Herbicide application strategies for wild radish management in Imidazolinone tolerant faba bean

Amali Upeksha Welgama

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School of Science, Psychology and Sports

PO Box 663

University Drive, Mount Helen

Ballarat Victoria 3353

Australia

ABSTRACT

The extensive and continual use of herbicides in cropping situations has inevitably led to the phenomenon of "herbicide-resistance" in weeds and this has become one of the most challenging issues in modern agriculture. Herbicide-tolerant crops (HTC) were introduced to diversify weed management practices, but the lack of integrated weed management strategies, along with the continuous use of the same herbicide mode of action (MOA) demanded by the HTC has continued to impose selection pressure on weeds to evolve with herbicide resistance. Consequently, this thesis has been focused on the introduction of herbicide MOA combinations into HTC systems in an attempt to reduce the rate of herbicide resistance evolution in weeds.

Raphanus raphanistrum is the number one broadleaf weed in Australia, and for this case study, the newly released ALS-inhibiting imidazolinone tolerant faba bean cultivar *PBA Bendoc* with its conventional cultivar, *PBA Samira,* were selected as the study species. ALS-inhibiting (imazamox + imazapyr and imazethapyr) and PSII-inhibiting (metribuzin) herbicides were used as the two herbicide MOAs. The herbicide sensitivity of *R. raphanistrum* was initially evaluated at different growth stages, in glasshouse studies using herbicide-resistant and susceptible biotypes to ALS-inhibiting herbicides. The highest susceptibility was observed at the earliest growth stage regardless of the biotype and Imazamox + imazapyr proved to be more effective in controlling both biotypes compared to imazethapyr.

The same two herbicides were tested on faba bean cultivars at different growth stages to assess crop tolerance and identify the herbicide application window. The field trials conducted in 2018 and 2019 showed increased ALS-inhibiting herbicide tolerance in *PBA Bendoc* compared to *PBA Samira* even at the most advanced growth stage. Both faba bean cultivars were then evaluated for their tolerance to metribuzin in-crop application at different herbicide rates. Both cultivars responded similarly, showing progressive herbicide damage with increasing application rates. However, the reduced pod number, even at the lowest rate used, flagged the possible yield penalties that may result in using in-crop metribuzin applications. It is thus suggested that metribuzin must be used post sowing pre-emergent (PSPE) respecting the label recommendations.

The potential herbicide combinations were then tested on herbicide-resistant *R. raphanistrum* and *PBA Bendoc* to evaluate their efficacies. Metribuzin was initially used as PSPE in all

combinations, and was to be followed by imazamox + imazapyr applications at the same growth stages of the weed and the crop as in previous experiments. However, 100% control of *R. raphanistrum* was achieved using metribuzin alone, and thus no second herbicide was required. All the assessed herbicide combinations were tolerated by *PBA Bendoc,* proving the suitability of these herbicide combinations for incorporation into the *PBA Bendoc* cropping system. These results led to two potential herbicide combination strategies: (i) herbicide rotations, with metribuzin as PSPE in one year along with another potential herbicide MOA in the following year, (ii) herbicide sequential application, with metribuzin applied at PSPE and imazamox + imazapyr applied at the 2-4 leaf stage if *R. raphanistrum* plants survived the metribuzin treatment.

A seed germination study was conducted under different temperature/photoperiods, pH levels, osmotic potentials, salinity and burial depths to identify the optimal germination conditions for *R. raphanistrum.* The optimum germination conditions for both herbicide-resistant and susceptible biotypes of *R. raphanistrum* were found to be 25ºC/15ºC temperature range under 24 hours complete dark. However, the significant interaction between photoperiod and temperature indicated that the seed germination under higher temperatures is less favoured by 24 hours dark conditions regardless of the biotype. An increased moisture stress tolerance in herbicide-resistant seeds was observed, whilst both biotypes reacted similarly to different pH levels and burial depths.

In summary, this thesis has elucidated the effectiveness of two herbicide MOAs in controlling *R. raphanistrum* while addressing the crop tolerance to these herbicide MOA combinations. These findings will help in setting up stewardship guidelines to be used with the *PBA Bendoc* faba bean cultivar to mitigate the misuse of herbicides, thus ensuring their sustainable application. In addition, the demonstration of differential seed germination requirements of resistant and susceptible *R. raphanistrum* seeds has provided further information to help with its systematic management. Overall, this study can be used as a case study to investigate herbicide options that can be used in different HT crop cultivars to control a range of weed species.

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This thesis is dedicated to my loving family to make them proud!

DECLARATION

I, Amali Upeksha Welgama hereby declare that this thesis titled “Herbicide application strategies for wild radish management in imidazolinone tolerant faba bean” comprises only my original work towards the degree of Doctor of Philosophy and has not been submitted either in whole or in part for another degree or diploma. Due acknowledgement has been made in the text to all other material used. The thesis does not exceed 100,000 words and fulfills the requisites set out for the degree of Doctor of Philosophy by the Federation University.

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Amali Upeksha Welgama

24 November, 2020

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CHAPTER 1 – General introduction

1.1 Background and context

Agriculture is the backbone of the Australian economy, contributing AUD $59 billion per year to the Gross National Product (Australian Bureau of Statistics, 2019). Within this sector, the contribution of the Australian grain industry is a critical element, having a total crop value of AUD $29 billion (Australian Bureau of Statistics, 2019), representing 26% of all agricultural produce. In this crop category, the highest contribution is from wheat, followed by barley, canola and pulses. In this thesis, the focus will be on pulses, which are now beginning to play a major and increasingly important role, in Australian agriculture and the economy.

Pulses are leguminous broad-acre crops, harvested for their dried grains. The collective term 'pulse' is used for chickpeas, field peas, lentils, lupins and faba beans, which are the five major pulse crops grown in Australia. Faba bean is a winter growing pulse, originating in the Middle-east (Caracuta *et al.*, 2015; Stoddard, 1991). Conditions within Victoria are ideal for cultivation of this bean, and it is rapidly becoming an integral part of the state's agronomy strategy. According to Australian Bureau of Statistics 2017 data, faba beans contributed AUD $137 million to the grain industry, making it the second largest contributor to the value of produce by pulse crops in Victoria. Consequently, any impediments to the growth and productivity of these broad-acre plants must be given timely and appropriate investigation, in order to protect the current economic status of the industry and to preserve the opportunities for future generations to benefit from its economic production.

In this respect, "weeds" are aggressive plants that have become a serious problem in the grain industry across Australia, and they currently pose severe constraints to the health and productivity of all major crops including grain legumes (Rubiales *et al.*, 2006). The effects of weeds on broad-acre crops are numerous. They (i) rob soil moisture and nutrients, (ii) cause issues at sowing, including restricted access for planting rigs, (iii) cause problems at harvest through contamination of the grains with weed seeds, (iv) prevent some crops being grown where in-crop herbicide options are limited, (v) can be toxic to stock, and (vi) also carry diseases and host pest insects. Each year, millions of dollars are expended by the farmers to control these weeds in their productive fields. Moreover, it has been claimed that the total crop loss due to weed competition throughout the world as a whole, is greater than the combined effect of insects, pests and diseases

(Amare *et al.*, 2014). At the local level, it has been estimated that the total cost of managing weeds and yield loss due to weeds to Australian grain growers is at AUD $3.3 billion (Llewellyn *et al.*, 2016). As a consequence, weed management has become an urgent priority in farming Australian winter crops (Niknam *et al.*, 2002). To preserve the integrity of the agricultural sector and to generate substantial economic benefits to both the growers and the industry, sustainable weed management practices must be conducted.

In terms of weed management, it is now well accepted that the use of herbicides has become necessary in today's agriculture due to the laborious, tiresome and expensive nature of physical removal methods (Marwat *et al.*, 2008). However, as a consequence of over-use of herbicides in previous crop management practices, it has been found that there are 48 weeds in Australia which have been identified as herbicide resistant (Croplife Australia, 2018; Heap, 2017). Herbicide resistance is known to be a result of inherited ability to survive under the normal doses of herbicide, due to the continuous and repeated use of the same herbicide for a long period of time (Moss, 2017). To reduce the possible side effects of the over-use and reliance on herbicides, an integrated weed management (IWM) system is being introduced. This system is a 'mixed method' weed control approach, which integrates a suite of weed control methods, including chemical and physical methods, or chemical and cultural methods (Harker & O'donovan, 2013).

