-response studies were conducted to determine the level of resistance to ACCase and ALS-inhibitors. A known ACCase-inhibitor resistant population (FP) and a standard susceptible population (Yaninee) were used as resistant and susceptible standards for these experiments[21].

2.2 Seed germination and plant growth

Seeds for all experiments were germinated in plastic trays (33 cm x 28 cm x 5 cm) containing standard cocoa peat potting mix[22]. Germinated seedlings at Z11 stage[23] were transplanted into 9.5 cm x 8.5 cm x 9.5 cm punnet pots (Masrac Plastics, SA) containing standard potting mix. There were seven to nine seedlings per pot and the pots were arranged in a randomized complete block design with three replications for each herbicide dose. The pots were watered as required and maintained outdoors during the normal growing season in 2014 and 2015.

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Table 1. Geographical locations of the tested *H. glaucum* populations.

Population	Location	Coordinates
UN4	Mambray Creek, SA	32º49'S, 137º57'E
UN6	Mambray Creek, SA	32º50'S, 137º55'E
UN10	Port Germein, SA	33º02'S, 138º03'E
UN13	Baroota, SA	32º54'S, 137º58'E
UN15	Mambray Creek, SA	32º48'S, 137º57'E
M146	Rosedale, SA	34°34'S, 138°50'E
M83	Yorketown, SA	35°00'S, 137°37'E
M87	Sheok Flat, SA	34°42'S, 137°51'E
FP	Baroota, SA	32º55'S, 137º59'E
Yaninee	Yaninee, SA	32°56'S, 135°14'E

2.3   Dose-response experiments

To determine the level of resistance to ACCase and ALS-inhibitors among resistant
biotypes of *H. glaucum*, dose-response experiments were conducted during May-
September in 2014 and 2015. At Z12-13 stage, commercial formulations of herbicides
from subgroups of ACCase and ALS-inhibitors were applied with a moving-boom
laboratory twin-nozzle cabinet sprayer delivering herbicide in 121 L ha^{-1} water at a
pressure of 250 kPa and a speed of 1 m s^{-1}. Plants were returned and maintained outdoors
after herbicide application. At 28 days after treatment (DAT), visual survival assessment
was made and above ground shoot biomass of surviving plants was harvested. Plants with
new green leaf tissue after herbicide application were recorded as resistant (R), and plants
showing severe chlorosis, stunting and mortality were considered as susceptible (S). The
harvested plants were oven dried at 80°C for 72 h, and weighed. The details of ACCase
and ALS-inhibitors used in this study are given below.

2.3.1 ACCase-inhibitors

Commercial formulations of two APP herbicides: quizalofop (Targa[®], 99.5 g L^{-1}) and haloxyfop (Verdict[®], 520 g L^{-1}); and one CHD herbicide: clethodim (Select[®], 240 g L^{-1}) were applied. Herbicides as commercial formulations were applied with adjuvants as specified on the labels: 0.2% v/v BS1000, a biodegradable wetting and spreading agent, for quizalofop and 1% v/v Hasten™, a blend of esterified vegetable oil and non-ionic surfactants, for haloxyfop and clethodim. Seedlings were sprayed with a range of herbicide doses (minimum six): quizalofop was applied from 0 to 199 g ha^{-1}, haloxyfop from 0 to 312 g ha^{-1} and clethodim from 0 to 480 g ha^{-1}. The recommended field rate for quizalofop, haloxyfop and clethodim in Australia is 25, 39 and 60 g ha^{-1}, respectively.

2.3.2 ALS-inhibitors

Commercial formulations of two SU herbicides mesosulfuron (Atlantis[®], 30 g L^{-1}) and sulfometuron (Oust[®], 750 g kg^{-1}) and one IMI herbicide imazamox plus imazapyr (Intervix[®], 33 g L^{-1} and 15 g L^{-1}) were applied. Herbicides as commercial formulation were applied with adjuvants as specified on the labels: 1% v/v Hasten™ for mesosulfuron and 0.2% v/v BS1000 for imazamox plus imazapyr. Seedlings were sprayed with mesosulfuron from 0 to 40 g ha^{-1}, sulfometuron from 0 to 30 g ha^{-1} and imazamox plus imazapyr from 0 to 79 plus 36 g ha^{-1}. The recommended field rate for mesosulfuron, sulfometuron and imazamox plus imazapyr in Australia is 9.9, 15 and 19.8 plus 9 g ha^{-1}, respectively.

2.4 Synergistic effect of malathion

Malathion is a non-systemic, contact, organophosphate insecticide and acaricide known to inhibit cytochrome P450 monooxygenases[24, 25]. Seedlings were sprayed with malathion at 1000 g ha^{-1}, 30 min prior to the herbicide application as described above. ACCase-inhibitors were applied with or without malathion at untreated control and field rate,

whereas, ALS-inhibitor mesosulfuron was applied with or without malathion at all herbicide rates. Assessments were taken at 28 DAT as described above.

2.5   Sequencing of ACCase and ALS gene

At 28 DAT, fresh leaf material plant material (100mg) from the new or youngest green leaf tissue of R and S plants (5 individuals per population) was collected from the dose-response experiments. Fresh leaf tissue thus obtained was snap frozen in liquid nitrogen and stored at -20 °C. As per manufacturer's instructions, DNeasy Plant Mini Kit (Qiagen) was used to extract DNA from 50 to 100 mg plant tissue under liquid nitrogen. For *ACCase* gene, polymerase chain reactions (PCR) were performed using two sets of previously described primers [16, 26], which amplified nearly the entire carboxyl transferase (CT) domain of the plastidic *ACCase* gene. For ALS gene, PCR were performed using two sets of primers designed on the basis of homologous regions of ALS gene sequences of *Arabidopsis thaliana* (AY042819) *and Raphanus raphanistrum* (AJ344986). Primers used were: ALS-1R (5'-CAAGCTGTTGCTGAATATC-3'), ALS-1F (5'-TTCATCTCCCGATACGCTCCC-3'), ALS-3R (5'-TCAATACTAAGTGCTACCATC-3') and ALS-3F (5'-GGAGAAGCCATTCCTCC-3'). MyFi DNA polymerase kit (Bioline) was used to run a PCR reaction of 25 µL, which contained $1 \times$ MyFi reaction buffer, 80-100 ng DNA template, 0.8µM primers each and 2 units of DNA polymerase. An automated DNA thermal cycler (Bioer) was used for DNA amplification with PCR conditions as follows: 3 min denaturing at 94 ºC, 40 cycles of 30s denaturation at 94 ºC, 30s annealing at 56 ºC and 2 min elongation at 68 ºC, and a final extension for 7 min at 68 ºC.

PCR products were prepared using $1 \times$ Ficoll loading dye [15% (w/v) Ficoll 4000, 0.25% (w/v) bromophenol blue, 0.25% (w/v) xylene cyanol FF] and visualised on 1.5% agarose gels stained with $1 \times$ SYBR® Safe DNA gel stain. Samples were electrophoresed in $1 \times$ TAE Buffer (40 mM Trizma base, 1 mM Na2EDTA, pH to 8 with glacial acetic

acid) at 100 V and photographed under UV light (λ = 302 nm). DNA fragment sizes were estimated by comparing their mobility to bands of known sizes in a low mass molecular weight marker (EasyLadder; Bioline). PCR products were sequenced by Australian Genome Research Facility (AGRF) Ltd., Australia using same primers described previously for *ACCase* gene[16, 26]; ALS gene was sequenced using the same primers as for amplification. All the sequences were visually rechecked using the chromatogram files and DNA sequence data were assembled, compared and analyzed using Geneious® v8.1 (Biomatters).

2.6   Statistical analysis

Plant survival was pooled and analysed using probit analysis for binary data [27] and the dose of herbicide required to kill 50% of the plants (LD_{50}) were obtained. Dose-response curves were obtained by plotting probits and actual data using GraphPad Prism v6.0. The level of resistance from the dose-response was derived by calculating resistance index (RI), i.e. the ratio between the LD_{50} of R population and the LD_{50} of the S control. The RI of LD_{50} values was used to classify the herbicide resistance level for the R populations. The populations were rated as having high (RI > 12), moderate (RI > 6 to 12) or low (RI = 2 to 6) resistance [28, 29].

Shoot dry biomass data from the dose-response experiment were transformed as percent of untreated control before regression analysis. Data were pooled and a non-linear, log-logistic regression model (Equation 1) was fitted to the data using GraphPad Prism v.6.0. Herbicide dose required to inhibit plant growth by 50% (GR_{50}) with respect to the untreated control were calculated for each population, and RI was computed as GR_{50} (R)/GR_{50} (S). The model fitted was

$$y = \frac{100}{1 + 10^{(\log IC_{50} - x) \times b}} \tag{1}$$

where, y is the plant biomass (% of control), x is the log-dose of the herbicide used, IC_{50} is the dose of herbicide required to inhibit plant growth by 50%, and b is the slope of the curve.

3. RESULTS AND DISCUSSION

3.1 Dose-response to ACCase-inhibitors

Dose-response experiments showed high level of resistance in *H. glaucum* populations collected from UN region and low level of resistance in populations collected from MN (Table 2). Sole application of malathion at 1000 g ha^{-1} did not affect survival or biomass of either R or S populations compared to untreated control (data not shown). Moreover, no synergistic effect of malathion was observed when applied in addition to ACCase-inhibitors (data not shown). Similar non-synergistic effect was observed when malathion was used in combination of diclofop and tralkoxydim in *Lolium rigidum* Gaudin[30]. At 28 DAT, the standard S population (Yaninee) was controlled and the standard R population (FP) survived (74 to 100% survival) at the recommended field rates of all the ACCase-inhibitors used, i.e. quizalofop at 24.9 g ha^{-1}, haloxyfop at 39 g ha^{-1} and clethodim at 60 g ha^{-1} (Figure 1).

During both the experimental runs, all the populations tested were highly resistant (RI > 12) to quizalofop except M146, which had low resistance (RI = 6). The quizalofop LD_{50} for the standard R and S populations (F.P and Yaninee) was 178.8 and 2.5 g ha^{-1}, respectively, with a RI of 71.3 (Table 2). One population, UN15, exhibited little mortality even at 199.2 g quizalofop ha^{-1} (8-fold the recommended field rate) (Figure 1A), therefore, the LD_{50} for this population could not be predicted. Based upon the maximum rate used (199.2 g ha^{-1}), LD_{50} was >199.2 g ha^{-1}, which was >79.7-fold greater than S population (Table 2). For the remaining five R populations, the quizalofop LD_{50} ranged from 15.0 to

Table 2. Estimated LD_{50}, GR_{50} and resistance index (RI) values for *H. glaucum* populations treated with quizalofop, haloxyfop and clethodim. Values in parenthesis are 95% confidence intervals. Data are means of two experiments.

Population	Quizalofop				Haloxyfop				Clethodim			
	LD_{50}	RI	GR_{50}	RI	LD_{50}	RI	GR_{50}	RI	LD_{50}	RI	GR_{50}	RI
UN4	165.9 (144.2, 199.1)	66.1	68.1 (51.4, 90.3)	26.2	117.1 (106.9, 129.3)	27.7	57.7 (45.7, 73.0)	14.7	79.5 (65.9, 95.7)	8.0	62.7 (41.2, 95.4)	8.3
UN6	149.3 (126.9, 184.2)	59.5	60.7 (44.7, 82.7)	23.4	88.6 (78.6, 99.9)	21.0	58.2 (45.9, 73.8)	14.9	89.9 (76.2, 106.1)	9.1	42.8 (34.2, 53.5)	5.7
UN10	186.6 (161.3, 231)	74.4	74.4 (55.9, 99.1)	28.7	74.4 (67.3, 82.2)	17.6	66.2 (53.8, 81.4)	16.9	102.2 (87.4, 119.5)	10.3	101.5 (76.5, 134.7)	13.4
UN13	139.5 (110.1, 194.2)	55.6	80.5 (55.5, 116.7)	31.0	100.6 (67.7, 170.1)	23.8	63.8 (46.1, 88.3)	16.3	36.8 (32, 42.2)	3.7	39.2 (30.9, 49.7)	5.2
UN15	>199.2	>79.7	>199.2	>76.6	>312	>74.3	>312	>80	35.0 (29.9, 40.6)	3.5	20.3 (15.4, 26.7)	2.7
M146	15 (7.8, 22.8)	6.0	17.8 (13.6, 23.4)	6.8	-	-	-	-	22.0 (0.9, 44)	2.2	21.3 (17.9, 25.4)	2.8
FP	178.8 (160.2, 200.8)	71.3	87.6 (58.5, 131.1)	33.7	86.9 (77.2, 97.8)	20.6	65.6 (55.8, 77.1)	16.7	96.3 (81.8, 113.4)	9.7	52.5 (42.7, 64.7)	6.9
Yaninee	2.5 (2.5, 2.5)	-	2.6 (2.4, 2.8)	-	4.2 (3.4, 5.1)	-	3.9 (3.2, 4.8)	-	9.9 (8.2, 12.1)	-	7.6 (6.1, 9.5)	-

Resistance index (RI) was calculated as the ratio between the LD_{50} or GR_{50} of each population and the LD_{50} or GR_{50} of the susceptible control. The recommended field rate for quizalofop, haloxyfop and clethodim is 24.9, 39 and 60 g ha^{-1}, respectively.
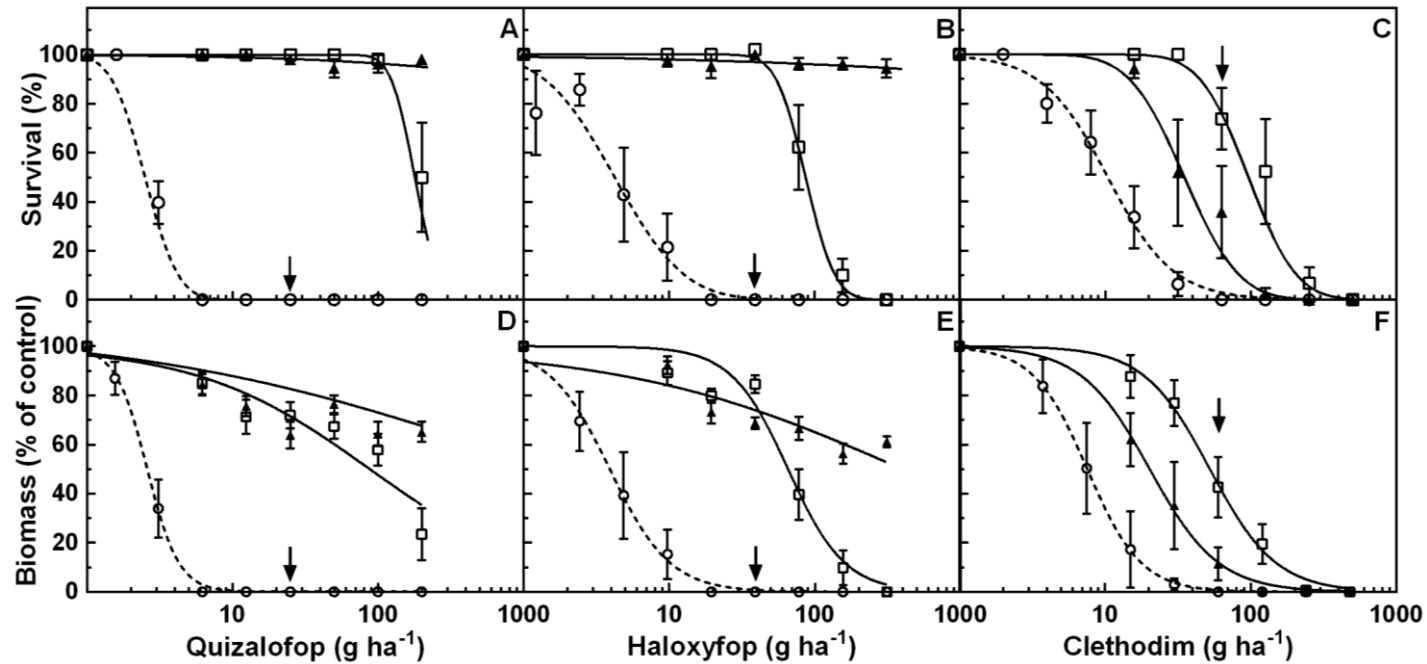
Figure 1. Dose-response for survival (A, B and C) and shoot dry matter (D, E and F) of one herbicide-resistant (UN15 ▲), standard resistant (FP □) and susceptible (Yaninee ○) population of *H. glaucum* treated with quizalofop (A and D), haloxyfop (B and E) and clethodim (C and F). Plants with new green leaf tissue were recorded as resistant, whereas, those that displayed severe chlorosis or no new growth were recorded as susceptible. Each data point represents the mean percentage survival ± SE or mean shoot dry weight expressed as a percentage of the untreated controls ± SE of the pooled data for both the experiments. Downward arrow (↓) indicates the recommended field rate of the herbicide used.

186.6 g ha^{-1}, which was 6 to 74.4-fold greater than the S population. Similarly, the quizalofop GR_{50} for the standard R and S populations was 87.6 and 2.6 g ha^{-1}, with a RI of 33.7 (Table 2). The shoot dry biomass production of UN15 population was reduced to 65% of untreated control at the maximum dose of quizalofop used. The quizalofop GR_{50} for UN15 population could not be predicted but was greater than the maximum dose (>199.2 g ha^{-1}) of the herbicide used, which was >76.6-fold greater than S population. The quizalofop GR_{50} for the remaining five R populations ranged from 17.8 to 80.5 g ha^{-1}, which was 6.8 to 31-fold greater than the S population. Varying levels of quizalofop resistance have previously been reported in *H. glaucum*, where the resistant populations were greater than 27-fold resistant to the herbicide[16]. Similarly, high level of quizalofop resistance was observed in other grasses, such as *Avena fatua* L.[31], *Echinochloa crusgalli* (L.) Beauv.[32, 33] and *Setaria faberi* Herrm[34].

Similar to quizalofop, all the resistant populations were highly resistant (RI > 12) to haloxyfop (Table 2). Although haloxyfop was also not effective in controlling R populations at the field rate (39 g ha^{-1}), it exhibited greater activity than quizalofop at higher herbicide rates. This is consistent with a previous study conducted on *H. glaucum* [16]. The haloxyfop LD_{50} for the standard R population was 86.9 g ha^{-1}, which was 20.6-fold greater than S population, with LD_{50} of 4.2 g ha^{-1} (Table 2). Similar to quizalofop, haloxyfop was ineffective in controlling UN15 population even at the maximum herbicide dose used (8-fold the recommended field rate) (Fig 1B). The predicted LD_{50} for UN15 was >312 g ha^{-1}, which was >74-fold greater than the S population. The remaining four R populations were 17.6 to 27.7-fold resistant to haloxyfop than S population, with LD_{50} ranging from 74.4 to 117.1 g ha^{-1}. For the standard R and S populations, the haloxyfop GR_{50} was 65.6 and 3.9 g ha^{-1}, with a RI of 16.7 (Table 2). Similar to quizalofop, shoot dry biomass reduction for UN15 population was 61% compared to the untreated control at the maximum haloxyfop dose used. Therefore, the GR_{50} could not be predicted, but was

greater than the maximum dose of haloxyfop used (>312 g ha^{-1}). The haloxyfop GR$_{50}$ values ranged from 57.7 to 66.2 g ha^{-1} for the remaining four R populations, which were 14.7 to 16.9-fold greater than the S population. Previously high level of resistance to haloxyfop (15 to 60-fold) has been reported in *H. glaucum*[16]. Similarly, *Alopecurus japonicus* Steud. populations from China were also found to be 12-fold resistant to haloxyfop[35].

