Clethodim Resistance in *Lolium rigidum* (Annual Ryegrass) and its Management in Broadleaf Crops

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ABSTRACT

Lolium rigidum is one of the most troublesome herbicide resistant weeds in Australia, which has so far evolved resistance to eleven major herbicide groups. Clethodim, an inhibitor of acetyl–coenzyme A carboxylase (ACCase), is a selective post-emergent herbicide used to control annual and perennial grasses in a wide variety of broadleaf crops and has been used by many farmers to manage *L. rigidum* in continuous cropping rotations. However, repeated use of this herbicide during the last two decades has resulted in the appearance of *L. rigidum* populations that are highly resistant to clethodim. Studies on the resistance mechanisms, genetics, and fitness of resistant alleles were undertaken to better understand the evolution of clethodim resistance in *L. rigidum.* Field studies were also undertaken to investigate the performance of alternative herbicides for the management of clethodim-resistant *L. rigidum* in faba bean and canola. Dose–response experiments were conducted on twelve populations of *L. rigidum* collected from different locations in Australia. All the populations were confirmed resistant to clethodim with resistance levels ranging from 3-34-fold as compared to the susceptible control. These resistant populations have also evolved cross-resistance to butroxydim. Sequencing of the target-site *ACCase* gene identified five known ACCase mutations (Leu-1781, Asn-2041, Gly-2078, Arg-2088, and Ala-2096) in these populations. In the highly clethodim-resistant populations, the level of clethodim resistance was influenced by the occurrence of frost close to herbicide application. A significant reduction in the level of clethodim efficacy was observed in resistant populations when plants were exposed to frost for three nights before or after clethodim application. However, there was no effect of frost on the response of the susceptible population suggesting that the mechanism present within the resistant populations interacts with frost to further reduce clethodim efficacy. The inheritance of clethodim resistance was investigated by cross pollinating the susceptible and five resistant populations. The results of the inheritance study showed different

patterns of inheritance of clethodim resistance in *L. rigidum*; which included a single gene, partially dominant, nuclear encoded trait, two different patterns of two-gene inheritance and an example of maternal inheritance of the resistance trait. The fitness of three resistant alleles (Leu-1781, Asn-2041, and Gly-2078) was also studied by determining the change in the frequency of resistant alleles in two generations of *L. rigidum* in the absence of clethodim use. The results of this experiment showed that there was no significant change in the frequency of Leu- 1781 and Asn-2041 alleles in *L. rigidum* populations from one generation to other but the frequency of Gly-2078 allele increased significantly (7 to 16%; $P \leq 0.05$). Studies were also undertaken to identify alternative herbicides for the control of clethodim resistant *L. rigidum* with a range of pre-emergent herbicides in broadleaf crops. In both faba bean and canola crops, pre-emergent herbicides alone were insufficient to effectively manage clethodim-resistant *L. rigidum.* The application of effective soil residual herbicides followed by the post-emergent tank-mixture of clethodim and butroxydim provided acceptable control of some clethodim resistant *L. rigidum* populations.

DECLARATION

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PUBLICATIONS ARISING FROM THIS THESIS

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Rupinder Kaur Saini, Jenna Malone, Christopher Preston, Gurjeet Gill (2015). Frost reduces clethodim efficacy in clethodim-resistant rigid ryegrass (*Lolium rigidum*) populations. Weed Science (Accepted).

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ACRONYMS

ACCase:	Acetyl coenzyme A carboxylase
AGRF:	Australian Genome Research Facility
AHAS	Acetohydroxyacid synthase
ALS	Acetolactate synthase
ANOVA	Analysis of variance
APP	Aryloxyphenoxypropionate
BC	Back cross
BCCP	Biotin carboxyl carrier protein
C	Celsius
CHD	Cyclohexanedione
CLF	Clearfield™
CT	Carboxyl transferase
EPSPS	5-enolpyruvylshikimate-3-phosphate
F_1	First filial generation
FAS	Frost after spray
fb	Followed by
FBS	Frost before spray
GR_{50}	Lethal dosage (herbicide dose causing 50% growth reduction of plants)
GSR	Growing season rainfall
KPa	Kilopascals

LD_{50}	Lethal dosage (dose required to control 50% of individuals in the population)
LSD	Least significant difference
N	Nitrogen
NF	No frost
NSW	New South Wales
P	Phosphorus
PCR	Polymerase chain reaction
POST	Post-emergence
PPI	Preplant incorporated
PPZ	Phenylpyraxoline
PSPE	Pre-sowing pre-emergence
R	Resistant
S	Susceptible
SA	South Australia
TT	Triazine tolerant
VIC	Victoria
WA	Western Australia
WAP	Weeks after planting
WAS	Weeks after sowing