In order to present alternative weed control options for improved reliability and convenience of use for farming systems, identification and development of herbicide-tolerant strains of commercial crops has become a major breeding priority. With the introduction of a herbicide-tolerant crop (HTC) for a particular herbicide mode of action (MOA), we are also demanding the use of that particular herbicide MOA rather than other chemical groups. As a consequence, a high level of selection pressure is instituted on the weed populations leading the weeds to evolve resistance over time. With the introduction of herbicide MOA combinations, other alternative herbicide options can be introduced to be used in the cropping system, reducing the risk of posing a biological selection pressure on weeds. Thereby we can enable the use of existing herbicides for a prolonged period of time without letting them be outdated. Therefore, before making full use of HTC, it will be essential to institute strict stewardship guidelines to oversee the development and widespread use of this approach. This will involve monitoring the development of alternative and effective weed control techniques, not only for the objective of sustainability of crop production but also for the preservation of current and future herbicide resources.

In summary, with this increased focus on the importance of strategic use of herbicides, it is timely to investigate new sustainable herbicide weed management strategies for use in broad-acre cropping systems (Green, 2018). Of particular interest to this thesis are concerns that arise from the limited selection of herbicides that farmers can use for pulse farming, which indicates that weed control using chemical treatment methods has become exceedingly problematic (Niknam *et al.*, 2002).

1.2 Herbicide Tolerant Crops (HTC)

HTCs play a vital role in crop production, as they have become a powerful tool to combat agricultural weeds (Kishore *et al.*, 1992; Lamichhane *et al.*, 2016; Moll, 1997; Peerzada *et al.*, 2019; Rasche & Gadsby, 1997). HTCs are of two types, depending on their origin. They can be either genetically modified where a foreign gene from another organism is introduced to the original genetic composition, or they can be conventionally bred by producing mutants using their existing germplasm (Knezevic & Cassman, 2003).

The first HTC cultivars, sulfonylurea-tolerant soybeans, were introduced in 1993 with an acquired tolerance to high rates of a number of registered sulfonylureas (Carpenter & Gianessi, 1999). Three years after their introduction, the glyphosate-tolerant soybean, cotton and maize cultivars were released to the market. These HTCs were rapidly adopted by farmers, since glyphosate is a non-selective herbicide and assured a broad spectrum of weed control (Carpenter & Gianessi, 1999; Ranjan *et al.*, 2020). Consequently, other HTCs have been released to the market, exhibiting increased tolerance levels towards different herbicide MOAs (Ranjan *et al.*, 2020). Within less than a decade of their introduction, HTCs have shown a steadily increased use in cropping systems, highlighting the popularity of this strategy (Knezevic & Cassman, 2003). Some HTC cultivars are developed with "stacked genes", where several new transgenes are introduced to the genome, to make the crop tolerant to multiple herbicide MOAs. This provides the crop manager with a range of herbicide options to apply to a crop (Knezevic & Cassman, 2003). Soybean cultivars developed with resistance to glyphosate, aryloxyalkanoate and glufosinate herbicides are one such example (Cui *et al.*, 2020).

However, in todays' agricultural armoury, HTCs have become a double-edged sword, as they evidence both positive and negative impacts on cropping systems (Lamichhane *et al.*, 2017). The most commonly addressed advantages of using HTCs include (i) the broad spectrum of weeds controlled, (ii) the reduction in crop injuries, (iii) new herbicide MOA incorporation into cropping areas, (iv) a reduction in the budget for herbicides, (v) more opportunities for no-tillage farming, (vi) simplicity of crop management, and (vii) reduced herbicide carryover. At the same time, the negative impacts imposed by this strategy include (i) weed population shifts due to continuous use of the same herbicide MOA, (ii) single selection pressure, (iii) adverse impacts on soil microflora and fauna, (iv) transgenic gene escape to the wild, (v) yield drag and lag due to the alterations in genomes, (vi) the age of the genome at the time of new gene insertion, (vii) organic crop contamination with gene flow, and (viii) herbicide drift to non-target sites (Das *et al.*, 2019; Knezevic & Cassman, 2003; Ranjan *et al.*, 2020). In essence, achieving a balance between these positive and negative issues in order to make this approach sustainable in cropping systems, an incorporation of mutual support from other weed control strategies is necessary.

1.3 Development of herbicide resistance in weeds

Herbicide resistance is a result of natural selection, which is a normal and predictable attribute of all plant species. When a herbicide is used at its normal rate in an agricultural situation, it is a plant population's innate capability to partially survive the herbicide and complete its life cycle. This ability, in a previously herbicide-susceptible weed population, is the cause of herbicide resistance (Heap, 2014). In weed populations prior to any herbicide exposure, there are always some rare mutants with naturally conferred herbicide resistance. Over time, with each continuous application of the herbicide, these mutant varieties increase in proportion in the population until they predominate, and the population thus becomes largely herbicide-resistant (Figure 1.1) (Heap, 2014).

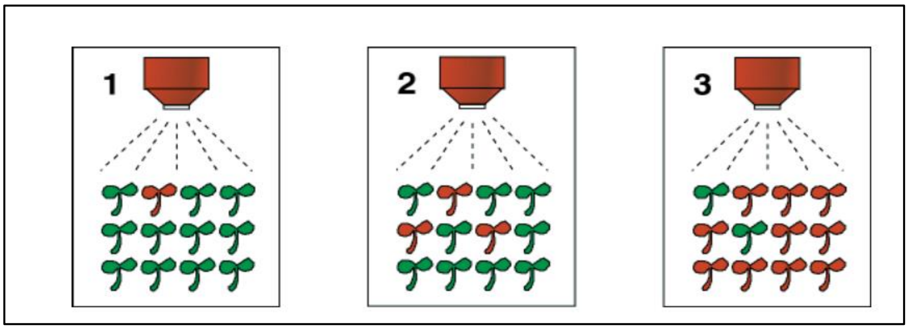


Figure 1.1. Steps showing the natural increase of a population of herbicide-resistant weeds in a field (Martin et al., 2000)

A herbicide-resistant weed is characterised by some specific features, which include: (i) the original or 'wild' population is susceptible to the herbicide of interest, (ii) within this species, herbicide-resistant plants are not controlled by this herbicide at the normal usage rate, and (iii) viable seeds are produced by these resistant plants, increasing their proportion in subsequent generations (Martin *et al.*, 2000).

Apart from the presence of resistant genes, there are other factors that have an influence on herbicide resistance including: (i) selection pressure imposed by frequency and efficacy of herbicide use, (ii) dominancy of resistant genes, (iii) rate of seed production, (iv) innate levels of seed dormancy, (v) soil seed bank persistence, and (vi) resistant trait vigour, all which have an influence on the degree of resistance evolution of a weed. When implementing weed management strategies to reduce the evolution of new herbicide-resistant weeds, selection pressure is the easiest aspect to manage among those factors (Heap, 2014).