APP-to-CHD herbicide cross-resistance was found in all the populations tested. In contrast to APP herbicides, all the populations tested with CHD herbicide clethodim had moderate (RI $>$ 6 to 12) or low resistance (RI = 2 to 6). Although, clethodim is not preferentially used by growers to control *H. glaucum* due to its lower activity compared to APP herbicides, it was more active on R populations at higher doses compared to APP herbicides (Figure 1). The clethodim LD$_{50}$ for the standard R and S populations were 96.3 and 9.9 g ha^{-1}, respectively, with a RI of 9.7 (Table 2). The R populations were approximately two to ten-fold more resistant to clethodim than the S population. Similarly clethodim was more effective in reducing shoot biomass compared to the APP herbicides tested. The clethodim GR$_{50}$ values for the standard R and S populations were 52.5 and 7.6 g ha^{-1}, respectively (Table 2). The clethodim GR$_{50}$ for the R populations ranged from 20.3 to 101.5 g ha^{-1}, which was 2.7 to 13.4-fold greater than the S population. APP-to-CHD herbicide cross-resistance in *H. glaucum* has not been reported in the literature yet. However, APP and CHD herbicide resistance has been previously reported in its closely related species *H. leporinum* from SA[36]. Studies conducted in other grass weed species, such as *L. rigidum*[29, 37-40], *L. perenne ssp. multiflorum* L.[41], *A. fatua* L.[28, 42], *Polypogon fugax*[43] and *Phalaris paradoxa*[44, 45] reported high level of clethodim resistance. Studies conducted on *P. paradoxa* showed APP-to-CHD cross-resistance patterns, with lower RI for CHD herbicides compared to APP herbicides[45]. The level of resistance to clethodim in

the current study is lower than that of APP herbicides and some of these populations can still be controlled at high clethodim rates.

3.2 Dose-response to ALS-inhibitors

Dose-response experiments showed that the *H. glaucum* populations collected from MN had low level (RI = 2 to 6) of resistance to the SU herbicide mesosulfuron, but were susceptible to the SU herbicide sulfometuron and the IMI herbicide imazamox plus imazapyr. As expected, mesosulfuron controlled the standard S population (Yaninee) at the recommended field rate of 9.9 g ha^{-1} (Figure 2). A greater dose of mesosulfuron was required to control the R populations with LD$_{50}$ ranging from 10.1 to 14.5 g ha^{-1}, which are approximately three to four-fold greater than the S population (Table 3). Although, the presence of resistance to ALS-inhibitors has been previously reported in *H. glaucum*[17] the level and mechanism of resistance has not been quantified till now. Resistance to SU herbicides has been previously reported in a closely related species *H. leporinum*[2, 19] and various other grass weeds, such as *L. rigidum*[46], *Bromus rigidus*[47] and *A. fatua*[25]. Application of malathion (1000 g ha^{-1}) 30 min prior to mesosulfuron application had a significant synergistic effect on R and S populations. The R populations became susceptible even at the lower herbicide rates and susceptibility of S population was further increased (Figure 2). In the presence of malathion, the mesosulfuron LD$_{50}$ of R populations was similar to the S population (Table 3). Synergistic effects of malathion and ALS-inhibitors have been well documented and literature suggests that malathion is an effective cytochrome P450 inhibitor[25, 30, 48]. This indicated that a non-target site mechanism that enhanced herbicide metabolism could be involved in conferring herbicide resistance to ALS-inhibitors.
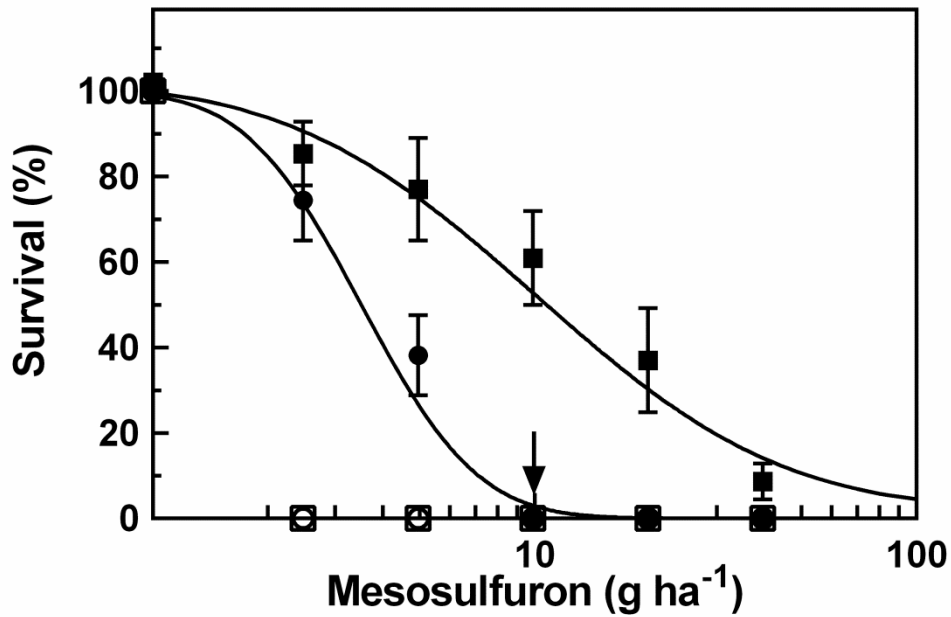
Figure 2. Dose–response curves for survival of one herbicide-resistant (M146 ■ or □) and susceptible (Yaninee ● or ○) *H. glaucum* populations treated with a range of mesosulfuron doses plus (open symbols) or minus (filled symbols) 1000 g ha^{-1} malathion. Each data point represents the mean percentage survival ± SE of the untreated controls ± SE of the pooled data for both the experiments. Downward arrow (↓) indicates the recommended field rate of the herbicide used.

Table 3. Estimated LD_{50} and resistance index (RI) values for *H. glaucum* populations treated with mesosulfuron and mesosulfuron + malathion. Data are means of two experiments.

| Population | Mesosulfuron | | | | Mesosulfuron + Malathion | | | |
| | LD_{50} | 95% C.I. | | RI | LD_{50} | 95% C.I. | | RI |
		Lower	Upper			Lower	Upper	
M146	10.1	7.8	13.2	2.9	0.04	0.04	0.04	1.0
M83	11.3	9.1	14.2	3.2	0.04	0.04	0.04	1.0
M87	14.5	11.7	18.3	4.1	0.04	0.04	0.04	1.0
Yaninee	3.5	2.9	4.1	-	0.04	0.04	0.04	-

Resistance index (RI) was calculated as the ratio between the LD_{50} of each population and the LD_{50} of the susceptible control. The recommended field rate for mesosulfuron is 9.9 g ha^{-1} and malathion was used at 1000 g ha^{-1}.

3.3   Multiple resistance in *H. glaucum*

During both experimental runs, the M146 population demonstrated low level (RI = 2 to 6) of resistance to both ACCase and ALS-inhibitors (Table 2 and 4). It was 6-fold resistant to quizalofop, 2.2-fold resistant to clethodim and 2.9-fold resistant to mesosulfuron compared to the S population. Similarly, the GR_{50} values were 17.8 and 21.3 g ha^{-1} for quizalofop and clethodim, respectively, which was 2.8 and 6.8-fold greater than the S population (Table 2). As described earlier, application of malathion prior to ACCase-inhibitors did not improve control, whereas survival was significantly reduced in the case of ALS-inhibitors. This study documents multiple resistance in a population of *H. glaucum* to both ACCase and ALS-inhibitors. However, multiple resistance to ACCase and ALS-inhibitors has been previously reported in other grass weed species such as *L. rigidum* [30, 49], *A. aequalis*[8] and *A. fatua* [25].

3.4 Resistance mechanisms: Target and non-target site

Among the *H. glaucum* populations resistant to ACCase-inhibitors, target-site mutations were identified in all resistant populations, except M146 population (Table 4). Sequencing of the CT domain of the plastidic *ACCase* gene revealed three known ACCase mutations (Ile-1781-Leu, Ile-2041-Asn and Gly-2096-Ala) in these populations (Table 4). The standard R population (FP) contained an amino acid substitution at 1781 position. Among five ACCase-inhibitor resistant populations containing mutations, Ile-2041-Asn mutation was detected in two populations (UN13 and UN15), and Ile-1781-Leu was identified in three other populations (UN4, UN6 and UN10) (Table 4). However, in UN13 multiple ACCase mutations were present, a codon change from GGC to GCC resulted in a predicted amino acid substitution of Gly-2096-Ala in four individuals, whereas, a codon change from ATT to AAT resulted in a predicted amino acid substitution of Ile-2041-Asn in one individual (Table 4). ACCase-inhibitor resistance in *H. glaucum* populations is likely due to altered target-site, i.e. amino acid changes at 1781, 2041 and 2096 positions. However, a non-target site mechanism of resistance to ACCase-inhibitors is suspected to confer resistance in M146, the multiple-resistant population. Amino acid substitution at 1781 position confers high levels of resistance to most ACCase-inhibitors and is the most common substitution found in grass weed species[50, 51]. However, 2041 and 2096 mutations provides moderate to high level of resistance to APP herbicides (reviewed in Beckie and Tardif[50]) and low to moderate level of resistance to CHD herbicides[29]. Amino acid substitutions at 1781 and 2096 loci have been recently reported to be the basis of high level of resistance to APP herbicides in *H. glaucum*[16], whereas 2041 mutation has not been previously reported in *H. glaucum*.

Of the three populations resistant to ALS-inhibitor mesosulfuron (Table 3), no mutations were found in any of the populations. It was observed that application of

Table 4. Comparison of nucleotide sequence and derived amino acid sequence of a highly conserved region of the ACCase enzyme from susceptible and resistant populations of *H. glaucum*.

Amino acid number	1781	1999	2027	2041	2078	2088	2096
Amino acid	Ile	Trp	Trp	Ile	Asp	Phe	Gly
Consensus sequence	ATA	TGG	TGG	ATT	GAT	TTC	GGC
UN4	CTA (5)	-	-	-	-	-	-
UN6	CTA (5)	-	-	-	-	-	-
UN10	CTA (5)	-	-	-	-	-	-
UN13	-	-	-	AAT (1)	-	-	GCC (4)
UN15	-	-	-	AAT (5)	-	-	-
M146	-	-	-	-	-	-	-
FP	CTA (5)	-	-	-	-	-	-
Yaninee	-	-	-	-	-	-	-

Hyphen (-) indicates identical codon to the consensus sequence. Figures in parentheses are number of individuals in which specific mutation(s) were identified.

malathion prior to herbicide application improved control of all these populations. Since malathion is a cytochrome P450 monooxygenases inhibitor, evidence suggests that cytochrome P450 plays a vital role in ALS-inhibitor resistance of these populations. Moreover, these populations were susceptible to sulfometuron, which also indicates the presence of non-target mechanism of resistance. Previous studies suggest that plants with non-target site mechanism of resistance are susceptible to sulfometuron[2]. The absence of mutations in ACCase and ALS enzyme of M146 population and reversal of SU resistance by malathion suggest that non-target site mechanisms i.e. enhanced metabolism by cytochrome P450 are involved in conferring resistance to ACCase and ALS-inhibitors.

Similar synergism of SU and malathion has been previously reported in *Alopecurus myosuroides*[52], *Vulpia bromides*[53] and *Lolium rigidum*[30].

4.   CONCLUSIONS

The selection pressure imposed by the application of ACCase and ALS-inhibitors has resulted in the evolution of resistance to both modes of action in *H. glaucum*. *H. glaucum* has evolved a high level of resistance to ACCase-inhibitors and a low level of resistance to ALS-inhibitors. This study has documented the first known case of field evolved APP-to-CHD herbicide cross-resistance, multiple-resistance to ACCase and ALS-inhibitors and also quantified the level of resistance to ALS-inhibitors in *H. glaucum*. Three previously known target-site mutations (1781, 2041, and 2096) conferring resistance to ACCase-inhibitors and a non-target site mechanism of resistance to ALS-inhibitors were identified in *H. glaucum* populations. Furthermore, we have also confirmed the first case of ACCase-inhibitor resistance due to amino acid substitution at 2041 in *H. glaucum*. Resistance to both groups of herbicides pose a serious management problem for growers. Increasing herbicide doses in the case of CHD and SU herbicides may improve control of this species, but this strategy is unlikely to be effective in the case of APP herbicides and may select for more highly resistant individuals. Weed management strategies should be diversified by using herbicides with different modes of action. Clearfield™ technology in wheat is currently being heavily used by Australian growers for the control ACCase-resistant *H. glaucum*. This practice is working well in most situations, but needs to be used with caution because resistance to ALS-inhibiting herbicides can develop rapidly compared to other herbicide groups [10, 50].

REFERENCES

1.   Preston C. Herbicide resistance: target site mutations. In *Weedy and Invasive Plant Genomics*, ed. by Stewart CN. Wiley-Blackwell: Oxford, UK, pp. 127-148 (2009).

2.   Yu Q, Nelson JK, Zheng MQ, Jackson M and Powles SB, Molecular characterisation of resistance to ALS-inhibiting herbicides in *Hordeum leporinum* biotypes. *Pest Manag Sci* **63**(9): 918-927 (2007).

3.   Powles SB and Yu Q, Evolution in action: Plants resistant to herbicides. *Annual Review of Plant Biology* **61**(317-347 (2010).

4.   Burton JD, Gronwald JW, Keith RA, Somers DA, Gengenbach BG and Wyse DL, Kinetics of inhibition of acetyl-coenzyme A carboxylase by sethoxydim and haloxyfop. *Pest Biochem Physiol* **39**(2): 100-109 (1991).

5.   Preston C and Mallory-Smith CA. Biochemical mechanisms, inheritance, and molecular genetics. In *Herbicide resistance and world grains*, ed. by Powles SB and Shaner DL. CRC Press: Florida, pp. 23-60 (2001).

6.   Sasaki Y, Konishi T and Nagano Y, The Compartmentation of Acetyl-Coenzyme A Carboxylase in Plants. *Plant Physiol* **108**(2): 445-449 (1995).

7.   McCourt JA and Duggleby RG, Acetohydroxyacid synthase and its role in the biosynthetic pathway for branched-chain amino acids. *Amino Acids* **31**(2): 173-210 (2006).

8. Guo W, Yuan G, Liu W, Bi Y, Du L, Zhang C, Li Q and Wang J, Multiple resistance to ACCase and AHAS-inhibiting herbicides in shortawn foxtail (*Alopecurus aequalis* Sobol.) from China. *Pest Biochem Physiol* **124**(66-72 (2015).

9. Corbett C-AL and Tardif FJ, Detection of resistance to acetolactate synthase inhibitors in weeds with emphasis on DNA-based techniques: a review. *Pest Manag Sci* **62**(7): 584-597 (2006).

10. Tranel PJ and Wright TR, Resistance of weeds to ALS-inhibiting herbicides: what have we learned? *Weed Sci* **50**(6): 700-712 (2002).

11. Heap I, The international survey of herbicide resistant weeds. www.weedscience.org [accessed September 3 2015].

12. Kaundun SS, Resistance to acetyl-CoA carboxylase-inhibiting herbicides. *Pest Manag Sci* **70**(9): 1405-1417 (2014).

13. Délye C, Gardin JAC, Boucansaud K, Chauvel B and Petit C, Non-target-site-based resistance should be the centre of attention for herbicide resistance research: *Alopecurus myosuroides* as an illustration. *Weed Res* **51**(5): 433-437 (2011).

14. Cocks PS, Boyce KG and Kloot PM, The *Hordeum murinum* complex in Australia. *Aust J Bot* **24**(651-662 (1976).

15. Fleet B and Gill G. Barley grass, an emerging weed threat in southern Australian cropping systems. In *15th Agronomy Conference*, ed. by Dove H and Culvenor R. Australian Society of Agronomy: Lincoln, New Zealand (2010).

16. Shergill LS, Malone J, Boutsalis P, Preston C and Gill G, Target-Site Point Mutations Conferring Resistance to ACCase-Inhibiting Herbicides in Smooth

Barley (Hordeum glaucum) and Hare Barley (Hordeum leporinum). *Weed Sci* **63**(2): 408-415 (2015).

17.   Shergill LS, Fleet B, Preston C and Gill G, Incidence of herbicide resistance, seedling emergence, and seed persistence of smooth barley (*Hordeum glaucum*) in South Australia. *Weed Technol* **29**(4): 782-792 (2015).

18.   Preston C, Holtum JAM and Powles SB, On the mechanism of resistance to paraquat in *Hordeum glaucum* and *H. leporinum* - delayed inhibition of photosynthetic O_2 evolution after paraquat application. *Plant Physiol* **100**(630-636 (1992).

19.   Owen MJ, Goggin DE and Powles SB, Identification of resistance to either paraquat or ALS-inhibiting herbicides in two Western Australian *Hordeum leporinum* biotypes. *Pest Manag Sci* **68**(5): 757-763 (2012).

20.   Fleet B and Gill G, Seed dormancy and seedling recruitment in smooth barley (*Hordeum murinum* ssp. *glaucum*) populations in southern Australia. *Weed Sci* **60**(3): 394-400 (2012).

21.   Shergill LS, Preston C, Boutsalis P, Malone J and Gill G. Amino acid substitutions in ACCase gene of barley grass (*Hordeum glaucum* Steud.) associated with resistance to ACCase-inhibiting herbicides. In *19th Australasian Weeds Conference*, ed. by Baker M. Tasmanian Weed Society Hobart, Tasmania, Australia, pp. 7-10 (2014).

22.   Boutsalis P, Gill GS and Preston C, Incidence of herbicide resistance in rigid ryegrass (*Lolium rigidum*) across southeastern Australia. *Weed Technol* **26**(3): 391-398 (2012).

23.   Zadoks JC, Chang TT and Konzak CF, A decimal code for the growth stages of cereals. *Weed Res* **14**(6): 415-421 (1974).

24.   Preston C, Herbicide resistance in weeds endowed by enhanced detoxification: complications for management. *Weed Sci* **52**(3): 448-453 (2004).

25.   Beckie HJ, Warwick SI and Sauder CA, Basis for herbicide resistance in Canadian populations of wild oat (*Avena fatua*). *Weed Sci* **60**(1): 10-18 (2012).

26.   Malone JM, Boutsalis P, Baker J and Preston C, Distribution of herbicide-resistant acetyl-coenzyme A carboxylase alleles in *Lolium rigidum* across grain cropping areas of South Australia. *Weed Res* **54**(1): 78-86 (2014).

27.   Sakuma M, Probit analysis of preference data. *Appl Entomol Zool* **33**(3): 339-347 (1998).

28.   Ahmad-Hamdani MS, Owen MJ, Yu Q and Powles SB, ACCase-inhibiting herbicide-resistant *Avena* spp. populations from the western Australian grain belt. *Weed Technol* **26**(1): 130-136 (2012).

29.   Saini RK, Malone J, Preston C and Gill G, Target enzyme-based resistance to clethodim in *Lolium rigidum* populations in Australia. *Weed Sci* **63**(4): 946-953 (2015).

30.   Preston C, Tardif FJ, Christopher JT and Powles SB, Multiple Resistance to Dissimilar Herbicide Chemistries in a Biotype of Lolium rigidum Due to Enhanced Activity of Several Herbicide Degrading Enzymes. *Pest Biochem Physiol* **54**(2): 123-134 (1996).

31.   Uludag A, Park KW, Cannon J and Mallory-Smith CA, Cross resistance of acetyl-coa carboxylase (ACCase) inhibitor–resistant wild oat (*Avena fatua*) biotypes in the Pacific Northwest. *Weed Technol* **22**(1): 142-145 (2008).

32.   Huan Z-B, Zhang H-J, Hou Z, Zhang S-Y, Zhang Y, Liu W-T, Bi Y-L and Wang J-X, Resistance level and metabolism of barnyard-grass (*Echinochloa crusgalli* (l.) beauv.) populations to quizalofop-p-ethyl in Heilongjiang Province, China. *Agri Sci China* **10**(12): 1914-1922 (2011).

33.   Huan Z, Xu Z, Lv D and Wang J, Determination of ACCase sensitivity and gene expression in quizalofop–ethyl-resistant and -susceptible barnyardgrass (*Echinochloa crusgalli*) biotypes. *Weed Sci* **61**(4): 537-542 (2013).

34.   Stoltenberg DE and Wiederholt RJ, Giant foxtail (*Setaria faberi*) resistance to aryloxyphenoxypropionate and cyclohexanedione herbicides. *Weed Sci* **43**(4): 527-535 (1995).

35.   Yang C, Dong L, Li J and Moss SR, Identification of japanese foxtail (*Alopecurus japonicus*) resistant to haloxyfop using three different assay techniques. *Weed Sci* **55**(6): 537-540 (2007).

36.   Matthews N, Powles SB and Preston C, Mechanisms of resistance to acetyl-coenzyme A carboxylase inhibiting herbicides in a *Hordeum leporinum* population. *Pest Manag Sci* **56**(441-447 (2000).

37.   Saini RK, Kleemann SGL, Preston C and Gill GS, Control of clethodim-resistant *Lolium rigidum* (rigid ryegrass) in triazine-tolerant canola (*Brassica napus* L.) in southern Australia. *Crop Prot* **78**(99-105 (2015).