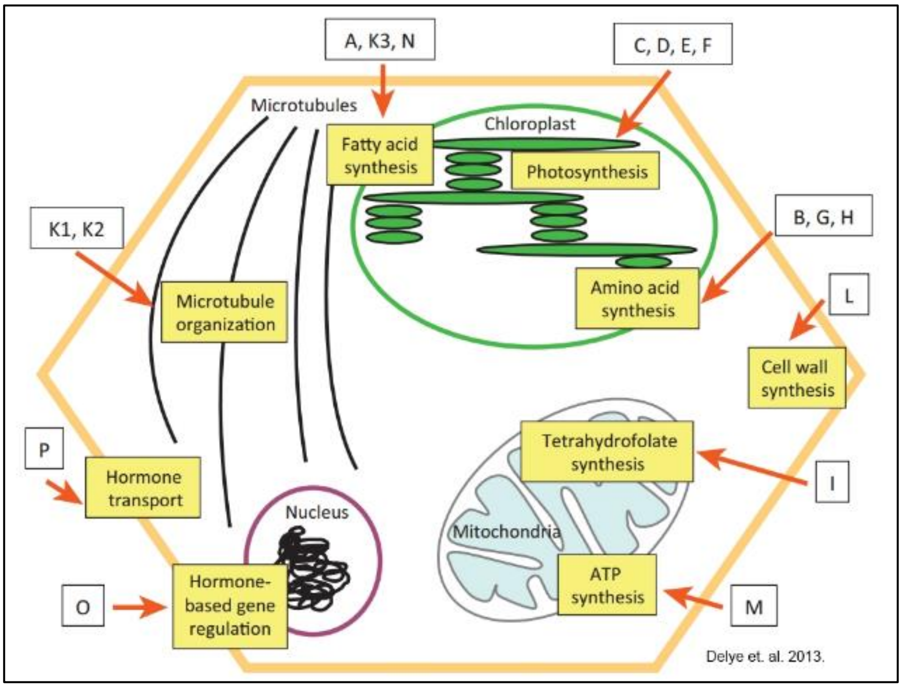


Figure 1.2. Cellular targets of different herbicide MOA (Délye *et al.*, 2013)

To address selection pressure on weeds, we have singled out two herbicide groups for further investigation in our project; the group B ALS-inhibiting herbicides and the group C PSII-inhibiting herbicides from the diverse array of herbicide groups (Figure 1.2), as these groups are currently being widely used in crop cultivations including pulses.

1.4 Details of plant species of concern to this study

1.4.1 *Raphanus raphanistrum* L.

Raphanus raphanistrum L. is known to be the principal broad-leaf weed species infesting Australia's winter cropping systems and is therefore of high interest to faba bean crop managers (Cheam *et al.*, 2008).

1.4.1.1 Plant description

Raphanus raphanistrum is a polymorphic annual or biennial plant which grows up to a height of one metre at maturity (Cheam & Code, 1995). The hairless petiole, which has a length of 10-20 mm, bears the cotyledon leaves of 8-15 mm in *R. raphanistrum* seedlings (Clapham *et al.*, 1990). The young prostrate rosette gives rise to erect hairy stems with alternate leaves. The lower leaves are 8-20 cm long and are deeply lobed with entire to slight indentations. The terminal leaf lobe is usually larger when compared to others on the plant (Holm, 1997).

The plant can be single or multiple-stemmed, depending on the conditions under which it grows. With favourable conditions, the rosette will result in multiple stems soon after emergence and will be expanded to varying lengths bearing inflorescence at the terminals (Cheam & Code, 1995). *R. raphanistrum* starts flowering between 4-12 weeks after seedling emergence (Figure 1.3). Depending mainly on day length and temperature, the flowering can take place for extended periods (12-42 weeks) (Reeves *et al.*, 1981). Flowers are in long raceme-like paniculate inflorescences at branch terminals. Petal colours of *R. raphanistrum* are usually yellow, white, brown or purple which determines the venation colour (Holm, 1997).

Raphanus raphanistrum pods are cylindrical and fleshy, bearing 2-10 seeds (Holm, 1997). Pods are usually 2-5 mm wide and 20-70 mm long, ending with a shallow beak without seeds. Upon maturity, pods develop with definite constrictions which form segments between seeds confining one yellow-to-brown colour globular seed in each segment (Holm, 1997). The hard pod and the seed coat enable a prolonged period of seed dormancy in *R. raphanistrum* (Cheam, 1986; Malik *et al.*, 2010; Young & Cousens, 1999). The main pollination agents of *R. raphanistrum* are insects such as bees and butterflies (Stanton, 1984) where the flowers are self-incompatible (Hill & Lord, 1987; Sampson, 1964).

Figure 1.3. *Raphanus raphanistrum* plant morphology (Friends of Queens Park Bushland, 2011)

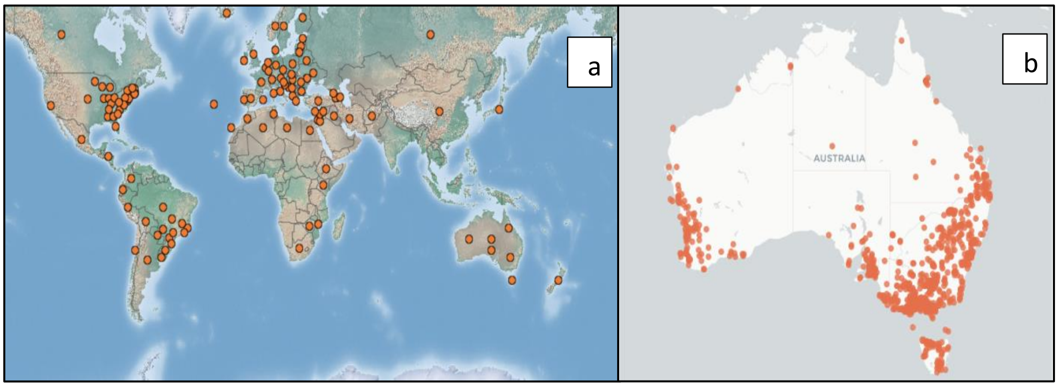


Figure 1.4. (a) The global distribution of *R. raphanistrum* (CABI, 2019) and **(b)** Distribution of *R. raphanistrum* in Australia (ALA, 2019)

1.4.1.2 Geographical distribution

Raphanus raphanistrum is a native to the Mediterranean region but is now a common weed in temperate countries including Europe, North America, South America, Africa, Australia and New Zealand (Figure 1.4 (a)) (Parsons & Cuthbertson, 2001). It is widespread and plentiful in temperate to sub-alpine climatic areas of the world. It is mainly associated with winter crop cultivations and in disturbed habitats (Holm, 1997), and is common throughout the cropping areas of Australia including Western Australia, Queensland, New South Wales, Victoria and South Australia (Figure 1.4 (b)) (Parsons & Cuthbertson, 2001).

Whilst occurring in most soil types in Australia (Borowiec *et al.*, 1972), it is known that in the United Kingdom it is not found in alkaline soils. It is not commonly found in pastures, as other invasive species are more successful competitors, and also the absence of the grazing stock results in reduced disturbances to promote seed germination (Cheam, 1986; Piggin *et al.*, 1978). The introduction of *R. raphanistrum* to Australia in the mid-19th century is regarded to be accidental, coming as a contaminant of agricultural produce (Donaldson, 1986).

1.4.1.3 Economic impact of *Raphanus raphanistrum*

Raphanus raphanistrum is known as an "economically damaging" weed in cropping systems across the world including Australia (Blackshaw *et al.*, 2002; Cheam & Code, 1995). Reduction in grain crop yield due to the crop-weed competition has been evident with *R. raphanistrum* populations present in the cropping fields (Blackshaw *et al.*, 2002; Code & Donaldson, 1996; Eslami *et al.*, 2006). A *R. raphanistrum* plant density of 200 plants/m^2 has been shown to contribute to a reduction in wheat yield by 50% (Cheam & Code, 1995) while low densities such as 10 plants/m^2 were responsible for 10% wheat yield reduction (Code & Donaldson, 1996). Reduction in wheat yield in the presence of *R. raphanistrum* has also been supported by Hashem and Wilkins (2002). An increased reduction in wheat yield has been shown to result when the *R. raphanistrum* plants emerge simultaneously with the crop or immediately after the crop emergence (Cheam & Code, 1995). In the study of Blackshaw *et al.* (2002), canola yield reduction due to *R. raphanistrum* populations with the densities of 4 and 64 plants/ m^2 was 11% and 91% respectively. Trials conducted in Western Australia have shown that the presence of 10 *R. raphanistrum* plants per square metre can reduce the yield of faba beans by 36% (GRDC, 2013). In pulses, the post-emergent herbicide options are limited and, particularly in faba bean cropping systems, no post-emergence herbicides have been registered for *R. raphanistrum* control (GRDC, 2017).