38. Saini RK, Kleemann SGL, Preston C and Gill GS, Alternative Herbicides for the Management of Clethodim-Resistant Rigid Ryegrass (Lolium rigidum) in Faba Bean (Vicia faba L.) in Southern Australia. *Weed Technol* **29**(3): 578-586 (2015).

39. Broster J, Koetz E and Wu H, Herbicide resistance levels in annual ryegrass (*Lolium rigidum* Gaud.) in southern New South Wales. *Plant Prot Q* **26**(1): 22-28 (2011).

40. Yu Q, Collavo A, Zheng M-Q, Owen M, Sattin M and Powles SB, Diversity of acetyl-coenzyme A carboxylase mutations in resistant *Lolium* populations: evaluation using clethodim. *Plant Physiol* **145**(2): 547-558 (2007).

41. Martins BAB, Sánchez-Olguín E, Perez-Jones A, Hulting AG and Mallory-Smith C, Alleles contributing to ACCase-resistance in an Italian ryegrass (*Lolium perenne ssp. multiflorum*) population from Oregon. *Weed Sci* **62**(3): 468-473 (2014).

42. Owen MJ and Powles SB, Distribution and frequency of herbicide-resistant wild oat (*Avena* spp.) across the Western Australian grain belt. *Crop Pasture Sci* **60**(1): 25-31 (2009).

43. Tang W, Zhou F, Chen J and Zhou X, Resistance to ACCase-inhibiting herbicides in an Asia minor bluegrass (*Polypogon fugax*) population in China. *Pest Biochem Physiol* **108**(0): 16-20 (2014).

44. Hochberg O, Sibony M and Rubin B, The response of ACCase-resistant Phalaris paradoxa populations involves two different target site mutations. *Weed Res* **49**(1): 37-46 (2009).

45. Cruz-Hipolito H, Domínguez-Valenzuela J, Osuna M and Prado R, Resistance mechanism to acetyl coenzyme A carboxylase inhibiting herbicides in *Phalaris paradoxa* collected in Mexican wheat fields. *Plant Soil* **355**(1-2): 121-130 (2012).

46. Christopher JT, Powles SB and Holtum JA, Resistance to acetolactate synthase-inhibiting herbicides in annual ryegrass (*Lolium rigidum*) involves at least two mechanisms. *Plant Physiol* **100**(4): 1909-1913 (1992).

47. Owen MJ, Goggin DE and Powles SB, Non-target-site-based resistance to ALS-inhibiting herbicides in six *Bromus rigidus* populations from Western Australian cropping fields. *Pest Manag Sci* **68**(7): 1077-1082 (2012).

48. Christopher JT, Preston C and Powles SB, Malathion antagonizes metabolism-based chlorsulfuron resistance in *Lolium rigidum. Pest Biochem Physiol* **49**(3): 172-182 (1994).

49. Tan MK, Preston C and Wang GX, Molecular basis of multiple resistance to ACCase-inhibiting and ALS-inhibiting herbicides in *Lolium rigidum. Weed Res* **47**(6): 534-541 (2007).

50. Beckie HJ and Tardif FJ, Herbicide cross resistance in weeds. *Crop Prot* **35**(0): 15-28 (2012).

51. Délye C, Weed resistance to acetyl coenzyme A carboxylase inhibitors: an update. *Weed Sci* **53**(5): 728-746 (2005).

52. Letouzé A and Gasquez J, Enhanced activity of several herbicide-degrading enzymes: a suggested mechanism responsible for multiple resistance in blackgrass (*Alopecurus myosuroides* Huds.). *Agronomie* **23**(7): 601-608 (2003).

53. Yu Q, Shane Friesen LJ, Zhang XQ and Powles SB, Tolerance to acetolactate synthase and acetyl-coenzyme A carboxylase inhibiting herbicides in *Vulpia bromoides* is conferred by two co-existing resistance mechanisms. *Pest Biochem Physiol* **78**(1): 21-30 (2004).

CHAPTER 5

INHERITANCE OF QUIZALOFOP RESISTANCE IN SMOOTH BARLEY

(*HORDEUM GLAUCUM*) BIOTYPE FROM SOUTH AUSTRALIA

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Contribution to the Paper	Did hybridization manually, planned and conducted the studies, data collection, data analysis, data interpretation and wrote manuscript.
Overall percentage (%)	85%
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By signing the Statement of Authorship, each author certifies that:

  i.   the candidate's stated contribution to the publication is accurate (as detailed above);
  ii.   permission is granted for the candidate in include the publication in the thesis; and
  iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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CHAPTER 6

FITNESS COSTS ASSOCIATED WITH 1781 AND 2041 ACCASE–MUTANT ALLELES CONFERRING RESISTANCE TO HERBICIDES IN *HORDEUM GLAUCUM* STEUD.

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By signing the Statement of Authorship, each author certifies that:

i.   the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

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Fitness costs associated with 1781 and 2041 ACCase–mutant alleles conferring resistance to herbicides in *Hordeum glaucum* Steud.

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Abstract

The amino acid substitutions that confer herbicide resistance may also involve fitness costs leaving mutant plants at a competitive disadvantage compared with wild–types. This research investigated the effect of two target–site point mutations of *ACCase* gene: Ile-1781-Leu/Val and Ile-2041-Asn on plant growth and fecundity of *Hordeum glaucum* biotypes grown in intraspecific competition and/or under interspecific competition in the field with wheat and lentil. The amino acid substitutions at 1781 position of *ACCase* gene did not impose any negative pleiotropic effects on relative growth rate (RGR), panicle emergence, plant height, total biomass and seed production in *H. glaucum* mutant plants. There was some evidence for fitness cost associated with Ile-2041-Asn mutation in terms of reduced RGR and reduced vegetative biomass and seed production when grown in competition with lentils. The absence of measurable negative pleiotropic effects on plant growth and fecundity associated with Ile-1781-Leu/Val ACCase mutations in *H. glaucum* suggest that the frequency of these alleles will not decline in the absence of herbicide selection pressure. However, the 2041-Asn allele should decrease in frequency in the absence of herbicide selection pressure.

Keywords: Smooth barley; *ACCase* gene; pleiotropic effect; seed production; evolution

1. Introduction

Acetyl coenzyme A carboxylase (ACCase)-inhibiting herbicides (hereafter called ACCase-inhibitors) are commonly used worldwide to selectively control grass weeds in a variety of crops. These herbicides inhibit plastidic ACCase enzyme, a key enzyme for fatty acid synthesis, in grass weeds (Preston & Mallory-Smith, 2001). Heavy reliance on these herbicides has led to a widespread evolution of resistance to ACCase-inhibitors in weeds. Until now, 47 grass weed species throughout the world have evolved resistance to ACCase-inhibitors (Heap, 2015). *Hordeum glaucum* (smooth barley) is a widespread problematic weed in the grain cropping regions of southern Australia (Cocks et al., 1976), which has also evolved resistance to ACCase-inhibitors (Shergill et al., 2015a, b). Target–site point mutations at amino acid position 1781 and 2096 in the CT domain of ACCase gene have been reported as the genetic basis of herbicide resistance in populations of *H. glaucum* (Shergill et al., 2015b).

It is widely accepted that evolution of herbicide resistance may involve fitness costs (Purba et al., 1996, Vila-Aiub et al., 2009, Powles & Yu, 2010). Fitness is the measure of survival and reproduction of a viable offspring in a given environment and plays a vital role in natural selection and adaptation (Orr, 2009). It is a relative term and if no differences in the fitness of two genotypes are found, their relative frequency will not be affected during reduced herbicide selection periods. However, if the resistant populations suffer a fitness penalty, the relative frequency of resistant genotypes will decrease in the absence of herbicide selection pressure. Therefore, in order to manage herbicide resistant populations and predict population dynamics, fitness costs need to be determined (Maxwell et al., 1990, Jasieniuk et al., 1996).

Control of genetic background is crucial for the quantification of fitness costs associated with the resistance genes (Vila-Aiub et al., 2005, Délye et al., 2013). To unequivocally quantify herbicide resistance fitness costs, relative fitness of individuals

with similar genetic background should be compared. Identification of genes conferring herbicide resistance is also important because different genes may be associated with the expression of different fitness costs (Roux et al., 2004). Resistance costs have proven difficult to identify in situations where the resistance mechanism is unknown or where the genetic background has not been controlled (Marshall et al., 1994, Gill et al., 1996, Purba et al., 1996).

Several studies of fitness in different weed species have used plant material of different origin, which exhibited genetic variability (Vila-Aiub et al., 2009). This is the first study in which fitness costs associated with ACCase-inhibitor resistance have been quantified in resistant and susceptible biotypes of *H. glaucum* selected from within each population. Previous studies conducted in *Alopecurus myosuroides* and *Lolium rigidum* have shown that different mutations in the *ACCase* gene have different pleiotropic effects (Délye et al., 2013, Vila-Aiub et al., 2015). Therefore, studies were designed to investigate the effect of different mutations in the populations of *H. glaucum*. Here we compare growth and fecundity of plants possessing Ile-1781-Leu/Val and Ile-2041-Asn alleles with susceptible plants co-existing in each population. Because fitness costs are influenced by environmental factors (Bergelson & Purrington, 1996), it has been acknowledged that expression of fitness costs under laboratory or glasshouse conditions may be quite different from that expressed in the field (Neve, 2007). Previous studies have also shown that ecological costs may become evident under different biotic interactions such as disease, predation and/or competition (reviewed in Vila-Aiub et al., 2009). Fitness costs should be more evident under resource competition because the mutated allele could impair the ability of the plant to efficiently capture or utilise the captured resources (Vila-Aiub et al., 2009). Therefore, fitness penalties associated with herbicide resistance were assessed in the field in two crop species of contrasting competitive ability.

2. Materials and Methods

2.1 Plant material

The ACCase-inhibitor resistant populations ($n = 3$) used in this study were collected in 2012 from Upper North and Eyre Peninsula regions of South Australia and were previously confirmed to be resistant to ACCase-inhibitors (Shergill et al., 2015a). Seeds were collected by two people moving in different directions; each followed an inverted W pattern through at least 1 ha of the field, beginning at least 20 m from the edge of the crop. Sampling was discontinued once seeds or panicles from a minimum of 100 plants were collected.

2.2 Selection and characterization of biotypes

Control of genetic background to estimate and interpret fitness costs associated with herbicide resistance has been emphasized in the literature (Vila-Aiub et al., 2009, Délye et al., 2013). In order to minimize the effect of genetic background on fitness, comparisons were made between the ACCase-inhibitor resistant and susceptible plants selected within each population of *H. glaucum*. As *H. glaucum* is a self-pollinated species, selected biotypes were confirmed to be homozygous (RR) for the specific ACCase-inhibitor resistance mutations or homozygous (rr) susceptible.

In 2013, seeds collected from the field were germinated in plastic trays (33 cm x 28 cm x 5 cm) containing standard cocoa peat potting mix. For each population, germinated seedlings (four per pot) at Z11 stage (Zadoks et al., 1974) were transplanted into 24 punnet pots (9.5 cm x 8.5 cm x 9.5 cm) (Masrac Plastics, South Australia) containing standard potting mix and were maintained outdoors during the normal growing season (April-October). At the Z23-24 stage, plants were uprooted and tillers were separated from the parent to form a clone. The tillers were carefully excised in order to retain some roots on each clone. Later, the parent and their respective clones were re-potted separately in a grid pattern to ensure subsequent identification. When the clones grew new green leaf

tissue, commercial formulation of quizalofop-p-ethyl (Targa®, 99.5 g L^{-1}) at 24.9 g a.i. ha^{-1} plus 1% v/v Hasten™ adjuvant was applied using a moving-boom laboratory twin nozzle cabinet sprayer (Tee-jet 110° flat fan spraying systems, Wheaton, IL) delivering herbicide in 121 L ha^{-1} water at a pressure of 250 kPa and a speed of 1 m s^{-1} to one set of clones and plants were returned and maintained outdoors. Previous research had shown that this rate of the herbicide is only lethal to susceptible plants. At 28 days after treatment (DAT), visual survival assessments were made and plants with new green leaf tissue were recorded as resistant (R), whereas those that displayed severe chlorosis or no new growth were recorded as susceptible (S) (Table 1). After the classification of clones as R or S, the unsprayed clones were repotted into 5 L pots and maintained outdoors till maturity. Plant material for DNA extraction and sequencing, as explained below, was collected from these plants (five individuals each) after R and S classification and sequences were compared to check that populations shared a similar genetic background. No separation with pollen proof bags was required since *H. glaucum* is a self-pollinated species (Cocks et al., 1976). Seeds from R and S plants were separately bulked to form a representative sample for each biotype of *H. glaucum* population. The seed obtained was stored in the laboratory under dry conditions at room temperature at The University of Adelaide, Waite Campus (34°58'S, 138°38'E), until February 2014 when they were threshed and cleaned manually.

Herbicide resistance status of the selected homozygous *H. glaucum* biotypes (R and S) of all the populations was confirmed by herbicide screening in 2014. Eight to nine seedlings per pot with three replicates for all biotypes were transplanted (Z11) and sprayed (Z12-13) at 24.9 g a.i. ha^{-1} with quizalofop-p-ethyl plus 1% v/v Hasten™ adjuvant. The plants were transplanted, sprayed and assessed (28 DAT) following the procedure explained above.

2.3 Sequencing of ACCase gene

To confirm the mechanism of herbicide resistance, plant material (100mg) from the youngest green leaf tissue of five resistant and susceptible plants of each biotype kept for seed production was collected. DNA was extracted and the presence of mutations in the CT domain of the *ACCase* gene was investigated by sequencing as described elsewhere (Shergill et al., 2015b). *H. glaucum* is a diploid and self-pollinated species, the target-site point mutations found were confirmed to be homozygous. The detailed information on *H. glaucum* populations used in this study is provided in Table 1.

Table 1

Information on the source of *Hordeum glaucum* populations used and survival assessment of clones and selected biotypes after quizalofop-p-ethyl application.

Population	Location	Previous crop	Biotype	Amino acid substitution	Frequency of clones[a] (%)	Survival of biotypes (%)
UN7	32°48'S	Wheat	R	Ile-1781-Leu	25 (6)	100
	137°56'E		S	-	75 (6)	0
UN14	32°51'S	Wheat	R	Ile-1781-Val	19 (3)	100
	137°58'E		S	-	81 (3)	0
EP37	33°35'S	Pasture	R	Ile-2041-Asn	93 (3)	100
	136°13'E		S	-	7 (3)	0

[a] values in parenthesis are ±SE

2.4 Pot experiment

2.4.1 Experimental design

The pot experiment was designed to assess growth and fecundity of R and S plants under intraspecific competition. Therefore in 2014, seeds (n = 10 pot^{-1} for UN7 and UN14; n = 15 pot^{-1} for EP7) of each homozygous *H. glaucum* biotype (R and S) were separately planted in 6 replicate pots (5 L) containing standard cocoa peat potting mix. At 21 days after planting (DAP), emerged seedlings were randomly thinned to 5 plants pot^{-1}. All pots

were arranged in a completely randomized design under natural conditions during April to October 2014. The pots were placed apart to avoid any pot to pot competition and were rearranged fortnightly to reduce any environmental bias due to pot position. Plants were watered as required and a recommended dose of fertilizer (1 scoop per five litre of water) (Peters Professional All-rounder; 20%N, 20% P_2O_5, 20% K_2O, 0.7% MgO, 1.5% SO_3, 0.02% B, 0.015% Cu, 0.12% Fe, 0.06% Mn, 0.010% Mo, 0.015% Zn) was applied twice during the experiment.

2.4.2 Plant growth assessments.

To estimate the relative growth rate (RGR), all plants from three replicates for each *H. glaucum* biotype (R and S) were harvested twice during the experiment. Mean plant weight per pot was used to estimate RGR. Above ground plant parts were initially harvested at Z14-15 with a subsequent final harvest at maturity. RGR was estimated using the unbiased formula proposed by Hoffmann and Poorter (2002) (equation 1). Variance of RGR was calculated using ln-transformed plant weights at different times according to Causton and Venus (1981). The formula used to estimate RGR is:

$$\text{RGR} = \frac{\left(\overline{\ln W_2} - \overline{\ln W_1}\right)}{(t_2 - t_1)} \qquad [2]$$

Where, $\overline{\ln W_2}$ is the mean of the natural logarithm transformed aboveground plant weights at harvest time t_2, $\overline{\ln W_1}$ is the mean of the natural logarithm transformed aboveground plant weights at harvest time t_1. The RGR estimates of each biotype (R and S) from a population were compared using Student's *t*-test (GraphPad Prism v6, GraphPad software, San Diego, CA).

2.4.3 Fecundity measurements.

To estimate the time of panicle emergence, panicles were counted from plants (n = 15) of each *H. glaucum* biotype (R and S) at weekly intervals till maturity, beginning with the emergence of the first panicle. Panicles were considered emerged when a panicle had fully

emerged from the leaf sheath. The panicle emergence data were fitted to a functional

three-parameter sigmoid model using SigmaPlot version 12.5. The model fitted was

$$E(\%) = \frac{E_{max}}{1 + \exp\left(-\left(\frac{x - t_{50}}{E_{rate}}\right)\right)} \qquad [3]$$

where E is the panicle emergence (%) at time x, E_{max} is the maximum panicle

emergence (%), t_{50} is the time (d) to reach 50% of maximum panicle emergence, and E_{rate}

indicates the slope around t_{50}.

At maturity, panicles were harvested and later threshed manually to extract seed.

Seeds were dried in an oven at 35 °C for 48 h, and weighed. To estimate seed production,

1 g seed for each biotype (R and S) was drawn and total seeds counted. The number of

seeds per gram for each biotype and the total weight of seeds harvested per plant were

used to calculate seed production per plant. Student's t-test using GraphPad Prism was

performed to compare panicle emergence and seed production estimates for all *H.*

glaucum biotypes (R and S).

2.5 Field experiment

2.5.1 Experimental site location and design.

The field experiment was designed to assess growth and fecundity of homozygous

ACCase-herbicide-resistant and susceptible biotypes of each population under

interspecific competition with wheat (high competition) and with lentils (low competition)

in a replacement series design. In 2014, *H. glaucum* biotypes (R and S) for each

population were grown in competition with wheat (cv. Shield) and lentils (cv. Nipper) at

three relative proportions of 100% R, 50% R: 50% S and 100% S in the field at

Roseworthy (34°31'S, 138°41'E), located in the Lower North region of South Australia.

The field experiment was arranged in a randomized complete block design with three

replicates for UN14 population and four replicates for UN7 and EP37 populations. The

soil type at the field site was sandy loam over medium calcareous clay with organic matter content 2 to 2.5% and a pH (water) of top-soil (0-20 cm) of 7 to 7.5. The crops (wheat and lentil) were sown on May 27, 2014 at a similar time (± 2 days) to *H. glaucum* biotypes sown in seedling trays (nursery).

Prior to sowing of the experiment, the field site was sprayed with glyphosate (Raze®, 510 g L^{-1}) at 1.5 kg a.i. ha^{-1} plus oxyfluorfen (Goal®, 240 g L^{-1}) at 19.2 g a.i. ha^{-1} for pre-plant weed control. Wheat at 90 kg ha^{-1} and lentils at 40 kg ha^{-1} were seeded in rows spaced 25 cm apart at a depth of 5 cm with a small-plot seeder fitted with knife-point opener and press-wheel closer. These crop seed rates represent commercial practice in the district. Diammonium phosphate (18 kg N and 20 kg P ha^{-1}) at 115 kg ha^{-1} was banded below the seed at sowing. Seeds for each *H. glaucum* biotype (R and S) were germinated in nursery trays containing standard cocoa peat potting mix and later transplanted in the field at the Z11 stage. Each plot (1 m long and 1 m wide) consisted of three rows of *H. glaucum* manually transplanted at 5 cm (plant-to-plant) between the rows of wheat and lentils. To delineate plant position in plots transplanted in 50R:50S proportion, plants in each row were transplanted in 1:1 ratio starting with R biotype and the first plant in each row was marked with a plastic tag. Each of the three proportions consisted of 60 plants plot^{-1} surrounded by 1 m crop buffer.