1.4.1.4 Management of *Raphanus raphanistrum*

1.4.1.4.1 Cultural methods

Common cultural practices include crop rotation, increased seeding rates, narrow row spacing, nutrient and irrigation management and cultivation (Colbach *et al.*, 2017; Norsworthy *et al.*, 2012). Crop rotation has been shown to reduce *R. raphanistrum* populations in cropping fields (Kebaso *et al.*, 2020). According to Newman (2003), a non-crop phase after every four years or two non-crop phases in five years is preferred to achieve a greater success. When using a crop rotation strategy, it is known that within a wheat phase it is relatively easy to control *R. raphanistrum* when compared to the pulse phase. It is claimed that this is due to the natural increased competitiveness and many herbicide options available for wheat compared to pulses (Hashem &

Wilkins, 2002). Adopting competitive cultivars in the cropping system is also evident to be successful in controlling *R. raphanistrum* (Hashem *et al.*, 2006). One such example is the increased competitive ability of the lupin variety Mandelup, which was more effective in suppressing weeds compared to the Belarsa and Tanjil varieties in a crop rotation (Hashem *et al.*, 2006).

Rouging is another effective cultural weed control practice, especially when the infestation is low in terms of weed density. This approach is known to reduce seed production in *R. raphanistrum* effectively when implemented before the flowers reach maturity (Madafiglio *et al.*, 2006). Windrow burning is also used in controlling *R. raphanistrum* as it ensures sufficient seed damage to prevent them from germinating (Walsh & Newman, 2007). The study of Walsh *et al.* (2005) has shown that the *R. raphanistrum* seeds can be destroyed by burning in narrow windrows for 10 s at 500°C and 20-30 s at 400°C, but escaping seeds from seed shattering is suggested to be a drawback of this method. Using mechanical seed destructive methods such as the Harrington Seed destructor ("Rotamill" developed by Harvestaire®) are also known to be effective in destroying *R. raphanistrum* seeds in the cropping fields (Newman, 2005; Walsh *et al.*, 2013).

Increased crop density is another cultural approach in controlling *R. raphanistrum*. Increased wheat densities have been shown to reduce *R. raphanistrum* seed production, leaf area index and dry matter (Blackshaw *et al.*, 2002; Minkey, 2006). This observation has also been supported by the study of Eslami *et al.* (2004), where an increase in wheat density from 100 to 300 plants/m^2 resulted in a 50% reduction in *R. raphanistrum* seed production. Incorporation of green or brown manure and hay freezing also can lead to a reduction in seed production when applied before the *R. raphanistrum* seed set (Cheam *et al.*, 2008).

Tillage can be implemented as a method to bring the *R. raphanistrum* seeds from deeper levels and thus enhance their germination, and allowing them to be controlled, before planting the crop (Code & Donaldson, 1996; Madafiglio *et al.*, 2006). *R. raphanistrum* germination and emergence is highly dependent on burial depth, with seeds buried between 1-2 cm below the soil surface having increased seedling emergence (Young, 2001). Though deep burial of *R. raphanistrum* (below 10 cm) has been shown to reduce the germination by 61% (Madafiglio *et al.*, 2006), a significant amount of seeds can remain viable, and impose a problem for long-term weed management, as the seeds may germinate once brought back to the soil surface (Cheam, 1986). In the study of Cheam (1986), two consecutive years of shallow cultivation is predicted to enhance the *R. raphanistrum* seed germination and thereby to achieve a rapid seed bank depletion.

1.4.1.4.2 Biological methods

Biological methods are significantly important in weed management as they have less impact on the environment, biodiversity and human health (Charudattan, 2005). The ecology, annual life cycle and the phenology of *R. raphanistrum* has made its control challenging using biocontrol agents (Scott *et al.*, 2012; Scott *et al.*, 2002). To overcome the complications related to closely related non-target plants of Brassicaceae family such as *Raphanus sativus* (edible radish) and *Brassica napus* (canola), increased levels of host specificity is essential when introducing a biological control (Kebaso *et al.*, 2020; Scott *et al.*, 2012). One such example is *Gephyraulus raphanistri* which feeds on *R. raphanistrum* seeds, but it also attacks *B. napus* plants, resulting in it being less attractive as a biocontrol agent for *R. raphanistrum* (Scott *et al.*, 2012).

The fungi *Alternaria raphani* and *Phoma lingam* have shown promising results as biocontrol agents for *R. raphanistrum* (Djebali *et al.*, 2009). The fungal infections on *R. raphanistrum* have not necessarily reduced the vegetative growth, but they do reduce the number of pods and weight due to increased sensitivity of the *R. raphanistrum* flowers to the fungal infections (Djebali *et al.*, 2009; Pathan *et al.*, 2006). Diseases affecting *R. raphanistrum* include black leg, white rust, black rot and downy mildew caused by *Leptosphaeria maculans, Albugo candida* Kuntze, *Xanthomonas campestris* Fr. and *Peronospora parasitica* Fr., respectively (Maxwell & Scott, 2008). In Western Australia, *Hyaloperonospora parasitica* has been identified as the most effective pathogen on *R. raphanistrum* (Maxwell & Scott, 2008). Pests include *Halotydeus destructor* (Cheam & Code, 1995) and *Urodon* spp. (Scott *et al.*, 2012) and are considered to be promising biocontrol agents for *R. raphanistrum*. When considering the problem of long-term weed control in fields, despite the initial introduction and perpetuation costs of biocontrol methods, they turn out more economical because of overall lower cost and minimum management costs when compared to mechanical and chemical methods (Chikwenhere & Keswani, 1997).

1.4.1.4.3 Chemical methods

Chemical use has become the most preferred method of controlling *R. raphanistrum* in almost all agricultural fields (Hashem *et al.*, 2006; Walsh *et al.*, 2006). The sensitivity of *R. raphanistrum* to herbicides is highly dependent on the weed growth stage, crop density and competitiveness, herbicide properties and abiotic conditions including humidity and temperature (Kudsk &

Kristensen, 1992; Madafiglio *et al.*, 2006). Some of the commonly used herbicides for *R. raphanistrum* control include diflufenican + bromoxynil, picolinafen in lupin (Cheam & Lee, 2004), soxaflutole in chickpeas, pyrasulfotole + bromoxynil, atrazine + mesotrione (Walsh *et al.*, 2012) and triasulfuron + MCPA in wheat (Madafiglio *et al.*, 2006). An increased seed set reduction is achieved in *R. raphanistrum* when a non-selective herbicide such as glyphosate is used (Newman, 2005; Newman & Adam, 2006). Herbicide combinations of triasulfuron (7.5 g/ha) + MCPA (350 g/ha), triasulfuron (15 g/ha), bromoxynil (140 g/ha) + MCPA (350 g/ha), flumetsulam (20 g/ha) and MCPA (700 g/ha) have showed promising results in the study of Madafiglio (2002), which was done under different abiotic conditions, growth stages, herbicide rates and geographical locations. Madafiglio's study also suggests a 100% control of seed set to be achieved when selective herbicides are applied within two weeks of flowering.

Among all the available herbicide options, using herbicide MOA combinations is highly recommended as it can reduce the herbicide-resistance evolution in *R. raphanistrum*. Depending on the herbicide resistant status, two herbicide applications are suggested to be effective in controlling *R. raphanistrum* in crop. Early post-emergent options include bromoxynil, picolinafen + pendimethilin + 1,2-benzisothiazole and bromoxynil + diflufenican tank-mixed with MCPA (GRDC, 2014). Avoiding the same MOA used in early post-emergent spray in the late application is useful in reducing the selection pressure on *R. raphanistrum* to evolve with herbicide resistance. Therefore, a tank mix of 2,4-D with an ALS-inhibiting herbicide is recommended as a commonly used late post-emergent application (GRDC, 2014). Pre-emergent herbicides including triasulfuron followed by post-emergent application of glyphosate and paraquat is predicted to reduce *R. raphanistrum* infestation (Newman, 2013). The study of Newman (2013) also reported the increased efficacy of *R. raphanistrum* control when diflufenican was applied at the two-leaf stage, followed by application of diflufenican + metribuzin at the 8 to 10-leaf stage, when compared to the sole application of those herbicides. Also, the isolated use of herbicides is not useful in long-term weed management (Kebaso *et al.*, 2020). Therefore, herbicide diversification in a cropping system is not only advantageous in achieving good control of *R. raphanistrum* but also in making the existing herbicides sustainable for their future use.

1.4.1.5 Herbicide resistance in *Raphanus raphanistrum*

Given that *R. raphanistrum* has been identified as one of the most troublesome broadleaf weeds in Australian winter cropping systems, its herbicide resistance to many herbicide MOAs has made its control even more difficult (M. J. Owen *et al.*, 2015; Walsh *et al.*, 2012). Cross pollination in *R. raphanistrum* plays a significant role in rapid herbicide resistance with gene flow from resistant plants survived from herbicide applications (Kebaso *et al.*, 2020).

According to Walsh (2004), the control of *R. raphanistrum* in Australia has been entirely herbicide dependent and as a result *R. raphanistrum* populations from the Northern Western Australian wheat belt have developed multiple herbicide resistances across four modes of action; ALS-inhibiting, PS II-inhibiting, carotenoid biosynthesis inhibitors and synthetic auxins. This study conducted by Walsh *et al.* (2007) in the Western Australian Wheat belt, also showed that 54% of the *R. raphanistrum* populations are resistant to ALS-inhibiting chlorosulfuron while 60% are resistant to synthetic auxin 2,4-D amine. This study has also revealed that 58% of the studied populations were multiple-resistant across at least two of the four herbicide modes of actions mentioned above. Similarly, in the survey of Walsh *et al.* (2001), it was confirmed that chlorosulfuron resistance occurred in *R. raphanistrum*. A similar case has been reported in the study of Smit and Cairns (2001) with a chlorosulfuron-resistant population in South African wheat fields. ALS-inhibiting Imazamox + imazapyr resistance in *R. raphanistrum* has also been found to be evident in the field surveys conducted in Southern New South Wales (Broster *et al.*, 2014). According to Hashem *et al.* (2001a), the (ALS)-inhibiting herbicides resistant biotypes are also cross resistant to chlorsulfuron and metosulam but susceptible to PS II-inhibiting metribuzin and synthetic auxin 2,4-D amine. The study of Hashem *et al.* (2001b), documented the first case of PS II-inhibiting triazine resistance in *R. raphanistrum* in Australia. The testing of known resistant *R. raphanistrum* populations have shown a high number of survivals of 57-97% towards a herbicide dosage rate of four times the recommended rate of metribuzin or atrazine, demonstrating their high tolerance to triazines. These findings have also been recorded in the international herbicide-resistant weed survey of Heap (2020), with confirmed cases reported of herbicide resistance in *R. raphanistrum* to ALS-inhibiting, PS II-inhibiting, synthetic auxins, carotenoid biosynthesis inhibitors and EPSP synthase inhibitors. The increase in herbicide resistant *R. raphanistrum* populations in the Western Australian cropping fields is clearly evident in the well-documented surveys carried out in the same area spaced over intervals during several years (Hashem *et al.*, 2001a; M. D. K. Owen *et al.*, 2015; Walsh *et al.*, 2001; Walsh *et al.*, 2007).

1.4.2 Faba bean

According to the United States Department of Agriculture (2017), the classification of Faba bean is (i) Kingdom: Plantae, (ii) Order: Fabales, (iii) Family: Fabaceae, (iv) Genus: *Vicia*, (v) Species: *Vicia faba.* It is also known as broad bean, fava bean, field bean, bell bean, English bean, horse bean and tick bean (Altuntaş & Yıldız, 2007).

1.4.2.1 Plant description

Faba bean is an erect plant which can grow up to 2 m at maturity but in Australia, the crop usually grows up to 1.5 m (Matthews & Marcellos, 2003). In Australia, the most commonly preferred seed size for sowing faba bean are the 'medium-sized' seeds, while in Europe they are the 'small-size' seeds (Matthews & Marcellos, 2003). Branches of the faba bean plants are produced from the base of the stem with compound leaves as two leaflets at the early growth stage and up to seven leaflets after the commencement of flowering (Figure 1.5).

Being a bean species, faba bean plants usually have shallow root systems with less roots mass than cereals, the tap root of which can penetrate the soil to around 60 cm with a profusion of fibrous roots in the top 30 cm of the soil (Matthews & Marcellos, 2003). In early cultivars, flowering begins at the 5-7th stem node but in late cultivars it is up to the 15th or higher leaf-bearing stem nodes. Comprising 3-8 flowers depending on the cultivar, inflorescences are produced in the axil at each node between the angle of stem and node (Matthews & Marcellos, 2003). Inflorescence moves successively up the stem for about 15 flowering nodes or as the new nodes are produced over a 4-6 week period. As with many other legumes, excess flowers are produced in faba bean but only around 15% will result in viable pods. In a well-grown faba bean crop, the pods bear 2-4 seeds, and the seed size varies with the cultivar. They are usually borne at a height of 20-30 cm above the ground level (GRDC, 2017). Upon maturity, pods turn black and eventually the stem and the leaves of the plant also start to turn black. Honeybees are the main pollinators of faba bean and aid in maintaining a cross-pollination rate of 25-30%. The bees, while feeding on the faba bean flower nectar, pick up and transfer pollen from one plant to another (Matthews & Marcellos, 2003). With the approach of daytime temperatures near to 30^{O}C, flowering of faba bean reaches its end but a few leaf-bearing nodes are still produced.

Figure 1.5. Faba bean plant morphology (Wells, 2017)

1.4.2.2 Geographical area

Agricultural land use practices have strongly influenced significant biosphere changes (Foley *et al.*, 2005). This has affected, worldwide, approximately 15 million km^2 and 31.5 million km^2 of natural vegetation which have been transformed into crop lands and pastures respectively (Ramankutty *et al.*, 2008). Estimates are that the nitrogen flux into the terrestrial biosphere has doubled with human activities, and a contributing factor is leguminous crops including faba bean. This has been ranked as the second most active species in this regard, with approximately 40 Tg N a^{-1} released per year, which is nearly a half of the amount due to nitrogen fertilizers (Monfreda *et al.*, 2008). Within these leguminous agricultural lands, the worldwide faba bean production is shown in tonnes in figure 1.6 (a). South Australia, Victoria and New South wales are the main regions in Australia for faba bean production with an average annual production exceeding 300 000 t (Skylas *et al.*, 2019) (Figure 1.6 (b)).

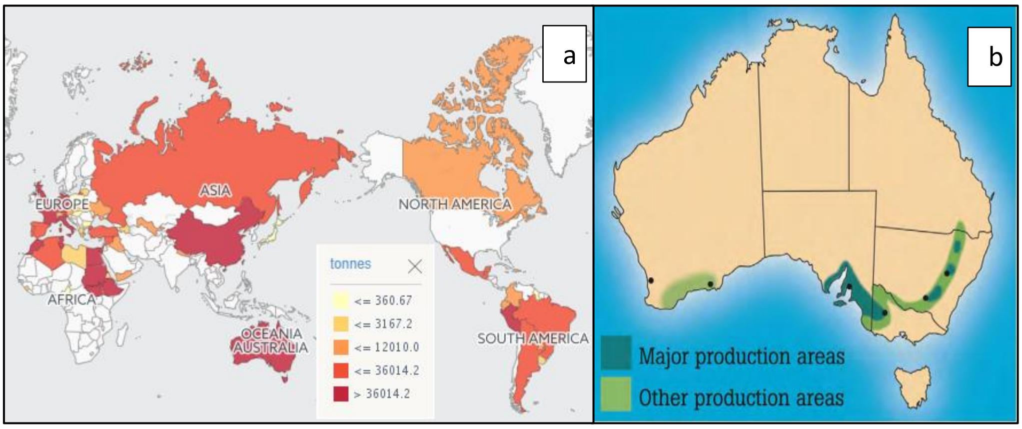


Figure 1.6.(a) The worldwide faba bean production (FAOSTAT, 2019) **(b)** Australian faba bean production areas (GRDC Grow Notes, 2017)