2.5.2 *Plant growth assessments.*

To estimate the seedling establishment of *H. glaucum* in the field, plants of each biotype of all the populations were individually counted 4 weeks after transplanting (WAT) from each plot. At the end of the experiment, when around quarter of the panicles had matured, two plant growth descriptors of ecological significance were estimated from the plants of each *H. glaucum* biotype (R and S) grown in each crop. The measurements included plant height, which reflects the capacity for inflorescences to appear above the crop canopy and total dry biomass, which reflects the competitive ability of the plant. Plant height was

determined by measuring the length of the longest flowering tiller of ten random plants in each plot. Total dry biomass was determined by harvesting and drying all plants of each *H. glaucum* biotype in a plot. The number of plants were counted and harvested individually by excision at the ground level and later weighed after drying them in an oven at 80°C for 72 h. Plants were separated into vegetative and floral components before drying and were weighed separately. Vegetative and panicle dry biomass were summed to calculate total dry biomass at harvest.

To quantify crop competitiveness, measurements such as crop density, plant height and total dry biomass at harvest were also recorded. At 4 weeks after sowing, crop density was determined by counting the number of plants along 0.6 m length of two adjacent crop rows at ten random locations in the experiment. At maturity, crop height was determined randomly from ten plants in each replicate and total crop dry biomass was determined by harvesting plants along 0.6 m length of two adjacent crop rows at a random location in each replicate. All the comparisons between homozygous ACCase-inhibiting herbicide-resistant biotypes (R) and susceptible (S) biotypes for each population were made by performing a Student's *t*-test using GraphPad Prism v6.

2.5.3   Fecundity measurements.

Fecundity measurements, such as time of panicle emergence and seed production, were recorded for each *H. glaucum* biotype (R and S) under different competition regimes. Time of panicle emergence was recorded as the date at which the first fully emerged panicle was seen. At maturity, panicles were harvested and weighed as described above. To estimate seed production, 1 g seed sample per plot for each biotype (R and S) was drawn and total seeds were counted. The number of seeds per gram for each biotype and the total panicle biomass per plant from each plot, and the number of plants per plot were used to calculate the seed production per plant. The seed production estimates for each *H.*

glaucum biotype (R and S) from a population were compared by performing a Student's t-test using GraphPad Prism v6.

3. Results

3.1 Characterization of selected biotypes

Herbicide resistance status of the selected *H. glaucum* biotypes (R and S) for each population produced in 2013 was confirmed by screening with quizalofop-p-ethyl in 2014. As expected, at 28 DAT plants of all the homozygous ACCase-inhibiting herbicide-resistant biotypes (R) survived (100% survival) quizalofop application at the recommended field rate (24.9 a.i. g ha^{-1}), whereas plants of all the susceptible biotypes (S) were killed (0% survival) at this rate (Table 1).

3.2 Sequencing of ACCase gene

DNA fragments (1600 bp) of the CT domain of the plastidic *ACCase* gene from 5 individuals of each R and S biotype were amplified by PCR and sequenced from both ends. This revealed a single nucleotide change among R and S biotype of each population, which resulted in a single amino acid modification conferring resistance to ACCase-inhibitors. In UN7-R biotype a codon change from ATA to TTA resulted in an amino acid substitution of Ile-1781-Leu, whereas, a codon change from ATA to GTA resulted in an amino acid substitution of Ile-1781-Val in UN14-R biotype (Table 1). Similarly, a codon change from ATA to AAT resulted in an amino acid substitution of Ile-2041-Asn in EP37-R biotype. No such substitution was observed in plants of S biotype of all the populations. Moreover, the comparison of 1600 bp of the CT domain of the plastidic *ACCase* gene of R and S biotypes revealed that biotypes of each population differed only for the mutation and there were no other nucleotide differences between the biotypes. However, there were several nucleotide differences among different populations. This confirms that the R and S biotypes share a similar genetic background and each population is different. Although, all individuals tested within each R biotype shared the same mutation, it does not preclude the

possibility that other individuals within the sample could have additional resistance mechanisms (either point mutation or non-target site).

3.3 Pot experiment

Growth analysis for homozygous R and S *H. glaucum* biotypes showed that plants homozygous for the mutation at 1781 position in UN7-R and UN14-R biotypes exhibited similar RGR ($P > 0.05$) compared to plants of their respective herbicide susceptible biotype (Fig. 1). On the other hand, plants homozygous for the mutation at 2041 position in the EP37-R biotype exhibited significantly reduced RGR (10%; $P < 0.05$) compared to plants of the herbicide susceptible biotype (Fig. 1).

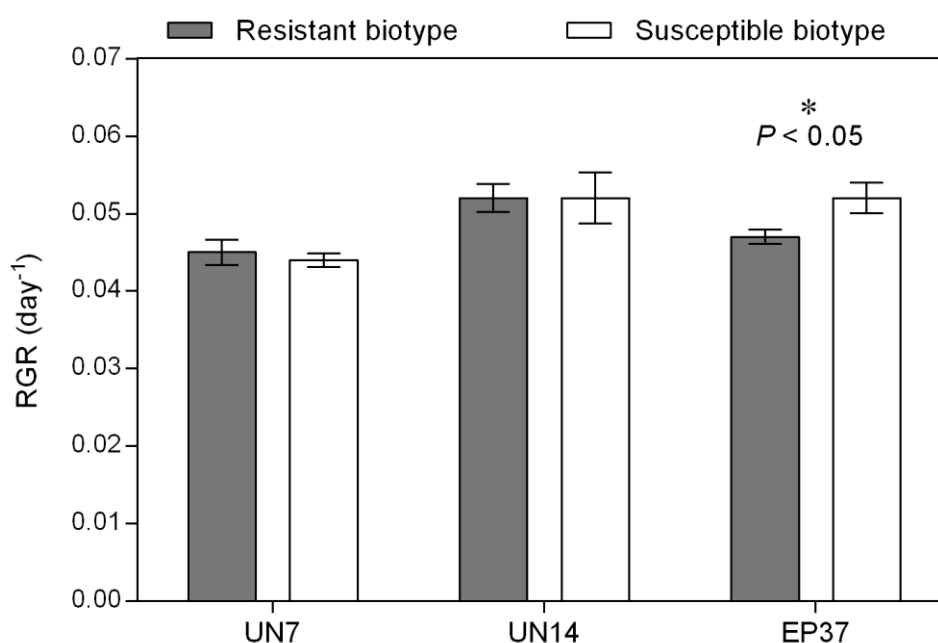**Fig. 1** Mean ± SE estimates of relative growth rate (RGR) of R and S biotypes of three *Hordeum glaucum* populations grown in pots. Asterisk indicates that values are significantly different within populations according to Student's *t*-test ($\alpha = 5\%$).

The functional three-parameter sigmoid model (Equation 2) provided a significant fit ($P < 0.0001$, $R^2 = 0.97$–0.99) to the panicle emergence data for all the homozygous R

and S *H. glaucum* biotypes. No significant differences ($P > 0.05$) were found in the time taken to reach 50% maximum panicle emergence (t_{50}) between plants of UN7-R and UN14-R biotypes, homozygous for the mutation at 1781 position, and plants of UN7-S and UN14-S biotypes, respectively (Fig. 2). Although, panicles in UN7-R biotype started emerging slightly later than the UN7-S biotype, both took a similar time ($P > 0.05$) to reach 50% maximum panicle emergence (Fig. 2a, Table 2). However, a significant difference ($P < 0.05$) in t_{50} estimates occurred for plants of the EP37-R biotype (125 days), homozygous for the mutation at 2041 position, and the EP37-S biotype (144 days) (Fig. 2c). Panicle emergence in EP37-S biotype started about 21 days later and took additional 19 days to reach 50% maximum panicle emergence compared with the EP37-R biotype (Fig. 2c, Table 2).
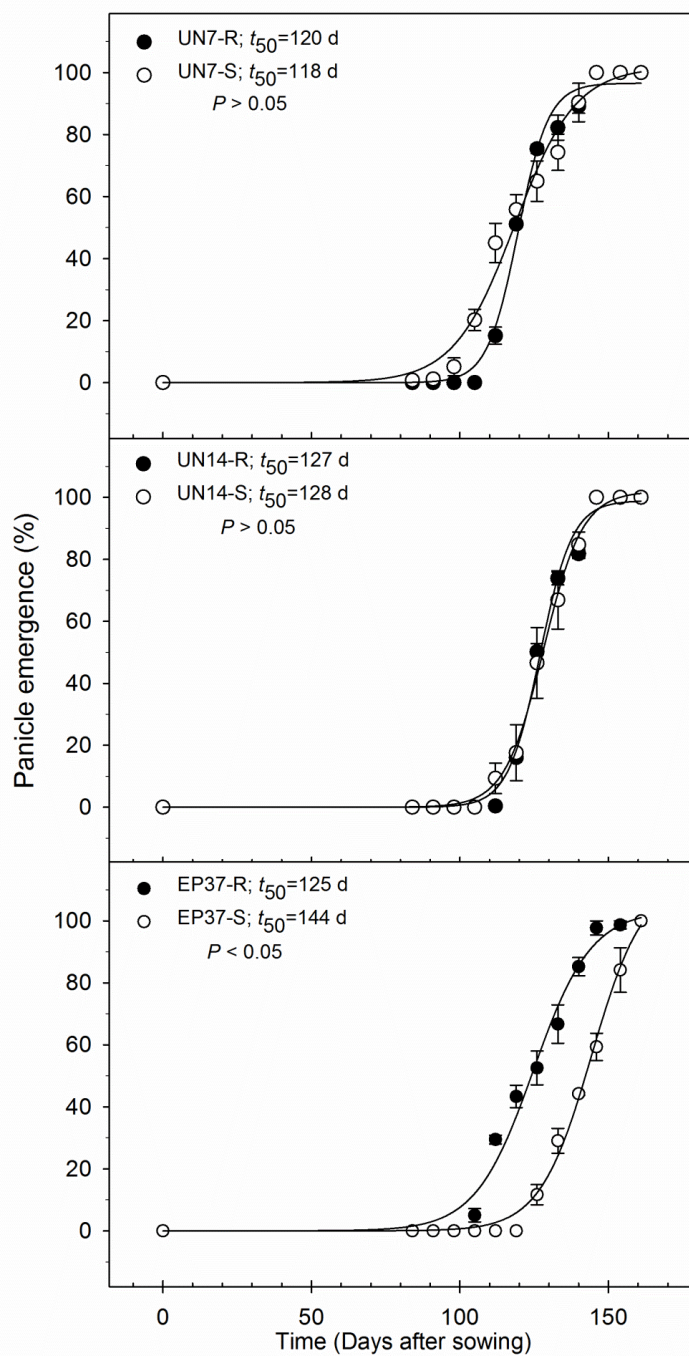
Fig. 2 Rate of panicle emergence of homozygous R (●) and S (○) biotypes sourced from three *H. glaucum* populations grown in pots: UN7 (top), UN14 (middle) and EP37 (bottom). Values are the mean ± SE for each recording date. Lines represent a functional three–parameter sigmoid model (Equation 1) fitted to the panicle emergence percent data for each biotype (R and S).
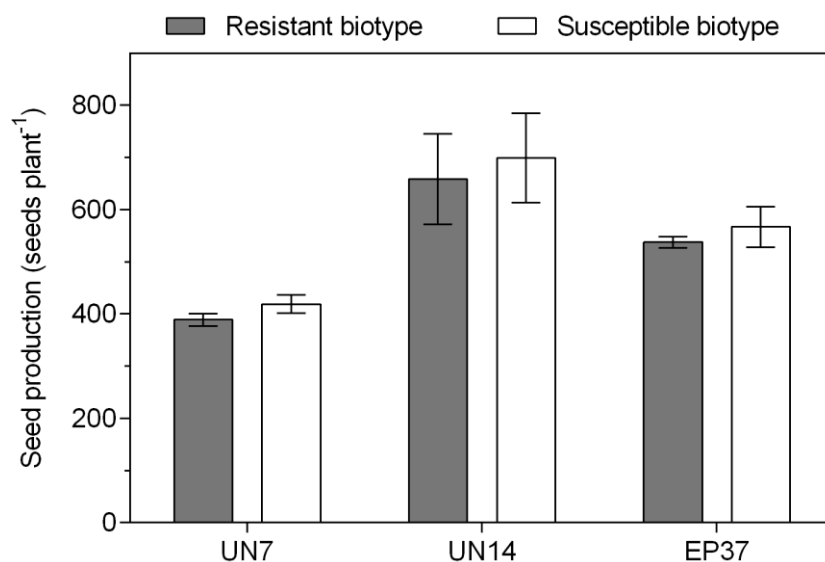
Fig. 3 Mean ± SE estimates of seed production of R (shaded) and S (clear) biotypes of three *Hordeum glaucum* populations grown in pots. The values are statistically similar within populations according to Student's *t*-test ($\alpha = 5\%$).

Table 2

Date of panicle emergence of different biotypes of *Hordeum glaucum* populations grown in pots and field.

Population	Biotype	Date of panicle emergence	
		Pots	Field
UN7	R	8 September 2014	9 September 2014
	S	11 August 2014	8 August 2014
UN14	R	8 September 2014	5 September 2014
	S	8 September 2014	5 September 2014
EP37	R	1 September 2014	5 September 2014
	S	22 September 2014	25 September 2014

Seed production estimates from plants under intraspecific competition (pot study) revealed that the amino acid substitutions at 1781 or 2041 positions had no effect on the capacity of *H. glaucum* plants to produce seed. At maturity, the plants of the homozygous R biotype of all the populations produced similar ($P > 0.05$) amount of seeds to the S biotype (Fig. 3).

3.4 Field experiment

To assess the ecological fitness costs under resource competitive conditions, homozygous R and S biotypes of each population were grown in competition with wheat and lentils. Wheat grown at a density of 261 plants m^{-2} provided a high level of competition against *H. glaucum*, producing a final dry matter of 7 t ha^{-1}. On the other hand, lentils grown at a density of 195 plants m^{-2} provided much lower competition against *H. glaucum* as indicated by the final lentil dry matter of 3 t ha^{-1}. The majority of the transplanted seedlings of *H. glaucum* biotypes (R and S) of all the populations established and developed successfully in the field until maturity. *H. glaucum* seedling establishment at 4 WAT showed that on average 75–82% seedlings of UN7-R biotype and 80–85% of UN7-S biotype established successfully in wheat and lentils (Fig. 4a). For UN14 population, average seedling establishment was 78–84% for the R biotype and 78–81% for the S biotype (Fig. 4b). Similarly, high seedling establishment in wheat and lentils occurred for the EP37-R (73–77%) and EP37-S (72–80%) biotypes (Fig. 4c).
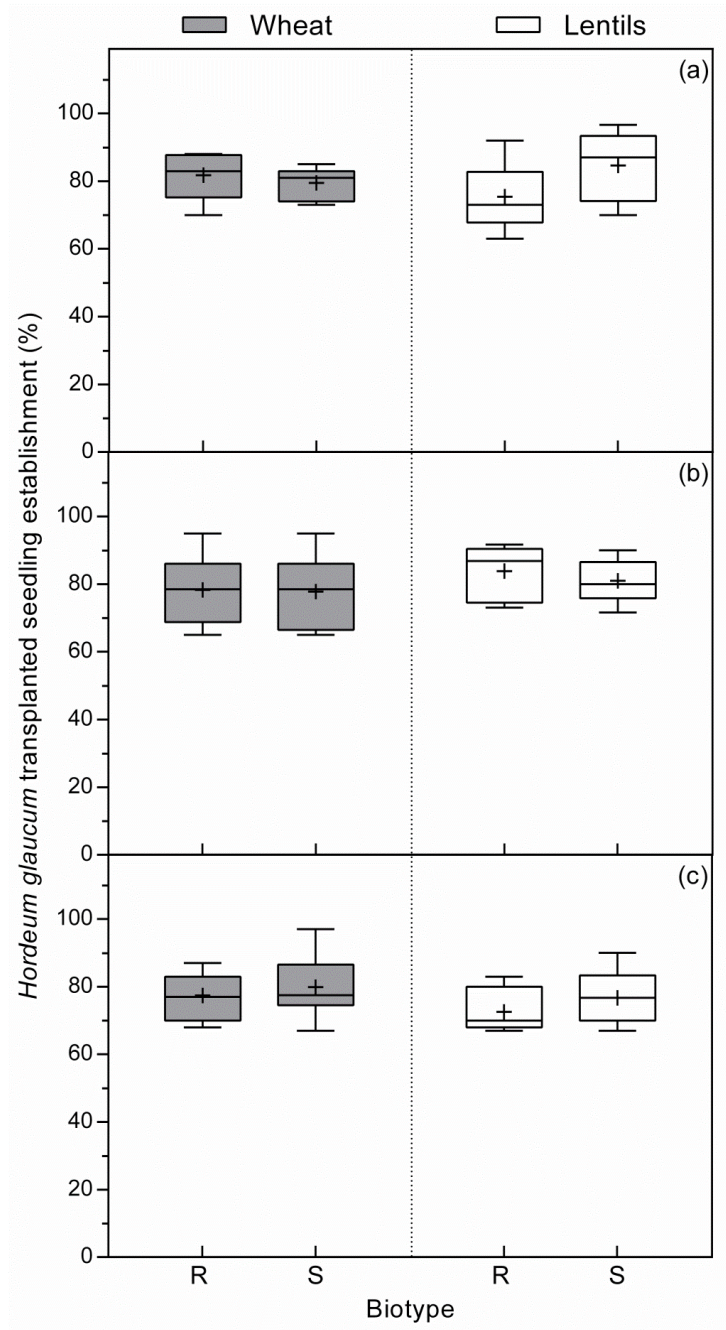
Fig. 4 Box and whiskers plot of transplanted seedling establishment in the field of resistant (R) and susceptible (S) biotypes of *Hordeum glaucum* populations (a) UN7, (b) UN14 and (c) EP37 in wheat and lentils. Lower and upper boxes represent the second and third quartiles, respectively. Line in the box represents median value. Plus '+' sign in the box represents the mean value. Lower and upper whiskers extend to the 10th and 90th percentiles of the data, respectively.

When plants of the R and S biotypes of each *H. glaucum* population were grown in monoculture (100R and 100S) or in a mixture (50R:50S) in competition with wheat or lentils, the plant height of R biotypes was similar ($P > 0.05$) to that of the S biotypes (Fig. 5). It was observed that *H. glaucum* plants of all the biotypes grown in competition with wheat were significantly taller ($P < 0.05$) than the *H. glaucum* plants grown in competition with lentils (Fig. 5). However, plants of all the *H. glaucum* biotypes were significantly taller ($P < 0.05$) than lentils and similar in height to wheat ($P > 0.05$). Time of panicle emergence of each *H. glaucum* biotype (R and S) grown in the field was similar to plants of the respective biotype in pots (Table 2). Panicles of UN7-R biotype started emerging approximately a month later than that of the UN7-S biotype but there was no difference in t_{50} between the R and S biotypes. In contrast, panicles of EP37-R biotype started emerging approximately 20 days earlier than that of S biotype in the field. There was no difference in the time of panicle emergence of UN14-R and UN14-S biotypes.