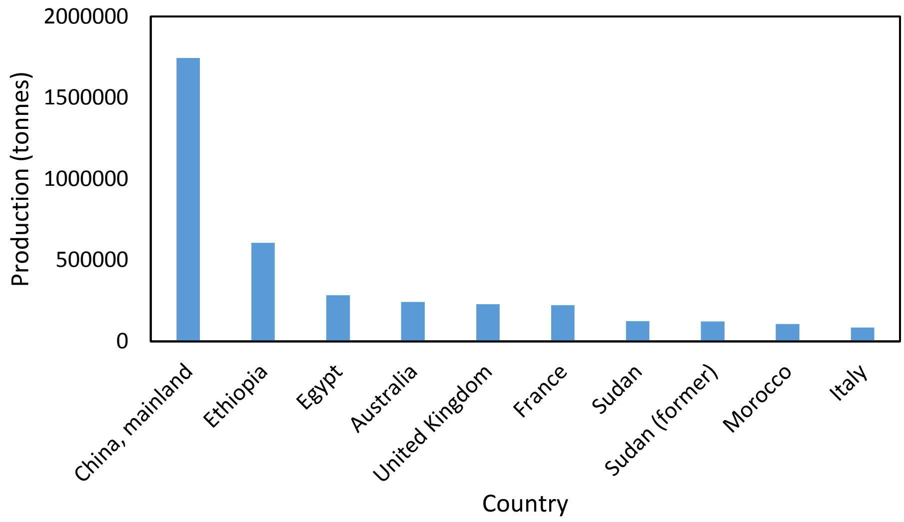


Figure 1.7. Average faba bean production in major faba bean-producing countries 1994-2018 (FAOSTAT, 2019)

In the early 1980s, faba bean was commercially grown for the first time in South Australia, with the release of the Fiord cultivar (GRDC, 2017). Prior to this release, from 1920s to 1970s it had been grown sporadically for the domestic horse-racing trade, for green manure crops or for export to the United Kingdom. With the development of this industry, faba bean production in Australia has steadily increased with South Australian production being stabilized in 1992-95. At that time, the production in Victoria was decreasing while it increased in New South Wales and Western Australia. Since 1995, the faba bean production area increased in all states, before it dropped in all states excluding South Australia in 2002. At the present time it is cultivated in South Australia, Victoria, New South Wales and Western Australia and to a smaller extent in Tasmania and Southern Queensland (GRDC, 2017). Faba beans grow much better in the eastern districts of the northern grain region, where the bean is well suited to the high rainfall and mild spring temperatures rather than the drier, hotter conditions in the western areas (GRDC, 2017).

1.4.2.3 Economic importance to agriculture in Australia

The world annual faba bean production has now exceeded 4.0 million tonnes, but still only 2% of this product is traded internationally (Figure 1.7) (GRDC, 2017). The major exporters of faba bean are Australia, France and the United Kingdom. China has now become a faba bean importer, notwithstanding its earlier history as a major exporter. Egypt dominates international trade in food-quality faba beans as a major importer while other countries also import faba beans in considerably large amounts for food. At the same time, several countries import faba bean as a livestock feed (GRDC, 2017).

All seed types of faba bean are used for human consumption or livestock feed as dry beans. For human consumption, the large seed beans are usually used as a green vegetable. In niche markets, canning, splitting, and preparation as snack foods are included as value-added faba bean products. In addition, faba beans have become an attractive rotation crop for cereal farmers, adding to its value as an export commodity. All Australian grown faba beans are used for human consumption markets. Major buyers of faba bean from Australia are the Middle East, specifically Egypt, Saudi Arabia and the United Arab Emirates (GRDC, 2017).

To achieve and sustain such success in the world faba bean market, producing a high-quality product is essential, and as a result Australia has become a major exporter of this high value product to food markets in the Middle-East. The Australian faba bean export market has grown steadily over the last decade before peaking in 2017 (FAOSTAT, 2019).

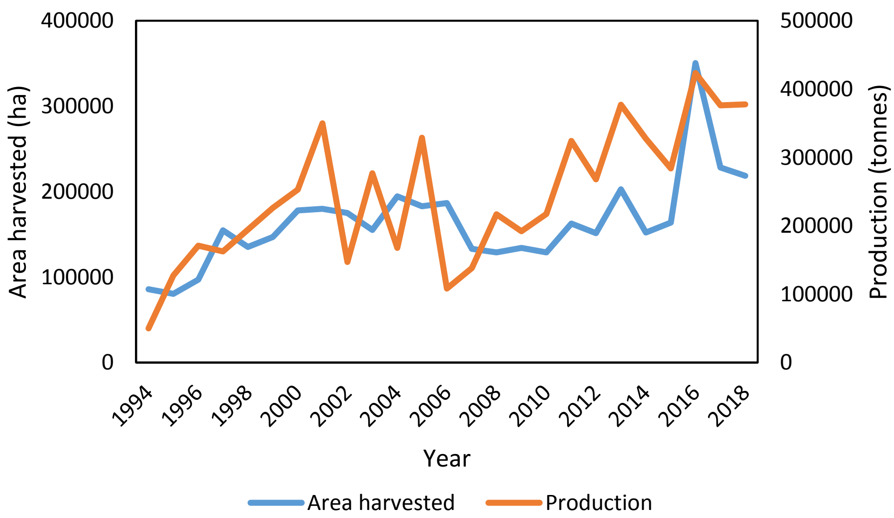


Figure 1.8. Australian faba bean production and harvest area from 1994-2018 (FAOSTAT, 2019)

With the introduction of the new faba bean cultivar 'Fiesta VF' to the market in 2004, faba bean production was predicted to be increased as the cultivar promised a better yield and resistance to Ascochyta blight (GRDC, 2003) (Figure 1.8). With droughts occurring in 2002 and 2006, Australia has lost some of its international export markets, particularly the loss of the UK and the French markets after the drought in 2002 (GRDC, 2017).

According to available statistics recently obtained relative to faba bean production, the current total area of land under faba bean cultivation in Australian is 313,000 ha, and the total annual faba bean production is 416 kt (ABARES, 2019). This is a significant increase compared with statistical estimates in 2015 which were 164,000 ha and 284 kt per annum of cultivation area and production respectively (ABARES, 2017). South Australia holds the position for the highest faba bean-sown area, yield and the greatest production compared to other states in Australia, followed by New South Wales and Victoria (ABARES, 2019).

1.5 Significance of the study

We have provided a justification for the claim that, for continued successful agricultural pulse production on an economic scale in a broad-acre milieu, effective weed management is critical. Further, we have indicated that whilst the use of herbicides has been the major method for controlling weeds in broad-acre intensive farming systems, due to inherent poor plant competitiveness of pulse species and the limited range of herbicide options, weeds pose a significant management problem. In this aspect, low safety margins between control of target weeds and the economic production of pulse crops advised for many registered herbicides, presents a further difficulty for commercial level pulse cultivation (McMurray *et al.*, 2016). Of particular concern to the pulse industry is that, because Australia is now the greatest global faba bean exporter (Pulse Australia, 2016), it is essential that the Agriculture industry places focused attention on weeds in faba bean cultivation, since, unchecked, they will increasingly contribute to a decrease in the commercial value of faba bean exports.

As we have previously discussed, *R. raphanistrum* is known to be the principal broad-leaf weed species infesting Australia's winter cropping systems (Cheam *et al.*, 2008). It has been specifically noted that, particularly in dicot crops, *R. raphanistrum* is known to be hard to control (Walsh, 2004). Indeed, in southern Australia, *R. raphanistrum* has become the most troublesome and widespread broadleaf weed found in cereal and grain legume crops over a range of soil types (Cheam & Code, 1995), and the revenue loss is AUD $4.9 million (GRDC, 2017). Because of the increased herbicide resistance of this weed, coupled with the rising cost of herbicides, this figure is predicted to increase.