No significant differences ($P > 0.05$) were identified in total dry biomass (Fig. 6), and seed production (Fig. 7) between the R and S biotypes of any of the populations when grown in monoculture or in a mixture in competition with wheat. Similarly, when plants of R and S biotypes of UN7 and UN14 populations were grown in competition with lentils, either in monoculture or in a mixture, there were no significant differences ($P > 0.05$) in total dry biomass production (Fig. 6) and seed production (Fig. 7) of each biotype. In contrast, plants of EP37-R biotype grown either in monoculture or in a mixture in competition with lentils produced significantly lower ($P < 0.05$) dry biomass (Fig. 6) and fewer seeds (Fig. 7) than plants of the EP37-S biotype.
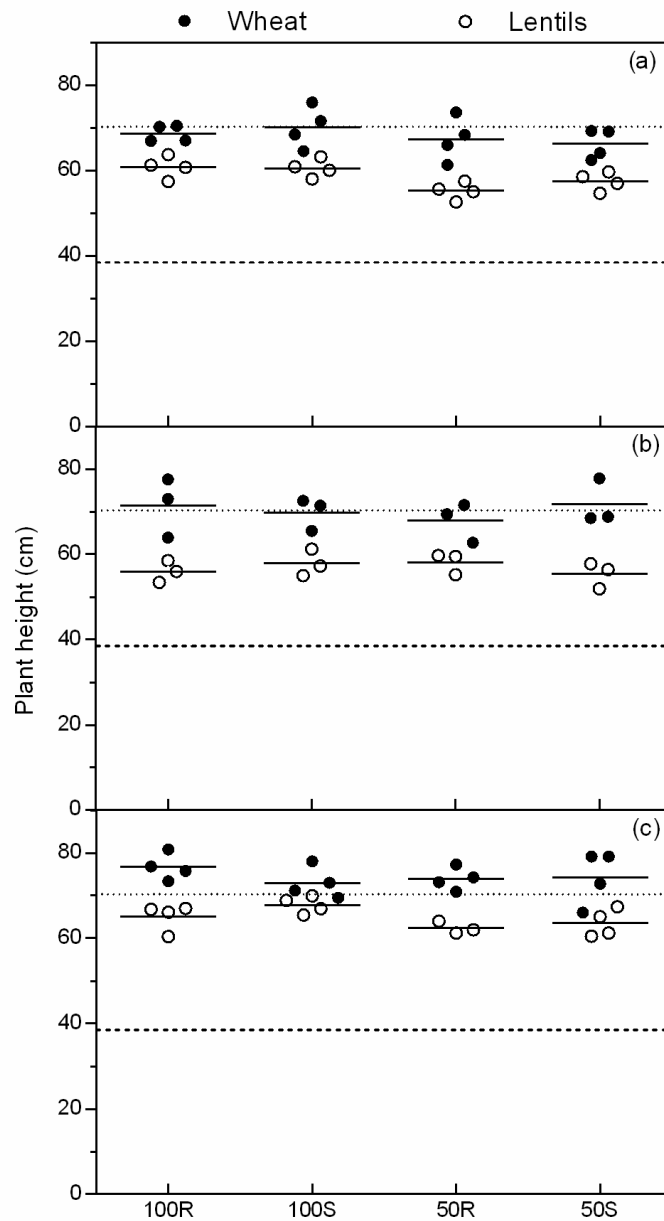
Fig. 5 Scatter–dot plot representing plant height of resistant (R) and susceptible (S) biotypes of three *Hordeum glaucum* populations (a) UN7, (b) UN14 and (c) EP37 grown in competition with wheat (closed circles) and lentils (open circles) at different ratios in field. Solid line near the dot plot represents the mean. Horizontal dotted line represents the plant height of wheat and horizontal dashed line represents the plant height of lentils.

Fig. 6 Mean ± SE estimates of total dry biomass of resistant (R) and susceptible (S) biotypes of three *Hordeum glaucum* populations (a) UN7, (b) UN14 and (c) EP37 grown in competition with wheat (shaded) and lentils (clear) at different ratios in the field. Asterisk indicates that values are significantly different within populations according to Student's *t*-test (α = 5%).

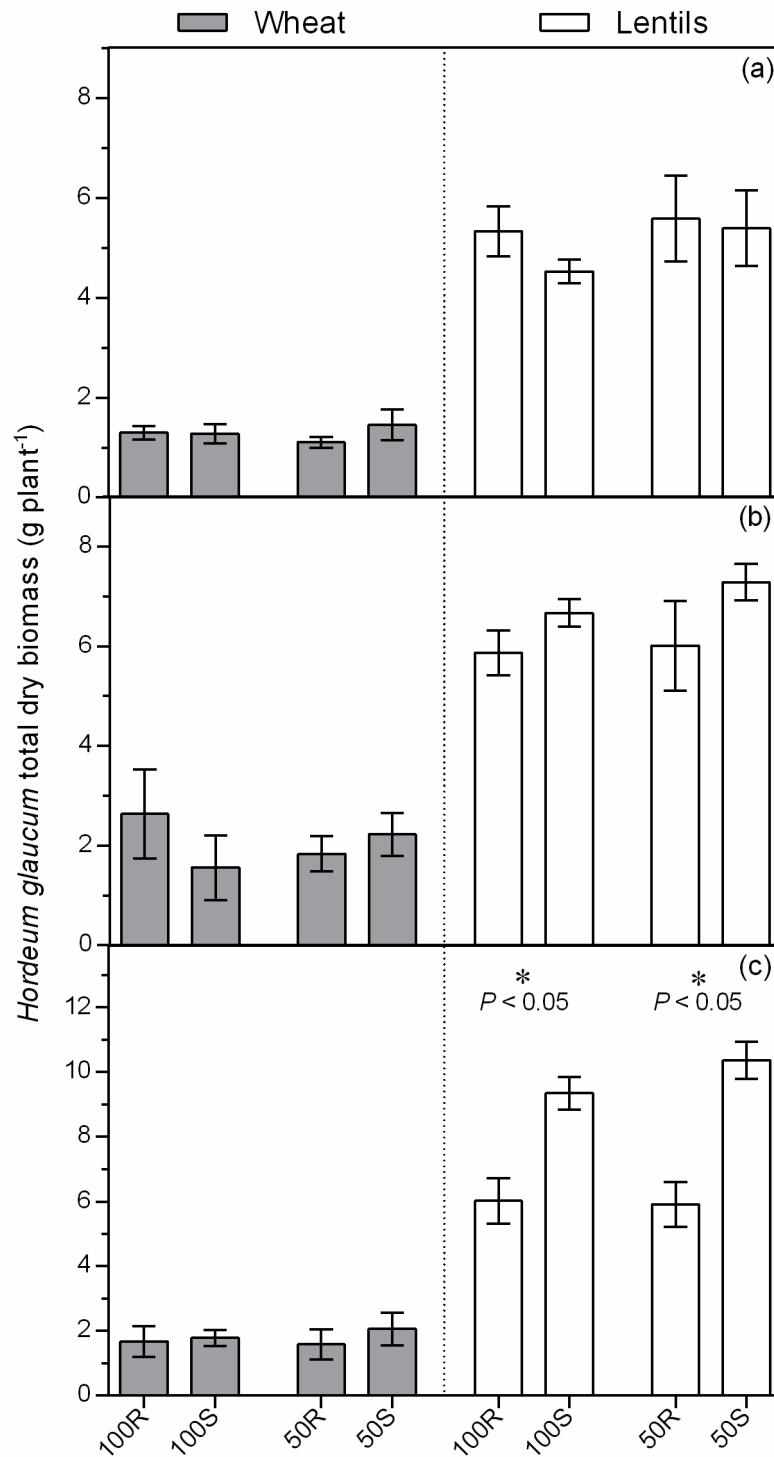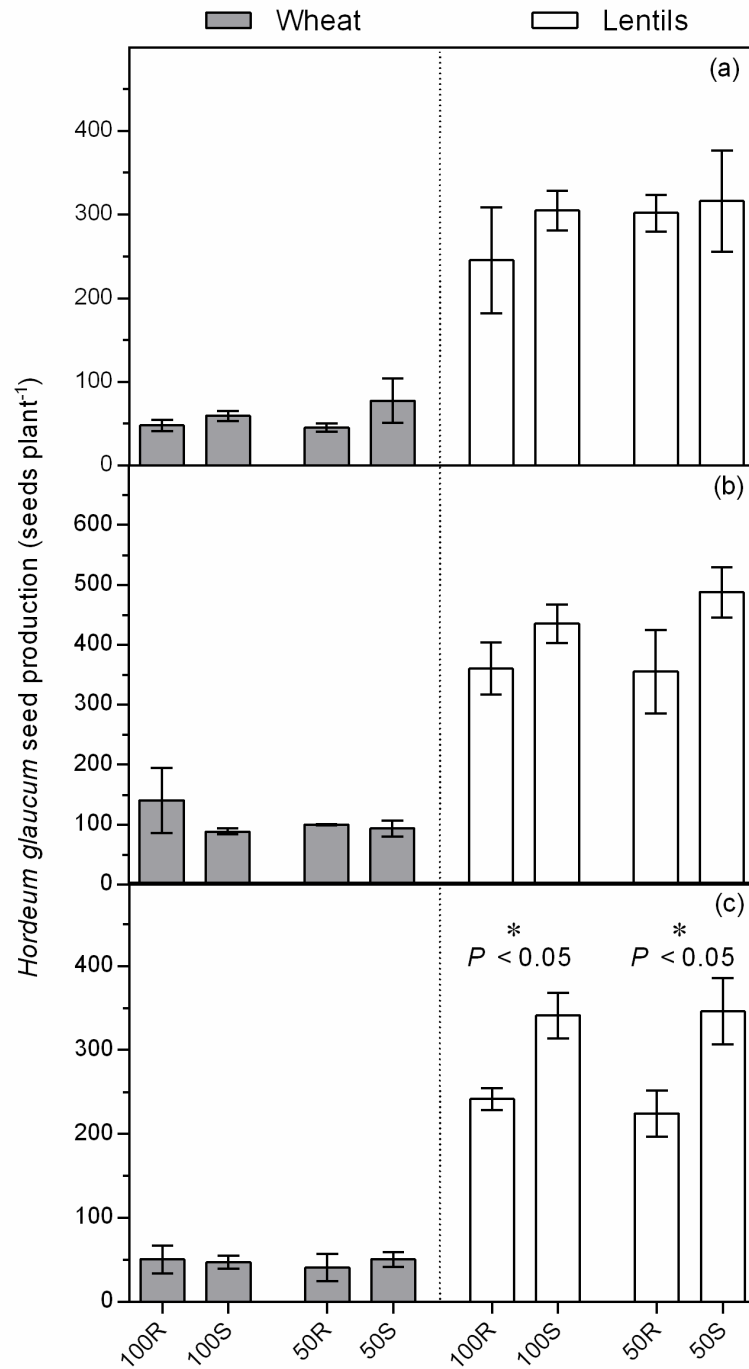**Fig. 7** Mean ± SE estimates of seed production of resistant (R) and susceptible (S) biotypes of three *Hordeum glaucum* populations (a) UN7, (b) UN14 and (c) EP37 grown in competition with wheat (shaded) and lentils (clear) at different ratios in the field. Asterisk indicates that values are significantly different within populations according to Student's *t*-test (α = 5%).

4. Discussion

We did not detect any fitness costs for the homozygous resistant Ile-1781-Leu/Val biotypes relative to the susceptible biotypes selected from the UN7 and UN14 populations. The results are in agreement with several previous studies on other grass weed species, which showed no fitness costs associated with the Ile-1781-Leu mutation in the absence of herbicide selection (Vila-Aiub et al., 2005, Menchari et al., 2008). Vila-Aiub et al. (2015) showed that *L. rigidum* plants segregating for Ile-1781-Leu mutation exhibited similar RGR and biomass accumulation compared to the susceptible plants. Similarly, Menchari et al. (2008) showed that vegetative biomass, plant height and seed production were similar among the *A. myosuroides* R biotypes segregating for Ile-1781-Leu mutation and S biotypes grown in the field. The absence of resistance cost associated with Ile-1781-Leu mutation in *H. glaucum* is likely due to lack of negative pleiotropic effect of this mutation on ACCase enzyme kinetics. Although ACCase enzyme activity was not studied, previous studies in other grass weed species suggest that mutant ACCase activity did not differ from that of wild-type ACCase (Délye et al., 2002, Vila-Aiub et al., 2015). The 1781-Leu allele has been shown to be fixed in the wild types of *Poa annua*, *Festuca rubra* and *F. bromoides* (Powles & Yu, 2010), which indicates little or no fitness penalty associated with this mutation. Furthermore, Ile-1781-Leu mutation is the most frequently reported mutation identified across resistant grass weed species (Délye, 2005, Beckie et al., 2012), which may reflect an evolutionary advantage related to this mutation relative to other mutations in ACCase. Wang et al. (2010) found that in the absence of herbicide selection pressure, *Setaria* mutants containing the ACCase 1781 allele were more fit than susceptible plants when grown in greenhouse or in the field.

Plants of the EP37-R biotype, with Ile-2041-Asn mutation, produced lower total dry biomass and seed production than the EP37-S biotype in lentils, which may be partly due to shorter vegetative phase of the R biotype. Weiner (1986) showed that large individual

plants obtain greater resources (water, light and nutrients) when compared to small individual plants, resulting in more seeds and biomass. Significant ($P < 0.05$) differences in biomass and seed production between plants with mutant Asn-2041 allele and the S biotype were only detected in the low competition environment of lentils. Even though a similar trend was observed in wheat, the differences in growth and fecundity between the R and S were non-significant ($P > 0.05$). As wheat was much more competitive against *H. glaucum*, it suppressed weed biomass by >72% and seed production by >79% compared to lentils. This is the likely reason that it was not possible to detect any fitness differences in the highly suppressive environment of wheat. Our study suggests that fitness costs could reduce the frequency of the R allele during an absence of herbicide selection, but only where the population experiences a low/moderate level of interspecific competition. Failure to detect any differences in seed production for plants homozygous for Ile-2041-Asn mutation when grown in pots is likely due to low intraspecific competition between R and S biotypes because of abundant supply of light, moisture and nutrients.

Reduced RGR detected in EP37-R biotype with 2041-Asn mutant allele is likely to reduce their fitness relative to the wild types of the same population, which could be partly related to reduced ACCase activity as reported in other species (Délye et al., 2003). However, the influence of early flowering on fitness could vary in different growing seasons in rainfed environments. In high rainfall seasons, plants with longer vegetative phase and later flowering are likely to produce greater biomass and seeds than plants that flower and mature early. In contrast, in a low rainfall situation, early flowering plants could escape terminal drought conditions and produce more seed than plants that flower later. The presence of earliness in the R biotype of EP37 may be related to the presence of this trait in the original individual in which the ACCase mutation occurred. As *H. glaucum* is a self-pollinated species, early flowering and the Ile-2041-Asn mutation would be expected to perpetuate together over multiple generations. Since the alteration in

flowering phenology could be a chance association with the 2041 mutation, the responses observed in our study may not be seen in other independent situations where this mutation has arisen. In contrast to our results, no differences in vegetative biomass, plant height and seed production were observed among the *A. myosuroides* R biotypes segregating for Ile-2041-Asn mutation and that of S biotypes, when grown in field (Menchari et al., 2008).

5. Conclusions

The results of the current study revealed that the target-site point mutation at 1781 position had no negative effects on the fitness of R biotypes of *H. glaucum*. In contrast, there was some evidence for a fitness cost associated with the Ile-2041-Asn in the EP37 population, which was expressed in the pot study as reduced RGR and in a lentil crop in the field as reduced vegetative biomass and seed production. However, some of this fitness penalty is likely to be caused by the shorter vegetative phase of the R biotype. Difference in the fitness of R and S biotypes has important implications for the management of herbicide resistance. Where a fitness cost exists for R biotypes, it means that over time R plants will be replaced to varying degrees by S individuals after herbicide selection pressure is removed (Maxwell et al., 1990). However, such selection against the R biotype is unlikely in the case of Ile-1781-Leu. In contrast, Ile-2041-Asn mutations could decrease in frequency in the absence of herbicide selection pressure, especially in high rainfall environments and/or in low/moderate level of interspecific competition.

References

BECKIE HJ, WARWICK SI & SAUDER CA (2012) Basis for herbicide resistance in Canadian populations of wild oat (*Avena fatua*). *Weed Science* 60, 10-18.

BERGELSON J & PURRINGTON CB (1996) Surveying patterns in the cost of resistance in plants. *The American Naturalist* 148, 536-558.

CAUSTON DR & VENUS JC (1981) *The biometry of plant growth*. Edward Arnold.

COCKS PS, BOYCE KG & KLOOT PM (1976) The *Hordeum murinum* complex in Australia. *Australian Journal of Botany* 24, 651-662.

DÉLYE C (2005) Weed resistance to acetyl coenzyme A carboxylase inhibitors: an update. *Weed Science* 53, 728-746.

DÉLYE C, MENCHARI Y, MICHEL S, CADET É & LE CORRE V (2013) A new insight into arable weed adaptive evolution: mutations endowing herbicide resistance also affect germination dynamics and seedling emergence. *Annals of Botany* 111, 681-691.

DÉLYE C, WANG T & DARMENCY H (2002) An isoleucine-leucine substitution in chloroplastic acetyl-CoA carboxylase from green foxtail (*Setaria viridis* L. Beauv.) is responsible for resistance to the cyclohexanedione herbicide sethoxydim. *Planta* 214, 421-427.

DÉLYE C, ZHANG XQ, CHALOPIN C, MICHEL S & POWLES SB (2003) An isoleucine residue within the carboxyl-transferase domain of multidomain acetyl-coenzyme A carboxylase is a major determinant of sensitivity to aryloxyphenoxypropionate but not to cyclohexanedione inhibitors. *Plant Physiology* 132, 1716-1723.

GILL GS, COUSENS RD & ALLAN MR (1996) Germination, growth, and development of herbicide-resistant and susceptible populations of rigid ryegrass (*Lolium rigidum*). *Weed Science* 44, 252-256.

HEAP I (2015) The international survey of herbicide-resistant weeds. Available at: www.weedscience.org (accessed September 3 2015).

HOFFMANN WA & POORTER H (2002) Avoiding bias in calculations of relative growth rate. *Annals of Botany* 90, 37-42.

JASIENIUK M, BRULE-BABEL AL & MORRISON IN (1996) The evolution and genetics of herbicide resistance in weeds. *Weed Science* 44, 176-193.

MARSHALL G, KIRKWOOD RC & LEACH GE (1994) Comparative studies on graminicide-resistant and susceptible biotypes of *Eleusine indica*. *Weed Research* 34, 177-185.

MAXWELL BD, ROUSH ML & RADOSEVICH SR (1990) Predicting the evolution and dynamics of herbicide resistance in weed populations. *Weed Technology* 4, 2-13.

MENCHARI Y, CHAUVEL B, DARMENCY H & DÉLYE C (2008) Fitness costs associated with three mutant acetyl-coenzyme A carboxylase alleles endowing herbicide resistance in black-grass *Alopecurus myosuroides*. *Journal of Applied Ecology* 45, 939-947.

NEVE P (2007) Challenges for herbicide resistance evolution and management: 50 years after Harper. *Weed Research* 47, 365-369.

ORR HA (2009) Fitness and its role in evolutionary genetics. *Nature reviews Genetics* 10, 531-539.

POWLES SB & YU Q (2010) Evolution in action: Plants resistant to herbicides. *Annual Review of Plant Biology* 61, 317-347.

PRESTON C & MALLORY-SMITH CA (2001) Biochemical mechanisms, inheritance, and molecular genetics. In: *Herbicide resistance and world grains*. (eds SB Powles & DL Shaner), 23-60. CRC Press, Florida.

PURBA E, PRESTON C & POWLES SB (1996) Growth and competitiveness of paraquat-resistant and susceptible biotypes of *Hordeum leporinum*. *Weed Research* 36, 311-317.

ROUX F, GASQUEZ J & REBOUD X (2004) The Dominance of the Herbicide Resistance Cost in Several *Arabidopsis thaliana* Mutant Lines. *Genetics* 166, 449-460.

SHERGILL LS, FLEET B, PRESTON C & GILL G (2015a) Incidence of herbicide resistance, seedling emergence and seed persistence of smooth barley (*Hordeum glaucum*) in South Australia. *Weed Technology*, In press.

SHERGILL LS, MALONE J, BOUTSALIS P, PRESTON C & GILL G (2015b) Target-Site Point Mutations Conferring Resistance to ACCase-Inhibiting Herbicides in Smooth Barley (Hordeum glaucum) and Hare Barley (Hordeum leporinum). *Weed Science* 63, 408-415.

VILA-AIUB MM, NEVE P & POWLES SB (2009) Fitness costs associated with evolved herbicide resistance alleles in plants. *New Phytologist* 184, 751-767.

VILA-AIUB MM, NEVE P, STEADMAN KJ & POWLES SB (2005) Ecological fitness of a multiple herbicide-resistant *Lolium rigidum* population: dynamics of seed germination and seedling emergence of resistant and susceptible phenotypes. *Journal of Applied Ecology* 42, 288-298.

VILA-AIUB MM, YU Q, HAN H & POWLES SB (2015) Effect of herbicide resistance endowing Ile-1781-Leu and Asp-2078-Gly ACCase gene mutations on ACCase kinetics and growth traits in *Lolium rigidum*. *Journal of Experimental Botany*, DOI: 10.1093/jxb/erv1248.

WANG T, PICARD J, TIAN X & DARMENCY H (2010) A herbicide-resistant ACCase 1781 *Setaria* mutant shows higher fitness than wild type. *Heredity* 105, 394-400.

WEINER J (1986) How competition for light and nutrients affects size variability in *Ipomoea tricolor* populations. *Ecology* 67, 1425-1427.

ZADOKS JC, CHANG TT & KONZAK CF (1974) A decimal code for the growth stages of cereals. *Weed Research* 14, 415-421.