Other than integrated weed management (IWM), introduction of herbicide-tolerant crops (HTC) to the cropping systems permits a wide range of selective and non-selective herbicide applications in cropping systems without causing injuries to the crops. At the same time, as *R. raphanistrum* is becoming resistant to most of the available herbicides, evaluating the efficacy of herbicide combinations will be a useful approach, allowing farmers to make the best use of so called 'out of date' herbicides. Thus, before using either these HTC or herbicide combinations, identification and development of strict stewardship guidelines on herbicide use is essential to validate not only the sustainable use of existing and future herbicides, but also the productivity of HTC.

In order to address these herbicide-resistant weed strategies in cropping systems, it is crucial to study the possible herbicide combinations to make sustainable use of existing herbicides and herbicide-tolerant crop cultivars.

1.6 Research questions

Farmers usually tend to apply herbicides at pre-planting and immediate post-planting due to difficulties in predicting the long-term effects of weeds in variable local situations (Kudsk, 2008). Until the last few decades, farmers were fortunate to receive a steady supply of new herbicides to deal with the predestined appearance of herbicide-resistant weeds. It is surprising and concerning to know that the release of new herbicide MOAs to the market has not occurred for three decades (Davis & Frisvold, 2017). To date, few new herbicides belonging to the already existing herbicide MOAs are in the process of registration to be released to the market in future. Other than these new herbicides, there is only a single new herbicide MOA in preparation to be introduced (Condon, 2020). A significant discouragement for chemical companies to invent new herbicide modes of action is the time and the cost of commercialization of a new herbicide. It is estimated to require 11 years of development and US $286 million dollars investment respectively (Duke, 2012; Green, 2018). This emphasises the highlighted importance of our study on reassessing the value of existing herbicides. Therefore, this project will critically address the issues related to (i) the development of an effective herbicide MOA combination for controlling *R. raphanistrum*, and (ii) the tolerance level of the herbicide-tolerant faba bean cultivar (*PBA Bendoc*) to these herbicide combinations.

Related to the significance, the overarching research question of this study will be on evaluating herbicide application strategies for wild radish management in Imidazolinone tolerant faba bean. Its purpose will be to generate an understanding of herbicide strategies which might be introduced for employing chemical weed control in order to make the sustainable use of existing and future herbicides possible. It will aim to avoid introducing a situation that encourages a biological selection pressure, which will inevitably lead to the evolution of more herbicide-resistant weeds. Therefore, identifying a suitable herbicide MOA combination will consist of a stepwise approach with the following objectives;

(i) To identify the most susceptible growth stage of *R. raphanistrum* to ALS-inhibiting herbicides;

(ii) To identify the flexibility of Imidazolinone tolerant *PBA Bendoc* and conventional *PBA Samira* faba bean cultivars to ALS-inhibiting herbicides;

(iii) To evaluate the PS II inhibiting herbicide tolerance in Imidazolinone tolerant *PBA Bendoc* and conventional *PBA Samira* faba bean cultivars;

(iv) To evaluate the efficacy of the sequential herbicide application of ALS-inhibiting and PS II-inhibiting herbicides in terms of *R. raphanistrum* susceptibility and faba bean crop safety;

(v) To evaluate the differential germination success of *R. raphanistrum* biotypes; resistant and susceptible to ALS-inhibiting herbicides.

1.7 Contribution to the existing knowledge

We have already emphasised the high and growing number of herbicide-resistant weed species that have been identified in Australia (Heap, 2017), which underscores the growing importance of addressing this problem of overuse and reliance on herbicides for weed management. Of these herbicide-resistant species, *R. raphanistrum* has been particularly singled out in our project. The herbicide treatment will combine group B; ALS-inhibiting herbicides and group C; PSII-inhibiting herbicides as a sequential application.

The current studies of herbicide resistance in *R. raphanistrum* are mainly concentrated in Western Australia. The urgency of this issue is shown by the observations of significant effects that have been evident from previous studies for the most widely and frequently used ALS-inhibiting herbicides and PS II-inhibiting herbicides (Hashem *et al.*, 2001a; Hashem *et al.*, 2001b; Walsh, 2004; Walsh *et al.*, 2007). To add to this existing knowledge, conducting a study to check the effect of herbicide MOA combinations on controlling herbicide-resistant *R. raphanistrum* will be an important contribution in decision making for their control.

In addition, once a germplasm has been developed for a new HTC cultivar, to make the maximum sustainable use of it, the strict adherence to 'stewardship guidelines' for such a product is essential. This refers to the carefully measured and applied dosages to a cultivated broad-acre area to control a weed infestation without challenging the crop resistance. Also, by carefully husbanding the herbicide dose, the possibility of building up resistance within a weed species is minimised. However, to identify and develop those stewardship guidelines, a carefully designed research program will need to be instituted. Experiments related to the effects of varying herbicide combinations and doses, as well as determining the optimum growth stage of the crop and the weed for herbicide application should be extensively studied.

1.8 Research Justification

Given that the grain industry, including pulse agronomy, contributes a large proportion of the profits to the Agricultural Industry, maintaining this status has become increasingly important. A considerable amount of money is already spent on weed control each year, as weeds markedly reduce the value and the quality of the grain crop. In addition to the costs of using extra integrated weed management practices, the estimated cost of controlling herbicide-resistant weeds is AUD $187 million (Llewellyn *et al.*, 2016). This is in a situation where the use of chemical weed control has become necessary, and therefore it is imperative that we make use of the existing and future herbicides in a sustainable and economical manner. In this context, it is timely to develop and implement new herbicide-resistant weed control approaches to make them more sustainable in future cropping systems.

Although some populations of *R. raphanistrum* are identified as resistant to ALS-inhibiting and PS II-inhibiting herbicides, it is beneficial to evaluate the effects of the sequential application of these compounds on its management. Therefore, in our project, we focus on the tolerance of faba beans to these herbicides and the possibility of introducing a sequential application combination to control *R. raphanistrum* plants in an ALS-inhibiting Imidazolinone tolerant *PBA Bendoc* faba bean context.

Upon completing this project, a sound background can be set for the control of herbicide-resistant *R. raphanistrum* with possible effective combinations of herbicide control options, and threats posed by this weed on faba bean cultivation. This will also beneficial in understanding the strength and flexibility of HTC in terms of herbicide application. This project will benefit not only the agricultural and weed management authorities but it will also be directly beneficial for local farmers and agronomists. It will also provide new leads for future research options in making weed herbicide control more beneficial and sustainable in the Agricultural Industry generally.

The proposed experimental design implemented to achieve the current project's objectives is described in the conceptual framework of this thesis shown below (Figure 1.9). Chapter Two is a detailed literature review that culminates in the identification of research gaps which will be addressed in later chapters. Chapters Three to Six are experimental chapters exploring the effectiveness of the proposed herbicide combination in a stepwise approach. Chapter Seven concentrates on the seed germination of herbicide-resistant and susceptible *R. raphanistrum* seeds to investigate their differential germination success under different abiotic conditions. A general combined discussion on the major findings of the experimental chapters and the recommendations and future research work for the agricultural industry to mitigate the problems associated with *R. raphanistrum* and to make HTCs more sustainable are provided in Chapter Eight.