CHAPTER 7

MANAGEMENT OF ACCASE-INHIBITING HERBICIDE-RESISTANT

SMOOTH BARLEY (*HORDEUM GLAUCUM*) IN FIELD PEA (*PISUM SATIVUM*)

WITH ALTERNATIVE HERBICIDES

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Overall percentage (%)	75%
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper
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Co-Author Contributions

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iii.   the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Management of ACCase-Inhibiting Herbicide-Resistant Smooth Barley (*Hordeum glaucum*) in Field Pea (*Pisum sativum*) with Alternative Herbicides

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Smooth barley is an annual weed species that is infesting crops and pastures in South Australia. Complicating control options is the presence of herbicide-resistant biotypes. A field trial was conducted to identify alternative herbicides for the management of acetyl coenzyme A carboxylase (ACCase)-inhibiting herbicide-resistant smooth barley in field pea. Pre-plant (PP) soil applications of pyroxasulfone; prosulfocarb plus S-metolachlor; dimethenamid-P; propyzamide; trifluralin alone or with triallate or with diuron; or imazamox applied POST were evaluated for their effectiveness and crop safety. Propyzamide, pyroxasulfone, or imazamox applied POST provided a high level of smooth barley control, did not cause any crop injury, and increased field pea grain or forage yield compared with the non-treated. Furthermore, propyzamide or pyroxasulfone reduced panicle density and seed production in smooth barley, whereas the effectiveness of POST imazamox varied over the two seasons. Dimethenamid-P reduced the impact of smooth barley on field pea yield, but cause stunting, and was less effective than propyzamide, pyroxasulfone and imazamox in reducing smooth barley seed production. Negative relationship between field pea yield and smooth barley panicle density indicated that smooth barley is highly competitive in field pea crops and can cause large yield losses. The results of this investigation suggest that propyzamide or pyroxasulfone applied

PP and imazamox applied POST could be used effectively in the field for the management of ACCase-inhibiting herbicide-resistant smooth barley in South Australia.

Nomenclature: Dimethenamid-P, diuron, imazamox, propyzamide, prosulfocarb plus S-metolachlor, pyroxasulfone, triallate, trifluralin, smooth barley, *Hordeum murinum* L. *ssp. glaucum* (Steud.) Tzvelev; field pea, *Pisum sativum* L.

Keywords: Barley grass, crop selectivity, herbicide efficacy, herbicide resistance, POST, post-emergence, PP, pre-plant.

Smooth barley is a problematic annual weed in Australia, typically occurring in areas with less than 425 mm rainfall (Cocks et al., 1976). Smooth barley is found in crop fields and pastures, as well as non-crop areas, such as roadside verges, sheep and cattle enclosures, building sites, and waste ground (Davison, 1977). It is valued as a source of animal feed in pastures early in the season, because it is readily grazed by animals in its vegetative stage. Smooth barley becomes problematic when it matures; its seeds have long barbed awns that irritate the mouth, eyes, and nose of the cattle or sheep, or get entangled in wool, resulting in loss of productivity and product quality (Campbell et al., 1972; Cocks et al., 1976). It can also serve as a host for various pathogenic fungi and nematodes in cereal-growing areas (Belair and Benoit, 1996; Vanstone and Russ, 2001).

Recent reports from growers and agricultural advisors in southern Australia have indicated an increase in the incidence of smooth barley in field crops. In a growers survey in low-rainfall districts of South Australia (SA), smooth barley was reported to be in the top five most problematic weeds (Fleet and Gill, 2010). Research by Fleet and Gill (2012) has also shown that weed management practices used in cropping systems of SA have increased seed dormancy in smooth barley populations, which might have contributed to its greater abundance in field crops. The presence of increased seed dormancy appears to have enabled smooth barley to escape pre-sowing control with non-selective herbicides

and herbicides with short to no residual control and establish after the crops have been planted. Local growers have relied heavily on POST herbicides for the control of these in-crop weed infestations. ACCase-inhibiting herbicides, due to their reliably high efficacy, are commonly used to control grass weeds in broadleaf crops (Preston, 2009). Increase in incidence of resistance to aryloxyphenoxypropionate ACCase-inhibiting herbicides in smooth barley populations has also been reported in SA (Shergill et al., 2015a). As a result, many growers are now growing Clearfield™ cereals and using imidazolinone herbicides to control ACCase-inhibiting herbicide-resistant smooth barley populations. There are now concerns about the evolution of resistance to ALS-inhibiting herbicides in smooth barley. Resistance to ALS-inhibiting herbicides is the most common form of resistance in weed populations across the globe (Heap, 2015) . Moreover, resistance to imidazolinone ALS-inhibiting herbicides in a closely related species, hare barley [*Hordeum murinum* L. ssp. *leporinum* (Link) Arcang.], has already been reported from Western Australia (Owen et al., 2012). The combination of herbicide resistance as well as high seed dormancy would make it increasingly difficult for Australian farmers to effectively control such problematic weed populations in their fields. Despite these concerns, some promising herbicide options are still available for the control of smooth barley.

In southern Australia, grain legume crops such as field peas are widely grown in rotations with cereals due to rotational benefits in terms of biological nitrogen fixation and reduced incidence of cereal root diseases. Field peas are widely grown for grain and sometimes for high-protein forage. However, grain legume crops tend to be less competitive with weeds than cereals. Hence, the control of ACCase-inhibiting herbicide-resistant smooth barley in field peas can be particularly difficult. Therefore, the objective of this study was to identify alternative herbicides for the management of ACCase-inhibiting herbicide-resistant smooth barley.

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Materials and Methods

Experimental Site Location and Design. Two field experiments were established in fields infested with ACCase-inhibiting herbicide-resistant smooth barley at Baroota and Mambray Creek near Port Germein (33.02°S, 138.00°E) in the Upper North region of South Australia during 2012 and 2014, respectively. Resistance to ACCase-inhibiting herbicides had been previously confirmed in smooth barley populations from these fields (Shergill et al., 2015b; 2014). The long-term average annual rainfall at Port Germein is 326 mm and average growing season rainfall (April to October) is 228 mm (Anonymous, 2015). Soil samples were analyzed by the soil and plant analysis laboratory of CSBP Fertilisers Ltd., Australia. Site characteristics are provided in Table 1.

Table 1. Summary of rainfall, soil characteristics, cropping history, planting dates and field pea cultivars at sites in Baroota and Mambray Creek near Port Germein, South Australia, in 2012 and 2014.

Year	Rainfall		Soil characteristics			Previous	Planting	Field pea
	Annual	GSR[a]	Texture	Organic carbon	pH	crop	date	cultivar
	——mm——			—%—				
2012	395	207	Loam	0.48	8.1	Wheat	May-09	Kaspa
2014	373	272	Clay loam	0.87	7.8	Barley	Apr-08	Kaspa

[a] Abbreviation: GSR, growing season (April to October) rainfall.

Prior to initiating the experiment, the field sites were sprayed with glyphosate (900 g ai ha^{-1}) for pre-plant (PP) weed control. Field pea (cv. Kaspa) were seeded at a depth of 5 cm with a no-till drill. The crop was sown at a seed rate of 90 kg ha^{-1} in rows 25 cm apart in 2012 and 23 cm apart in 2014. The experiments were established in a randomized

complete block design with four replicates, with plot size of 54 m^2 (4m by 13.5m) in 2012 to 60 m^2 (4m by 15m) in 2014. Soil fertility was adequate and supplemental fertilizer was applied as needed. Herbicide treatments (PP and POST) were included to evaluate alternative herbicides for control of smooth barley in field pea. The herbicides and their rates were selected according to the recommended field rates used for grass control in broadleaf crops (Table 2). PP treatments were applied to soil before planting and POST treatments were applied 6 to 7 weeks after planting (WAP) when field pea were at the two- to three-nodes stage and smooth barley was at the Z13 to Z20 stage (Zadoks et al., 1974). All herbicide treatments were applied using an all-terrain vehicle fitted with a spray boom with Hardi® flat fan ISO110-015 nozzle, delivering 100 L ha^{-1} water volume at a pressure of 200 kPa.

Data Collection. Field pea establishment was assessed at 5 to 6 WAP by counting the number of plants along 0.5 m length of two adjacent crop rows at four random locations in each plot. At 12 WAP, field pea plant height was determined by measuring two plants at four random locations in each plot. Smooth barley plant and panicle density were assessed in eight 0.09 m^2 (0.3m by 0.3m) randomly placed quadrats plot^{-1}. Smooth barley plant density assessments were taken at 12 WAP, whereas, panicle density was assessed at 18 to 21 WAP in September during both years, when all smooth barley panicles had emerged. To estimate smooth barley seed production, random samples of 3 plants plot^{-1} (i.e. 27 plants replicate^{-1}) were collected at maturity and panicle length and number of seeds plant^{-1} were counted. The data thus obtained were fitted to a functional two parameter linear model (Equation 1) with SigmaPlot version 12.5 (Systat Software Inc., Melbourne, Victoria, Australia), to derive a relationship between panicle length and number of seeds plant^{-1}. The model fitted was:

$$y = y_0 + bx \qquad\qquad (4)$$

where, y is the number of seeds in panicle length or density x, b is the slope of the line of best fit and y_0 is the intercept (set to 0).

Secondly, separate samples of 25 smooth barley plants $plot^{-1}$ were randomly collected at maturity and panicle length $plant^{-1}$ and number of panicles $plant^{-1}$ were measured. The panicle length $plant^{-1}$ obtained was converted to seeds $plant^{-1}$ using the relationship derived above (Equation 1). The seeds $plant^{-1}$ obtained were divided by number of panicles $plant^{-1}$ to derive seeds $panicle^{-1}$. Finally, seed production (seeds m^{-2}) was calculated by multiplying seeds $panicle^{-1}$ with panicle density data (panicles m^{-2}).

In 2012, field pea were harvested with a small plot harvester at maturity to determine grain yield at $\leq$ 14% moisture content, whereas in 2014, field pea were harvested at maturity for forage yield. Forage yield was determined by manually cutting field pea at ground level from an area of 4 m^2 in each plot and later weighed after drying them in an oven at 80 C for 72 h.

Statistical Analyses. All data were subjected to the ANOVA with the use of GenStat 15th edition (VSN International Ltd., Hemel Hempstead, UK). To meet assumptions of ANOVA, square-root transformations were used, which improved the normality and homogeneity of variance. Original means are reported, but the transformed means were separated using the Fisher's protected LSD at P = 0.05. SigmaPlot version 12.5 was used to perform regression analysis on the smooth barley panicle density, seed production, and field pea yield data.

Table 2. Effect of herbicide treatments on smooth barley plant and panicle density, and seed production in 2012 and 2014[a].

Treatments	Application timing	Rate	Plant density[b]		Weed control		Panicle density[c]		Seed production	
			2012	2014	2012	2014	2012	2014	2012	2014
		g ai ha^{-1}	—— plants m^{-2} ——		% of non-treated		—— panicles m^{-2} ——		—— seeds m^{-2} ——	
Non-treated	-	-	1291 d	464 d	-	-	1000 e	349 c	31515 d	14972 f
Pyroxasulfone	PP	100	163 ab	65 b	87	86	17 a	18 a	522 a	415 ab
Prosulfocarb plus S-metolachlor	PP	2000+300	672 cd	223 c	48	52	490 cd	257 bc	16785 c	5610 d
Dimethenamid-P	PP	720	658 cd	181 bc	49	61	181 b	132 b	4618 b	3503 c
Trifluralin	PP	960	624 cd	229 c	52	51	637 d	312 c	20802 cd	8346 e
Trifluralin plus triallate	PP	960+1000	480 bc	187 bc	63	60	544 cd	242 bc	19213 c	5539 d
Trifluralin plus diuron	PP	960+900	184 ab	172 bc	86	63	390 bc	279 bc	14206 c	8045 de
Propyzamide	PP	750	13 ab	5 a	99	99	8 a	3 a	258 a	69 a
Imazamox	POST	32	18 ab	76 bc	99	84	0.3 a	41 a	6 a	1710 b

[a] Means within the same column followed by the same letters are not significantly different according to Fisher's protected LSD at P ≤ 0.05. Data were square-root transformed before ANOVA. Non-transformed means are shown in the table.

[b] Data were taken at 12 weeks after planting (WAP).

[c] Data were taken at 18 and 21 WAP in 2012 and 2014, respectively.

Abbreviation: PP = Pre-plant.

Results and Discussion

Weed Control. During both years, all herbicide treatments reduced the density of ACCase-inhibiting herbicide-resistant smooth barley compared to the non-treated control (Table 2). The most effective treatments for reducing smooth barley density were propyzamide or pyroxasulfone applied PP, and imazamox applied POST; this resulted in 84 to 99% reduction in smooth barley density compared to the non-treated. Dimethenamid-P only gave modest reduction in plant density when evaluated shortly after emergence, but many smooth barley plants were severely stunted and died later. This reduction is especially evident in 2012, when panicle density in dimethanamid-P decreased about four-fold as compared to plant density recorded earlier in the season. In contrast, no such reduction was observed in other herbicides with lower efficacy. Prosulfocarb plus S-metolachlor, trifluralin alone, or trifluralin plus triallate provided around 50% reduction in weed density. Addition of diuron to trifluralin improved weed control relative to trifluralin alone in 2012 only. The lower activity of diuron in 2014 might be due to leaching of the herbicide by 67 mm of rainfall over four consecutive days after herbicide application, compared to no rainfall within this period in 2012 (Anonymous, 2015).

Differences in weed density between herbicide treatments were reflected in panicle density and seed production (Table 2). Propyzamide, pyroxasulfone, and imazamox reduced reproductive capabilities of smooth barley plants. Similar to the reduction in smooth barley plant density, these treatments significantly reduced panicle density and seed production compared with the non-treated. Imazamox applied POST was less effective against smooth barley in 2014 than in 2012, as indicated by lower weed control and higher panicle density and seed production. This difference in the level of weed control between the two years might be due to different environmental conditions between years or due to the evolution of resistance to ALS-inhibiting herbicides in the field

population, because it has already been reported in SA (Shergill et al., 2015a). Similar to the trend for weed control, prosulfocarb plus S-metolachlor, dimethenamid-P, and trifluralin alone or in combination with triallate or diuron were relatively ineffective in reducing smooth barley panicle density and seed production. Such low levels of weed control would likely result in increasing smooth barley future weed infestations, which will cause production problems in subsequent crops. High efficacy of pyroxasulfone on smooth barley has been previously reported in wheat by Fleet and Gill (2010). During both growing seasons, good soil moisture conditions due to excellent post-seeding rainfall could have favoured the activity of PPI herbicides on smooth barley. Thus, lack of control with some PPI herbicides (e.g. trifluralin and prosulfocarb plus S-metolachlor) was due to smooth barley tolerance to these herbicides. During both years of the study, there was a strong positive linear relationship between smooth barley panicle length and seeds plant^{-1} (Figure 1; R^2 = 0.99, 2012; R^2 = 0.98, 2014) and was used to calculate seed production. Quantification of seed production is critical to determine the success or failure of a weed management strategy, because it directly influences weed infestation in subsequent crops.
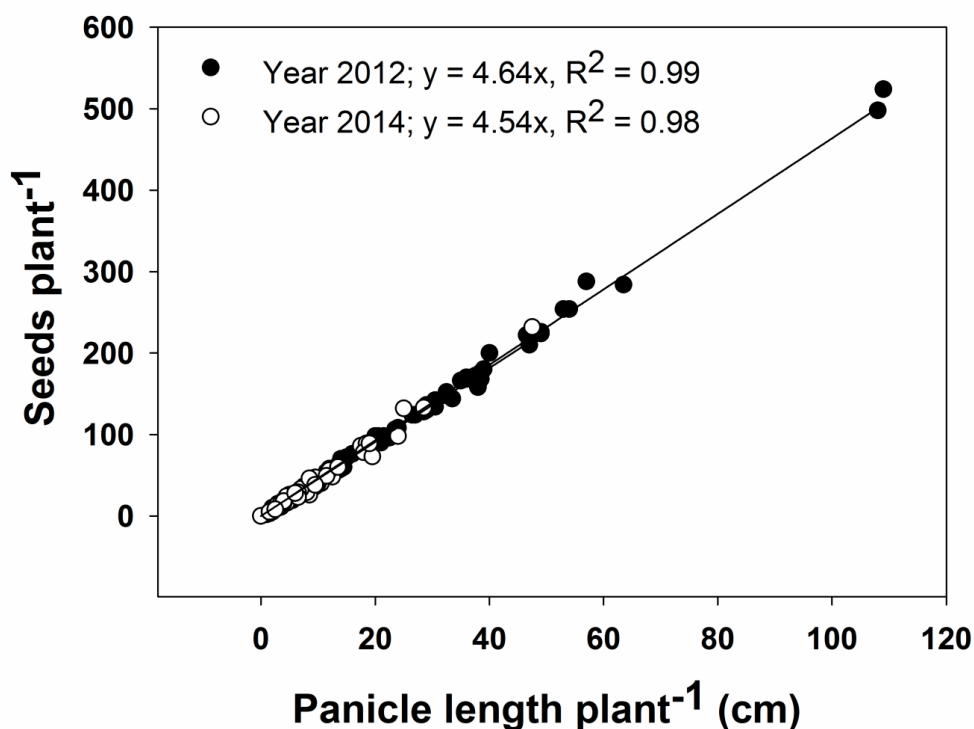
Figure 1. Relationship between total panicle length and total seeds produced plant[-1] of smooth barley during 2012 and 2014. Lines represent a functional two parameter linear model (equation 1) fitted to the individual data of panicle length and seeds plant[-1] ($n =$ 108); intercept (y_0) was set to zero.

Crop Response. Field pea height captured negative crop response (Table 3) better than chlorosis or reduction in early season crop establishment (data not shown). No crop injury was observed for most herbicides, however, reduction in plant height was observed with dimethenamid-P in both years, and in trifluralin plus diuron in 2012. The crop injury symptoms were more apparent in 2014 possibly due to better soil moisture conditions than in 2012.

The differences among herbicide treatments in field pea yield in 2012 and 2014 were highly influenced by smooth barley control. During both years, the highest yield (grain or forage) was recorded in plots treated with propyzamide, pyroxasulfone, dimethenamid-P, or imazamox, which were most effective against smooth barley (Table

3). In these treatments, grain yield increased over the non-treated check by >160% in 2012 and forage yield by >50% in 2014. Pyroxasulfone, propyzamide and imazamox have been successfully used to control other grass weed species in broadleaf crops (Blackshaw, 1998; Kleemann and Gill, 2012; Nelson and Renner, 1998; Tidemann et al., 2014). Dimethenamid-P was less effective in reducing smooth barley seed production but significantly increased grain and forage yields compared with the non-treated. Prosulfocarb plus S-metolachlor also increased grain (75%) and forage (24%) yield relative to the non-treated.

There was a negative linear relationship ($R^2 = 0.96$, 2012; $R^2 = 0.72$, 2014) between smooth barley panicle density and field pea yield (grain or forage) (Figure 2). The slope of the linear regression indicates that the impact of smooth barley on field pea yield was greater in 2012 than in 2014 (b = 0.02 versus 0.004). This could mean that grain yield (2012) of field pea was more sensitive to smooth barley competition than forage yield (2014) or it could be related to greater smooth barley plant density at the experimental site in 2012. The results indicate that smooth barley is highly competitive against field pea and unless effective control tactics are used, it can cause large yield losses (Table 3, Figure 2). It is widely known that field pea is less competitive than cereals (Lemerle et al., 1995) and large yield penalties due to grass weed competition have been previously reported in legume crops (Hashem et al., 2011; McDonald, 2003).
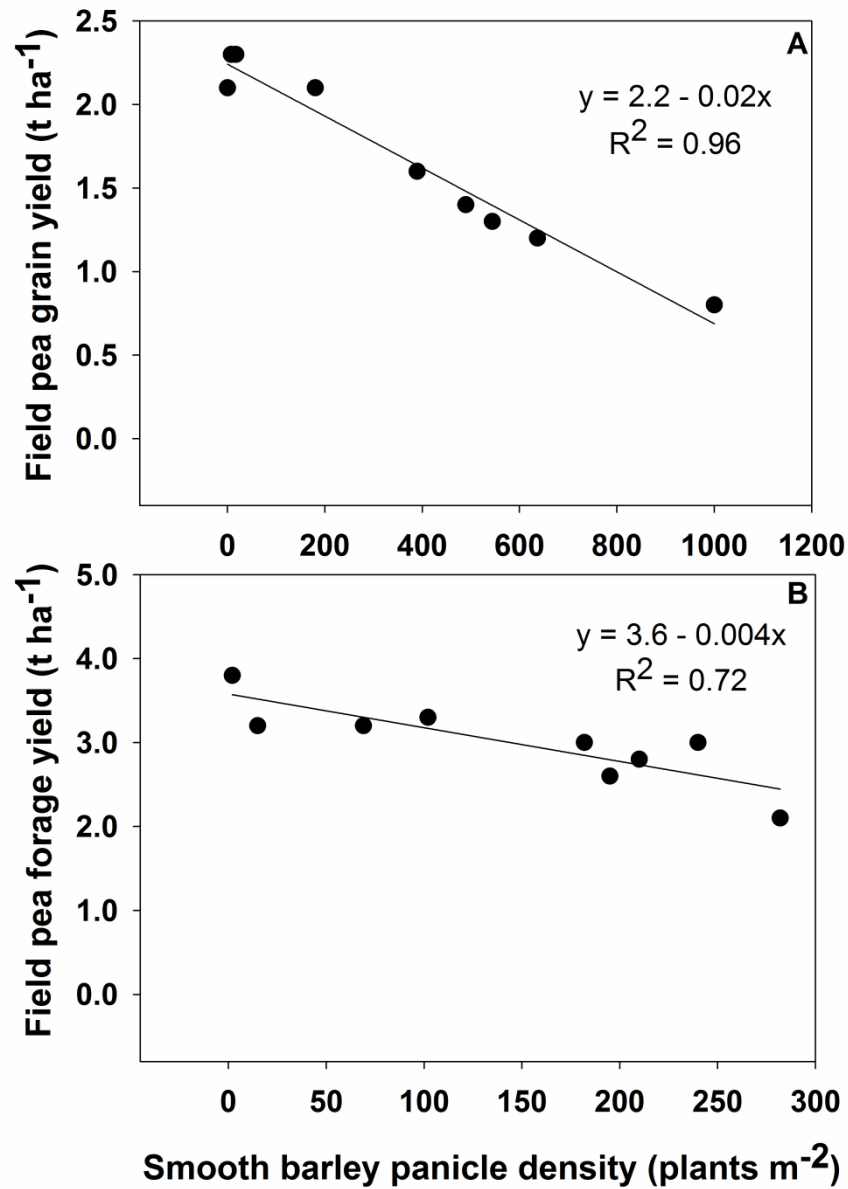
Figure 2. Effect of smooth barley panicle density on field pea grain yield (A) in 2012 and field pea forage yield (B) in 2014. Lines represent a functional two parameter linear model (equation 1) fitted to the mean of the smooth barley panicle density and field pea yield for individual herbicide treatments (n=9).

Table 3. Effect of herbicide treatments on field pea plant height, grain and forage yield in 2012 and 2014[a].

Treatments	Application timing	Rate	Plant height[b]		Grain yield		Gain in grain yield	Forage yield		Gain in forage yield
			2012	2014	2012			2014		
		g ai ha⁻¹	—— cm ——		— t ha⁻¹—		% increase of non-treated	—— t ha⁻¹—		% increase of non-treated
Non-treated	-	-	53 Bc	80 a	0.8	d	-	2.1	c	-
Pyroxasulfone	PP	100	52 Bc	82 a	2.3	a	188	3.2	ab	52
Prosulfocarb plus S-metolachlor	PP	2000+300	53 Bc	82 a	1.4	bc	75	2.6	bc	24
Dimethenamid-P	PP	720	48 C	60 b	2.1	a	163	3.3	ab	57
Trifluralin	PP	960	57 Ab	88 a	1.2	c	50	3	ab	43
Trifluralin plus triallate	PP	960+1000	57 Ab	85 a	1.3	bc	63	3	abc	43
Trifluralin plus diuron	PP	960+900	48 C	80 a	1.6	b	100	2.8	bc	33
Propyzamide	PP	750	60 A	85 a	2.3	a	188	3.8	a	81
Imazamox	POST	32	50 Bc	77 a	2.1	a	163	3.2	ab	52

[a] Means within the same column followed by the same letters are not significantly different according to Fisher's protected LSD at P ≤ 0.05.

[b] Data were taken at 12 weeks after planting.

Abbreviation: PP = Pre-plant.

Propyzamide and pyroxasulfone provided a high level of smooth barley control in field pea in both years of this study. Both of these herbicides are known to have residual soil activity, which might provide effective control of late-emerging seedling cohorts of smooth barley populations. Dimethenamid-P was effective in reducing the impact of smooth barley competition on field pea yield, but was less effective in reducing its seed production. Failure to reduce seed production will lead to a large build-up in weed infestations in future cropping seasons. Dimethenamid-P was the only herbicide to reduce field pea height for both years of this trial. Imazamox applied POST was effective in controlling smooth barley infestation, but weeds that survived this treatment in 2014 produced 1,700 seeds m^{-2}. Reasons for the differences in weed seed production in the imazamox treatment were not investigated, but resistance to ALS-inhibiting herbicides in smooth barley is suspected. However, field peas treated with imazamox produced similar yields to the herbicide treatments with the highest efficacy, which is likely due reduced competitiveness of smooth barley plants surviving an application of this herbicide. At present, Australian growers are relying heavily on imidazolinone herbicides in Clearfield^{TM} cereals for the control ACCase-inhibiting herbicide-resistant smooth barley. This practice is working well in most situations, but needs to be used with caution because resistance to ALS-inhibiting herbicides can develop rapidly compared to other herbicide groups (Beckie and Tardif, 2012; Tranel and Wright, 2002). Crop rotations including ALS-inhibiting herbicides such as imazamox should also include herbicides with different modes of action (e.g. propyzamide and pyroxasulfone). Propyzamide and pyroxasulfone are the best options to control ACCase-inhibiting herbicide-resistant smooth barley, because they reduce seed production, provide excellent crop safety and allowed for maximum yields. These products should be considered as part of a resistance management program because they provide an alternative mechanism of action and are highly effective on this species.

Literature Cited

Anonymous (2015) Climate Data Online: Australian Bureau of Meteorology. http://www.bom.gov.au/climate/data/index.shtml. Accessed March 21, 2015

Beckie HJ, Tardif FJ (2012) Herbicide cross resistance in weeds. Crop Prot 35:15-28

Belair G, Benoit DL (1996) Host suitability of 32 common weeds to *Meloidogyne hapla* in organic soils of southwestern Quebec. J Nem 28:643-647

Blackshaw RE (1998) Postemergence weed control in pea (*Pisum sativum*) with imazamox. Weed Technol 12:64-68

Campbell RJ, Robards GE, Saville DG (1972) The effect of grass seed on sheep production. Proc Aust Soc Anim Prod 9:225-229

Cocks PS, Boyce KG, Kloot PM (1976) The *Hordeum murinum* complex in Australia. Aust J Bot 24:651-62

Davison AW (1977) The ecology of *Hordeum murinum* L: III. Some effects of adverse climate. J Ecol 65:523-530

Fleet B, Gill G (2010) Barley grass, an emerging weed threat in southern Australian cropping systems. *in* Dove H, Culvenor R, eds. 15th Agronomy Conference. Lincoln, New Zealand: Australian Society of Agronomy

Fleet B, Gill G (2012) Seed dormancy and seedling recruitment in smooth barley (*Hordeum murinum* ssp. *glaucum*) populations in southern Australia. Weed Sci 60:394-400

Hashem A, Collins RM, Bowran DG (2011) Efficacy of interrow weed control techniques in wide row narrow-leaf lupin. Weed Technol 25:135-140

Heap I (2015) The international survey of herbicide resistant weeds. www.weedscience.org. Accessed September 3, 2015

Kleemann SGL, Gill GS (2012) Herbicide application strategies for the control of rigid ryegrass (*Lolium rigidum*) in wide-row faba bean (*Vicia faba*) in southern Australia. Weed Technol 26:284-288

Lemerle D, Verbeek B, Coombes N (1995) Losses in grain yield of winter crops from Lolium rigidum competition depend on crop species, cultivar and season. Weed Res 35:503-509

McDonald GK (2003) Competitiveness against grass weeds in field pea genotypes. Weed Res 43:48-58

Nelson KA, Renner KA (1998) Weed control in wide- and narrow-row soybean (*Glycine max*) with imazamox, imazethapyr, and CGA-277476 plus quizalofop. Weed Technol 12:137-144

Owen MJ, Goggin DE, Powles SB (2012) Identification of resistance to either paraquat or ALS-inhibiting herbicides in two Western Australian *Hordeum leporinum* biotypes. Pest Manag Sci 68:757-63

Preston C (2009) Herbicide resistance: target site mutations. Pages 127-148 *in* Stewart CN, ed. Weedy and Invasive Plant Genomics. Oxford, UK: Wiley-Blackwell

Shergill LS, Fleet B, Preston C, Gill G (2015a) Incidence of herbicide resistance, seedling emergence and seed persistence of smooth barley (*Hordeum glaucum*) in South Australia. Weed Technol: 29:782-792.

Shergill LS, Malone J, Boutsalis P, Preston C, Gill GS (2015b) Target-site point mutations conferring resistance to ACCase-inhibiting herbicides in smooth barley (*Hordeum glaucum*) and hare barley (*Hordeum leporinum*). Weed Sci 63:408-415

Shergill LS, Preston C, Boutsalis P, Malone J, Gill G (2014) Amino acid substitutions in ACCase gene of barley grass (*Hordeum glaucum* Steud.) associated with resistance to ACCase-inhibiting herbicides. Pages 7-10 *in* Proceedings of 19th Australasian Weeds Conference Hobart, Tasmania, Australia: Tasmanian Weed Society

Tidemann BD, Hall LM, Johnson EN, Beckie HJ, Sapsford KL, Raatz LL (2014) Efficacy of fall- and spring-applied pyroxasulfone for herbicide-resistant weeds in field pea. Weed Technol 28:351-360

Tranel PJ, Wright TR (2002) Resistance of weeds to ALS-inhibiting herbicides: what have we learned? Weed Sci 50:700-712

Vanstone VA, Russ MH (2001) Ability of weeds to host the root lesion nematodes *Pratylenchus neglectus* and *P. thornei*. I. Grass weeds. Australas Plant Pathol 30:245-250

Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. Weed Res 14:415-421

CHAPTER 8

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

1. General Discussion

Weeds pose one of the most significant agronomic problems associated with crop production. They compete with crop plants for nutrients, moisture and sunlight and negatively impact crop yields and quality (reviewed in Slaughter et al. 2008). Herbicides due to their reliably high efficacy and economical weed control have emerged as a primary method of weed control during the last 65 years (Heap 2014). Heavy reliance on herbicides has led to widespread evolution of herbicide resistance in weeds (Heap 1997; Powles and Yu 2010). Currently, there are more than 247 herbicide-resistant weed species with resistance to 157 different herbicides in 66 countries of the world (Heap 2015). Herbicide resistance is an evolutionary phenomenon in plant species (Maxwell and Mortimer 1994). Many factors contribute to the evolution of herbicide resistance, which include the frequency of resistant alleles in a natural population, the intensity of selection, the mode of inheritance of resistance, gene flow within and between populations, the nature and extent of herbicide use, and the relative fitness of susceptible and resistant biotypes in the presence and absence of herbicide (Diggle et al. 2003; Jasieniuk et al. 1996; Powles et al. 1997).

Hordeum glaucum Steud. is a problematic annual weed in field crops and pastures of Australia (Cocks et al. 1976; Smith 1972). Previous studies have shown that this species has short-lived innate dormancy and was unlikely to infest crops because the majority of its seeds germinated with early autumn rains (Davison 1971; Harris 1961; Smith 1968) and the seedbank did not persist from one year to the next (Popay 1981). However, recent

reports from growers and agricultural advisors in southern Australia have indicated an increase in the incidence of *H. glaucum* in field crops. Research by Fleet and Gill (2012) has shown that weed management practices used in cropping systems of SA have selected for increased seed dormancy in *H. glaucum* populations, which may have contributed to its greater abundance in field crops. In the past, non-dormant populations of *H. glaucum* could be easily controlled with the use of burndown herbicides applied in late autumn. But this change in weed biology has increased the selection pressure on post-emergent herbicides, possibly contributing to the evolution of herbicide resistance. Therefore, seed dormancy and suspected herbicide resistance maybe associated with the increase in abundance of *H. glaucum* in cropping fields in SA. Furthermore, previous investigations of populations of a closely related species *H. leporinum* had confirmed resistance to ACCase-inhibiting herbicides in SA and Tasmania (TAS) (Broster et al. 2012; Matthews et al. 2000), to ALS-inhibiting herbicides in Western Australia (WA) (Owen et al. 2012a; Yu et al. 2007) and to bipyridyliums across southern Australia (Hidayat 2004; Owen et al. 2012a; Powles 1986; Preston et al. 1992). The combination of increased seed dormancy with herbicide resistance would make it very difficult for Australian farmers to effectively manage this weed species in cropping systems. Therefore, there was an urgent need to undertake a comprehensive study on characterisation and management of herbicide resistance in this weed species.

Initially, seeds of *H. glaucum* and *H. leporinum* populations were collected from fields where growers had reported control failure with ACCase-inhibiting herbicides. Seeds of three *H. glaucum* and two *H. leporinum* populations were tested for herbicide resistance and confirmed varying levels of resistance to quizalofop and haloxyfop (Chapter 2). Sequencing the CT domain of the ACCase gene from resistant plants of different populations confirmed the presence of previously known mutations Ile1781Leu and Gly2096Ala. This is the first known case of field evolved target-site resistance to

ACCase-inhibiting herbicides in *H. glaucum*. Resistance to ACCase-inhibiting herbicides has been reported in 47 grass weed species around the world (Heap 2015) and resistance to this group in *Lolium rigidum* (Boutsalis et al. 2012; Broster et al. 2012; Zhang and Powles 2006), *Bromus diandrus* (Boutsalis and Preston 2006), *Avena sterilis* L. (Ahmad-Hamdani et al. 2012; Maneechote et al. 1994; Owen and Powles 2009) has been reported previously in Australian cropping systems.

Following the confirmation of resistance in *H. glaucum* and *H. leporinum* to ACCase-inhibiting herbicides (Chapter 2), an extensive random field survey was conducted in 2012 across the grain cropping regions of SA to quantify the occurrence of herbicide resistance, determine the level of variation in seed dormancy, seed persistence and examine any relationship between seed dormancy and herbicide resistance in *H. glaucum* populations (Chapter 3). The survey showed that 14% of the populations tested with quizalofop exhibited some level of resistance. Resistance to ALS-inhibiting herbicides was low (3% populations to imazamox + imazapyr and 12% to sulfosulfuron) for the herbicides tested and no multiple resistance patterns were observed. In contrast, surveys conducted in southern New South Wales (NSW) in 2007 and Western Australia (WA) in 2005 found no resistance to ACCase-inhibiting herbicides in *H. glaucum* populations (Broster et al. 2010; Owen et al. 2012a). However, a survey of the cropping region of Tasmania (TAS) in 2010, identified resistance to APP herbicide haloxyfop in one population of a closely related species, *H. leporinum* (Broster et al. 2012). Although, resistance to ALS-inhibiting herbicides is the most common form of resistance in weed populations (both monocots and dicots) across the globe (Heap 2015), it appears to be still relatively uncommon in Australia in *H. glaucum*.

The majority of *H. glaucum* populations emerged rapidly (median T_{50} = 8d), but some populations displayed an extremely slow emergence pattern with T_{50} of > 20 d. There was no direct linkage between seed dormancy and herbicide resistance. The

majority of *H. glaucum* populations showed a low level or no seedbank persistence, but a few populations showed up to 20% seedbank persistence from one year to the next. The negative association between *H. glaucum* seedling emergence and the level of seed persistence indicated that greater seed dormancy could be responsible for the extended persistence of the seedbank of this weed species. Short-lived innate dormancy, rapid emergence after autumn rains and low level of seed persistence has been shown to be typical behaviour of *H. glaucum* reported in the Australian literature (Cocks and Donald 1973; Peltzer and Matson 2002; Smith 1968). In contrast, the results of the present study clearly indicate that some *H. glaucum* populations have adequate seedbank persistence to reinfest crops in the next season.

H. glaucum populations which were classified as resistant (> 20% survival) in the survey screening (Chapter 3) and three other populations obtained from the Mid-North (MN) region in 2013, were further studied to quantify the level of resistance and also characterize the molecular basis of resistance to ACCase and ALS-inhibiting herbicides (Chapter 4). Dose-response experiments showed that *H. glaucum* populations from the upper-north region were highly resistant (RI > 12) to the APP herbicides quizalofop and haloxyfop and had low to moderate resistance (RI = 2 to 12) to CHD herbicide clethodim. Although, clethodim is not a preferred option for growers to control *H. glaucum* due to its lower activity compared to APP herbicides, it was more active on R populations at higher doses compared to APP herbicides. Studies conducted in other grass weed species, such as *Echinochloa crus-galli* L. (Huan et al. 2013), *Setaria faberi* Herrm (Stoltenberg and Wiederholt 1995), *Alopecurus japonicus* Steud (Yang et al. 2007), *L. rigidum* (Broster et al. 2011; Saini et al. 2015a, b, c), *L. perenne* ssp. *multiflorum* L.(Martins et al. 2014), *A. fatua* L. (Ahmad-Hamdani et al. 2012; Uludag et al. 2008) and *Phalaris paradoxa* (Cruz-Hipolito et al. 2012; Hochberg et al. 2009) also reported varying level of resistance across ACCase-inhibiting herbicides.

Malathion reversible, low level of resistance (RI = 2 to 6) to SU herbicide mesosulfuron was also observed in populations collected from the mid-north region, with one population having multiple resistance to both ACCase and ALS-inhibiting herbicides. Resistance to SU herbicides has been previously reported in a closely related species *H. leporinum* (Owen et al. 2012a; Yu et al. 2007) and various other grass weeds, such as *L. rigidum* (Christopher et al. 1992), *B. rigidus* (Owen et al. 2012b) and *A. fatua* (Beckie et al. 2012).

Gene sequencing confirmed the presence of previously known mutations Ile-1781-Leu, Ile-2041-Asn and Gly-2096-Ala in the *ACCase*-gene of some populations. Amino acid substitution at 1781 position confers high levels of resistance to most ACCase-inhibitors and is the most common substitution found in grass weed species (Beckie and Tardif 2012; Délye 2005). However, 2041 and 2096 mutations provide moderate to high level of resistance to APP herbicides (reviewed in Beckie and Tardif 2012) and low to moderate level of resistance to CHD herbicides (Saini et al. 2015c). No amino acid substitution was found in *ALS*-gene of resistant populations, but the use of malathion (a cytochrome P450 inhibitor) in combination with mesosulfuron enhanced the activity of herbicide. Synergistic effects of malathion and ALS-inhibitors have been well documented and literature suggests that malathion is an effective cytochrome P450 inhibitor (Beckie et al. 2012; Christopher et al. 1994; Preston et al. 1996). These populations were also susceptible to the SU herbicide sulfometuron and IMI herbicide imazamox plus imazapyr. The reversal of SU resistance by malathion and susceptibility to sulfometuron suggests that non-target site mechanisms (herbicide metabolism by cytochrome P450) conferred resistance to ALS-inhibitors in these populations. This study documented first known case of field evolved APP-to-CHD herbicide cross-resistance and multiple-resistance to ACCase and ALS-inhibiting herbicides in this weed. However, APP-to-CHD herbicide

resistance has been previously reported in a closely related species *H. leporinum* from SA (Matthews et al. 2000). This is the first report of Ile-2041-Asn mutation in *H. glaucum*.

To better understand the evolution and spread of resistance, greater knowledge of inheritance patterns of resistance traits is required (Maxwell and Mortimer 1994). The majority of previous studies have shown that resistance to ACCase-inhibiting herbicides is conferred by a single nuclear gene, either dominant or partially-dominant. The current study showed that mode of inheritance of resistance of a highly resistant population (>20-fold to quizalofop), due to insensitive target-enzyme (Ile-1781-Leu mutation), was due to a single gene with partially-dominant allele (Chapter 5). Initially, the resistant plants (pollen donor) were crossed with susceptible plants (pollen acceptor) to generate an F_1 generation. The F_1 individual was confirmed to be heterozygous by the sequencing of DNA, indicating that the inheritance of resistance is nuclear encoded. The F_1 hybrid was selfed to produce the F_2 generation. A detailed dose-response analysis of the F_2 population to quizalofop confirmed that ACCase-inhibiting herbicide resistance in *H. glaucum* is conferred by a single gene with partially-dominant allele. The F_2 plants segregated in a 3:1 ratio when treated with 6.2 g quizalofop ha^{-1}, which is consistent with a single major gene model. Sequencing of the CT domain of the ACCase gene in individuals of the F_2 population also confirmed that resistance alleles segregated in 1:2:1 ratio, as expected for single-gene inheritance. These results are in agreement with several other studies of grass weed species, which showed that resistance to ACCase-inhibitors is conferred by a single, nuclear and partially-dominant gene (Betts et al. 1992; Tal and Rubin 2004; Tardif et al. 1996; Volenberg and Stoltenberg 2002).

It is widely accepted that the evolution of herbicide resistance may involve a fitness costs (Powles and Yu 2010; Purba et al. 1996; Vila-Aiub et al. 2009). Fitness is the measure of survival and reproduction of a viable offspring in a given environment and plays a vital role in natural selection and adaptation (Orr 2009). We investigated the effect

of two target–site point mutations of ACCase gene: Ile-1781-Leu/Val and Ile-2041-Asn on plant growth and fecundity of *H. glaucum* biotypes grown in intraspecific competition and/or under interspecific competition in the field with wheat and lentil crops (Chapter 6). The amino acid substitutions at 1781 position of ACCase gene did not impose any negative pleiotropic effects on relative growth rate (RGR), panicle emergence, plant height, total biomass and seed production in *H. glaucum* mutant plants. The results are in agreement with several other studies of other grass weed species, which showed no fitness costs associated with the Ile-1781-Leu mutation in the absence of herbicide selection (Menchari et al. 2008; Vila-Aiub et al. 2005; Vila-Aiub et al. 2015). There was some evidence for fitness cost associated with Ile-2041-Asn mutation in terms of reduced RGR and reduced vegetative biomass and seed production when grown in competition with lentils. Reduced RGR detected in a biotype with 2041-Asn mutant allele is likely to reduce their fitness relative to the wild types of the same population, which could be partly related to reduced ACCase activity, as reported in other species (Délye et al. 2003). Panicles of the R biotype with 2041-Asn mutant allele started emerging approximately 20 days earlier than that of S biotype in the field. However, the influence of early flowering on fitness could vary over different growing seasons in rainfed environments. In high rainfall seasons, plants with a longer vegetative phase and later flowering are able to produce greater biomass and seeds than plants that flower and mature early. In contrast, in a low rainfall situation, early flowering plants could escape terminal drought conditions and produce more seed than plants that flower later. The absence of measurable negative pleiotropic effects on plant growth and fecundity associated with Ile-1781-Leu/Val ACCase mutations in *H. glaucum* suggest that the frequency of these alleles will not decline in the absence of herbicide selection pressure. However, the 2041-Asn allele should decrease in frequency in the absence of herbicide selection pressure, especially in high rainfall environments.

Studies were conducted in the field to identify alternative herbicides for the management of ACCase-inhibiting herbicide-resistant *H. glaucum* (Chapter 7). Pre-plant (PP) soil applications of pyroxasulfone, prosulfocarb plus S-metolachlor, dimethenamid-P, trifluralin alone or with triallate or with diuron, or propyzamide, or imazamox applied POST were evaluated for their effectiveness and crop safety. Propyzamide, pyroxasulfone, or imazamox applied POST provided a high level of *H. glaucum* control, did not cause any crop injury, and increased field pea grain or forage yield compared with the non-treated control. Furthermore, propyzamide or pyroxasulfone reduced *H. glaucum* panicle density and seed production consistently, whereas the effectiveness of POST imazamox varied over the two seasons. Pyroxasulfone, propyzamide and imazamox have been successfully used to control other grass weed species in broadleaf crops (Blackshaw 1998; Kleemann and Gill 2012; Nelson and Renner 1998; Tidemann et al. 2014). Dimethenamid-P reduced the impact of *H. glaucum* on field pea yield, but was less effective than propyzamide, pyroxasulfone and imazamox in reducing *H. glaucum* seed production. A negative relationship between field pea yield and *H. glaucum* panicle density indicated that *H. glaucum* is highly competitive in field pea crops and can cause large yield losses. The results of this investigation suggest that propyzamide or pyroxasulfone applied PP and POST imazamox should be considered as part of a resistance management program because they provide an alternative mechanism of action and are highly effective on this species in field pea.

2. Conclusions

In conclusion, this research provides valuable insights into the general pattern of herbicide resistance, seedbank behaviour, level and mechanisms of herbicide resistance, mode of inheritance of ACCase-inhibiting herbicide resistance, fitness penalties associated with herbicide resistance. This study also identified effective management options for the control of ACCase-inhibiting herbicide-resistant field populations of *H. glaucum* in SA. It

also reports the first known instances of resistance to ACCase and ALS-inhibiting herbicides in *H. glaucum* due to target and non-target site mechanisms. Although, the overall occurrence of resistance on farms across the two regions was low, 39% of the fields in the UN region had detectable level of resistance to the ACCase-inhibiting herbicide quizalofop. Evidence presented suggests that crop management practices used by the growers in the cropping fields has selected for greater seed dormancy and a persistent weed seedbank in some populations. The study also reveals that a large proportion of cropping land still contain herbicide susceptible *H. glaucum* populations, where rotations including ACCase and ALS-inhibiting herbicides will still provide effective weed control. This study determined that quizalofop resistance in a population of *H. glaucum* is controlled by a single, nuclear gene, encoding a mutation within ACCase that is dominant at the field rate of quizalofop. In the absence of ACCase-inhibiting herbicide selection pressure, the amino acid substitutions at 1781 position of ACCase gene did not impose any fitness costs, whereas, there was some evidence for fitness cost associated with Ile-2041-Asn mutation in *H. glaucum*. To control ACCase-inhibiting herbicide-resistant *H. glaucum* in field pea, propyzamide and pyroxasulfone appear to be the best options at present, as they reduce seed production, provide excellent crop safety and increased crop yield.

3. Recommendations for Future Research

Based on the findings of this thesis, one area requiring further research is studying the non-target site mechanisms for both ACCase and ALS-inhibiting herbicides. Malathion was used as a synergist to provide evidence for non-target site resistance to ALS-inhibiting herbicides. However, malathion is not effective for this purpose on ACCase-inhibiting herbicides (Preston et al. 1996). There is a need to conduct enzyme assays to study the sensitivity of ACCase and ALS enzyme in populations that did not show target site mutations. The survey showed multiple resistance to ACCase and ALS-inhibiting

herbicides and low level of resistance to ALS-inhibiting herbicides, which is a concern because of current heavy reliance on Clearfield™ technology to control ACCase-inhibiting herbicide-resistant *H. glaucum*. Therefore, further studies are required to find effective alternatives for the management of this weed species. The survey also showed that growers in the cropping fields have selected for greater seed dormancy and a persistent weed seedbank. Further investigations are required to study the segregation of dormancy alleles over multiple generations, which would help to develop better management practices. The fitness studies revealed that the expression of fitness cost is influenced by the environment in which they are tested (lentil versus wheat). Therefore for future studies, fitness costs should be determined under different environments and different levels of resource competition.

4. References

Ahmad-Hamdani MS, Owen MJ, Yu Q, Powles SB (2012) ACCase-inhibiting herbicide-resistant *Avena* spp. populations from the western Australian grain belt. Weed Technology 26:130-136

Beckie HJ, Tardif FJ (2012) Herbicide cross resistance in weeds. Crop Protection 35:15-28

Beckie HJ, Warwick SI, Sauder CA (2012) Basis for herbicide resistance in Canadian populations of wild oat (*Avena fatua*). Weed Science 60:10-18

Betts KJ, Ehlke NJ, Wyse DL, Gronwald JW, Somers DA (1992) Mechanism of inheritance of diclofop resistance in italian ryegrass (*Lolium multiflorum*). Weed Science 40: 184-189.

Blackshaw RE (1998) Postemergence weed control in Pea (*Pisum sativum*) with imazamox. Weed Technology 12:64-68

Boutsalis P, Gill GS, Preston C (2012) Incidence of herbicide resistance in rigid ryegrass (*Lolium rigidum*) across southeastern Australia. Weed Technology 26:391-398

Boutsalis P, Preston C (2006) Resistance to acetyl-coenzyme A carboxylase (ACCase)-inhibiting herbicides in *Bromus* spp. in Australia. Pages 538-540 *In* Proceedings of the 15th Australian Weeds Conference. Adelaide, South Australia: Weed Management Society of South Australia

Broster J, Koetz E, Wu H (2011) Herbicide resistance levels in annual ryegrass (*Lolium rigidum* Gaud.) in southern New South Wales. Plant Protection Quarterly 26:22-28

Broster J, Koetz E, Wu H (2012) Herbicide resistance frequencies in ryegrass (*Lolium* spp.) and other grass species in Tasmania. Plant Protection Quarterly 27:36

Broster JC, Koetz EA, Wu H (2010) A survey of southern New South Wales to determine the level of herbicide resistance in brome grass and barley grass populations. Pages 274-277 *In* Proceedings of the 17th Australasian Weeds Conference. Christchurch, New Zealand: New Zealand Plant Protection Society

Christopher JT, Powles SB, Holtum JA (1992) Resistance to acetolactate synthase-inhibiting herbicides in annual ryegrass (Lolium rigidum) involves at least two mechanisms. Plant Physiology 100:1909-1913

Christopher JT, Preston C, Powles SB (1994) Malathion Antagonizes Metabolism-Based Chlorsulfuron Resistance in Lolium rigidum. Pesticide Biochemistry and Physiology 49:172-182

Cocks P, Donald C (1973) The germination and establishment of two annual pasture grasses (*Hordeum leporinum* Link and *Lolium rigidum* Gaud.). Australian Journal of Agricultural Research 24:1-10

Cocks PS, Boyce KG, Kloot PM (1976) The *Hordeum murinum* complex in Australia. Australian Journal of Botany 24:651-62

Cruz-Hipolito H, Domínguez-Valenzuela J, Osuna M, Prado R (2012) Resistance mechanism to acetyl coenzyme A carboxylase inhibiting herbicides in Phalaris paradoxa collected in Mexican wheat fields. Plant and Soil 355:121-130

Davison AW (1971) The ecology of *Hordeum Murinum* L.: II. The ruderal habit. Journal of Ecology 59:493-506

Délye C (2005) Weed resistance to acetyl coenzyme A carboxylase inhibitors: an update. Weed Science 53:728-746

Délye C, Zhang XQ, Chalopin C, Michel S, Powles SB (2003) An isoleucine residue within the carboxyl-transferase domain of multidomain acetyl-coenzyme A carboxylase is a major determinant of sensitivity to aryloxyphenoxypropionate but not to cyclohexanedione inhibitors. Plant Physiology 132:1716-1723

Diggle AJ, Neve PB, Smith FP (2003) Herbicides used in combination can reduce the probability of herbicide resistance in finite weed populations. Weed Research 43:371-382

Fleet B, Gill G (2012) Seed dormancy and seedling recruitment in smooth barley (*Hordeum murinum* ssp. *glaucum*) populations in southern Australia. Weed Science 60:394-400

Harris G (1961) The periodicity of germination in some grass species. New Zealand Journal of Agricultural Research 4:253-260

Heap I (1997) 'The occurence of herbicide-resistant weeds worldwide'. Pesticide Science 51:235-243

Heap I (2014) Global perspective of herbicide-resistant weeds. Pest Management Science 70:1306-1315

Heap I (2015) The international survey of herbicide resistant weeds. http://weedscience.org/. Accessed October 21, 2015

Hidayat I (2004) Evolution and Spread of Paraquat-Resistant Barley Grasses *Hordeum glaucum* Steud. and *H. leporinum* Link. Ph.D dissertation. Adelaide, Australia: The University of Adelaide. 176 p

Hochberg O, Sibony M, Rubin B (2009) The response of ACCase-resistant Phalaris paradoxa populations involves two different target site mutations. Weed Research 49:37-46

Huan Z, Xu Z, Lv D, Wang J (2013) Determination of ACCase sensitivity and gene expression in quizalofop–ethyl-resistant and -susceptible barnyardgrass (*Echinochloa crusgalli*) biotypes. Weed Science 61:537-542

Jasieniuk M, Brule-Babel AL, Morrison IN (1996) The evolution and genetics of herbicide resistance in weeds. Weed Science 44:176-193

Kleemann SGL, Gill GS (2012) Herbicide application strategies for the control of rigid ryegrass (*Lolium rigidum*) in wide-row faba bean (*Vicia faba*) in southern Australia. Weed Technology 26:284-288

Maneechote C, Holtum JA, Preston C, Powles SB (1994) Resistant acetyl-CoA carboxylase is a mechanism of herbicide resistance in a biotype of *Avena sterilis* ssp. ludoviciana. Plant and Cell Physiology 35:627-635

Martins BAB, Sánchez-Olguín E, Perez-Jones A, Hulting AG, Mallory-Smith C (2014) Alleles contributing to ACCase-resistance in an Italian ryegrass (*Lolium perenne ssp. multiflorum*) population from Oregon. Weed Science 62:468-473

Matthews N, Powles SB, Preston C (2000) Mechanisms of resistance to acetyl-coenzyme A carboxylase inhibiting herbicides in a *Hordeum leporinum* population. Pest Management Science 56:441-447

Maxwell BD, Mortimer AM (1994) Selection for herbicide resistance. Pages 1-26 *in* Powles SB, Holtum JAM, eds. Herbicide Resistance in Plants: Biology and Biochemistry. Boca Raton, FL: Lewis Publishers

Menchari Y, Chauvel B, Darmency H, Délye C (2008) Fitness costs associated with three mutant acetyl-coenzyme A carboxylase alleles endowing herbicide resistance in black-grass *Alopecurus myosuroides*. Journal of Applied Ecology 45:939-947

Nelson KA, Renner KA (1998) Weed control in wide- and narrow-row soybean (*Glycine max*) with imazamox, imazethapyr, and CGA-277476 plus quizalofop. Weed Technology 12:137-144

Orr HA (2009) Fitness and its role in evolutionary genetics. Nature reviews Genetics 10:531-539

Owen MJ, Goggin DE, Powles SB (2012a) Identification of resistance to either paraquat or ALS-inhibiting herbicides in two Western Australian *Hordeum leporinum* biotypes. Pest Management Science 68:757-63

Owen MJ, Goggin DE, Powles SB (2012b) Non-target-site-based resistance to ALS-inhibiting herbicides in six Bromus rigidus populations from Western Australian cropping fields. Pest Management Science 68:1077-1082

Owen MJ, Powles SB (2009) Distribution and frequency of herbicide-resistant wild oat (*Avena* spp.) across the Western Australian grain belt. Crop and Pasture Science 60:25-31

Peltzer SC, Matson PT (2002) How fast do the seedbanks of five annual cropping weeds deplete in the absence of weed seed input? Pages 553-555 *In* Proceedings of the 13th Australian Weeds Conference. Perth, Western Australia: Plant Protection Society of WA Inc.

Popay A (1981) Germination of seeds of five annual species of barley grass. Journal of Applied Ecology:547-558

Powles SB (1986) Appearance of a biotype of the weed, *Hordeum glaucum* Steud., resistant to the herbicide paraquat. Weed Research 26:167-172

Powles SB, Preston C, Bryan IB, Jutsum AR (1997) Herbicide Resistance: Impact and Management. Pages 57-93 *in* Donald LS, ed. Advances in Agronomy: Academic Press

Powles SB, Yu Q (2010) Evolution in action: Plants resistant to herbicides. Annual Review of Plant Biology 61:317-347

Preston C, Holtum JAM, Powles SB (1992) On the mechanism of resistance to paraquat in *Hordeum glaucum* and *H. leporinum* - delayed inhibition of photosynthetic O_2 evolution after paraquat application. Plant Physiology 100:630-636

Preston C, Tardif FJ, Christopher JT, Powles SB (1996) Multiple Resistance to Dissimilar Herbicide Chemistries in a Biotype of Lolium rigidum Due to Enhanced Activity of Several Herbicide Degrading Enzymes. Pesticide Biochemistry and Physiology 54:123-134

Purba E, Preston C, Powles SB (1996) Growth and competitiveness of paraquat-resistant and susceptible biotypes of *Hordeum leporinum*. Weed Research 36:311-317

Saini RK, Kleemann SGL, Preston C, Gill GS (2015a) Alternative Herbicides for the Management of Clethodim-Resistant Rigid Ryegrass (Lolium rigidum) in Faba Bean (Vicia faba L.) in Southern Australia. Weed Technology 29:578-586

Saini RK, Kleemann SGL, Preston C, Gill GS (2015b) Control of clethodim-resistant Lolium rigidum (rigid ryegrass) in triazine-tolerant canola (Brassica napus L.) in southern Australia. Crop Protection 78:99-105

Saini RK, Malone J, Preston C, Gill G (2015c) Target enzyme-based resistance to clethodim in *Lolium rigidum* populations in Australia. Weed Science 63:946-953

Slaughter DC, Giles DK, Downey D (2008) Autonomous robotic weed control systems: A review. Computers and Electronics in Agriculture 61:63-78

Smith DF (1968) The growth of barley grass (*Hordeum leporinum*) in annual pasture. 1. Germination and establishment in comparison with other annual pasture species. Australian Journal of Experimental Agriculture and Animal Husbandry 8:478-483

Smith DF (1972) *Hordeum* species in grasslands. Herbage Abstracts 42:213-223

Stoltenberg DE, Wiederholt RJ (1995) Giant foxtail (*Setaria faberi*) resistance to aryloxyphenoxypropionate and cyclohexanedione herbicides. Weed Science 43:527-535

Tal A, Rubin B (2004) Molecular characterization and inheritance of resistance to ACCase-inhibiting herbicides in *Lolium rigidum*. Pest Management Science 60: 1013-8.

Tardif F, Preston C, Holtum J, Powles S (1996) Resistance to acetyl-coenzyme A carboxylase-inhibiting herbicides endowed by a single major gene encoding a resistant target site in a biotype of *Lolium rigidum*. Functional Plant Biology 23: 15-23.

Tidemann BD, Hall LM, Johnson EN, Beckie HJ, Sapsford KL, Raatz LL (2014) Efficacy of fall- and spring-applied pyroxasulfone for herbicide-resistant weeds in field pea. Weed Technology 28:351-360

Uludag A, Park KW, Cannon J, Mallory-Smith CA (2008) Cross resistance of acetyl-coa carboxylase (ACCase) inhibitor–resistant wild oat (*Avena fatua*) biotypes in the Pacific Northwest. Weed Technology 22:142-145

Vila-Aiub MM, Neve P, Powles SB (2009) Fitness costs associated with evolved herbicide resistance alleles in plants. New Phytologist 184:751-767

Vila-Aiub MM, Neve P, Steadman KJ, Powles SB (2005) Ecological fitness of a multiple herbicide-resistant *Lolium rigidum* population: dynamics of seed germination and seedling emergence of resistant and susceptible phenotypes. Journal of Applied Ecology 42:288-298

Vila-Aiub MM, Yu Q, Han H, Powles SB (2015) Effect of herbicide resistance endowing Ile-1781-Leu and Asp-2078-Gly ACCase gene mutations on ACCase kinetics and growth traits in *Lolium rigidum*. Journal of Experimental Botany 66: 4711-4718.

Volenberg DS, Stoltenberg DE (2002) Giant foxtail (*Setaria faberi*) outcrossing and inheritance of resistance to acetyl-coenzyme A carboxylase inhibitors. Weed Science 50: 622-627.

Yang C, Dong L, Li J, Moss SR (2007) Identification of japanese foxtail (*Alopecurus japonicus*) resistant to haloxyfop using three different assay techniques. Weed Science 55:537-540

Yu Q, Nelson JK, Zheng MQ, Jackson M, Powles SB (2007) Molecular characterisation of resistance to ALS-inhibiting herbicides in *Hordeum leporinum* biotypes. Pest Management Science 63:918-27

Zhang X-Q, Powles SB (2006) Six amino acid substitutions in the carboxyl-transferase domain of the plastidic acetyl-CoA carboxylase gene are linked with resistance to herbicides in a *Lolium rigidum* population. New Phytologist 172:636-645