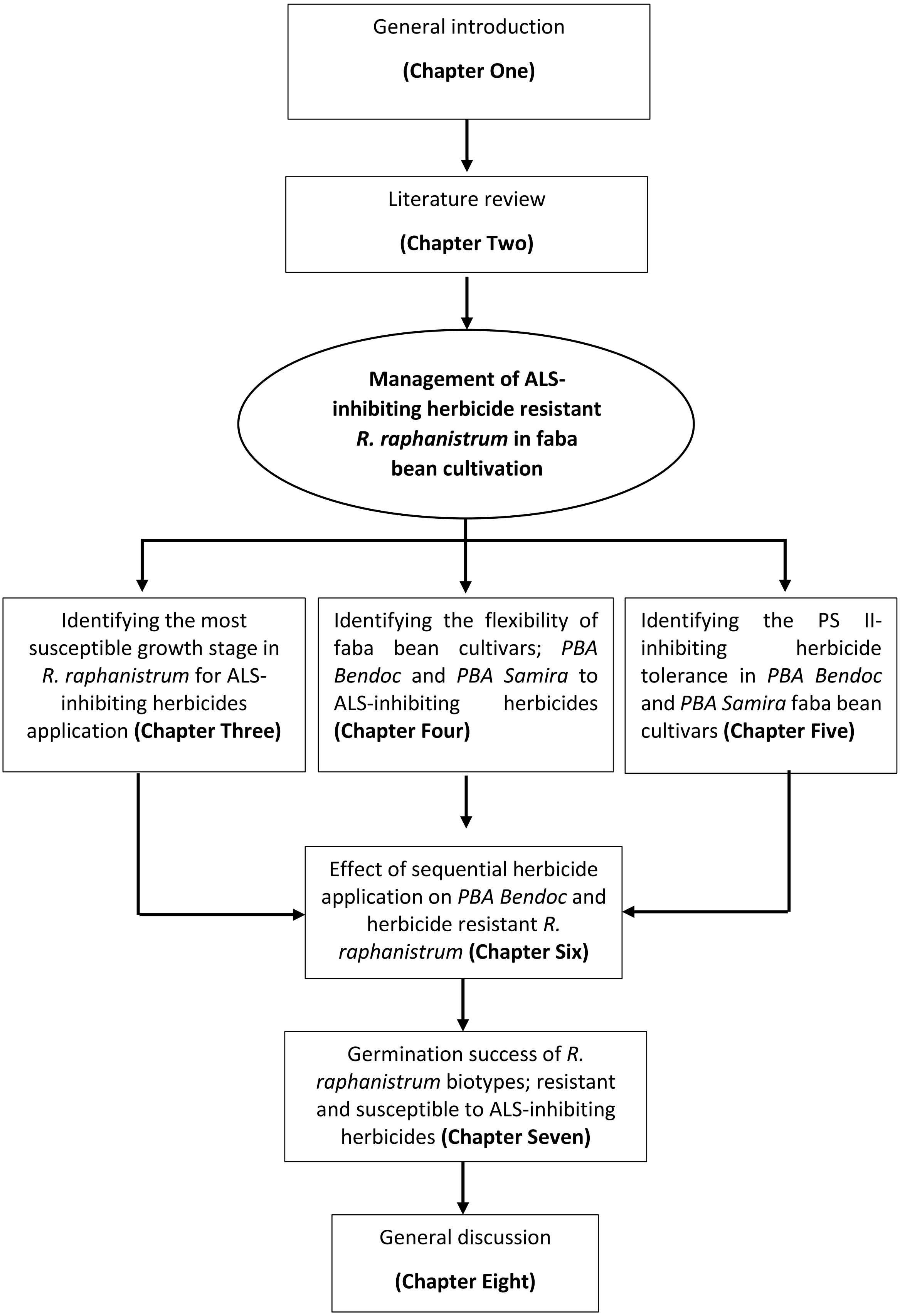


Figure 1.9. Conceptual framework of the thesis

CHAPTER 2 – Literature review

Integrating herbicide mode-of-action combinations and herbicide-tolerant crops to reduce herbicide-resistant weeds' evolution.

2.1 Introduction

Prior to the introduction of herbicides, traditional methods and biological control methods played a key role in weed control strategies used in agriculture (Heap, 2014). The first modern herbicides were synthetic auxins (2,4-D and MCPA) which were introduced to kill broadleaf weeds in cereal crops. These were developed during World War II and were first commercially marketed in 1944 (Heap, 2014). Due to their high reliability and reasonable price, the crop yield of many cropping systems was significantly increased, and this began the success of this revolutionary chemical approach which has continued over the last 65 years. Since they were first introduced, more than 300 herbicide-active ingredients have been brought to the market by agricultural chemical companies (Heap, 2014). Since the late 1960s, herbicides have been the main, and almost exclusive, worldwide weed control strategy (Perotti *et al.*, 2020)

However, the evolution of herbicide resistance (HR) in weeds has become the most threatening and challenging impediment for this successful chemical weed control approach. Though scientists did forecast this scenario soon after the introduction of chemical herbicides (Harper, 1956), the first case of HR weeds was reported in 1957 in a *Daucus carota* (wild carrot) population, which was found to be resistant to 2,4-D (Switzer, 1957; Whitehead & Switzer, 1963). Since then, 498 unique cases of HR weeds have been reported globally to date (Figure 2.1). Of particular concern has been the unavailability of any new herbicide mode of action (MOA) to the market for three decades. This has made farmers cognisant of the upcoming challenges related to the sustainable and integrated use of existing herbicides, since the increasing number of HR weeds can no longer be dealt with by 'single shot' chemical materials (Davis & Frisvold, 2017).

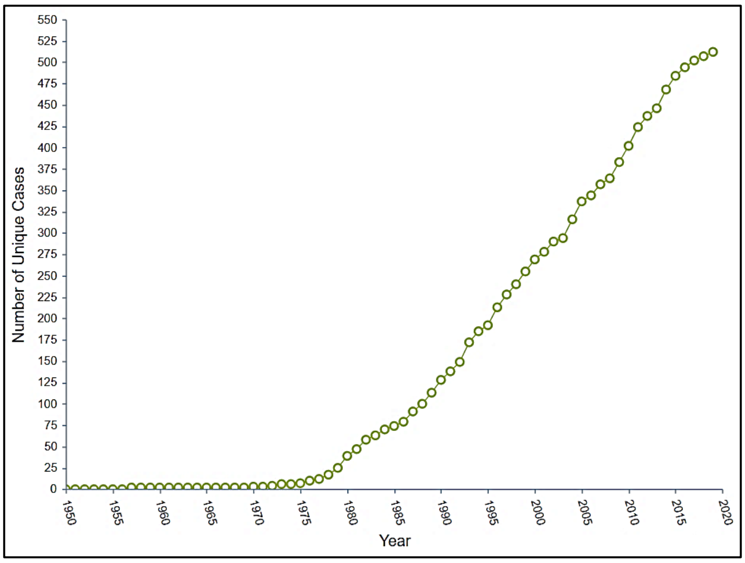


Figure 2.1. Chronological increase of unique herbicide resistant cases globally (Heap, 2019).

With the introduction of HTCs, farmers have been given multiple herbicide options to control weed populations in cropping systems without imposing a crop injury. The low cost of this broad herbicide spectrum, with its reduced crop injury and the ability to attune with no-tillage and reduced-tillage systems, has made the use of HTCs extremely popular in current agricultural practice (Lamichhane *et al.*, 2017). It should be noted, however, that there is a 'safe herbicide spectrum', where the herbicide options that can be used with HTCs are restricted to particular mode of actions (MOAs) for which the HTC has been developed to be resistant. Therefore, the adoption of HTC practices, which require specific materials, may involve unanticipated in-weed population shifts coupled with increasing selection pressure to evolve herbicide resistance in the weeds (Owen & Zelaya, 2005). Studies have also shown that there is controversy regarding whether HTCs increase or decrease the need for herbicide usage (Benbrook, 2001; Champion *et al.*, 2003; Phipps & Park, 2002). However, it appears that with the current promising outcomes in terms of dealing with weed infestations, the broad adoption of HTCs with the continuous use of the recommended herbicides seems quite certain. It is nevertheless of concern that this practice may unavoidably lead to the ultimate result of herbicide resistance in a range of weeds.

Another area of unease is that, given that there are no current indications of an introduction of new herbicide MOAs to the existing herbicide array, coupled with the concerning rapid herbicide-resistant changes occurring in agricultural weeds, it will be essential to consider new weed management tactics and strategies to deter the rate of herbicide-resistant evolution in weeds. As these trends have not been predicted to slow down in the immediate future, identifying and implementing the most suitable strategies is critical to maintaining the sustainability of HTC in the existing herbicide context. One such attempt, which is the integration of HTCs and herbicide MOA combinations, has been discussed in this review as a HR weed control strategy. However, it is worth mentioning that these chemical herbicide strategies will not solve the problem of HR weed evolution in the future but will significantly reduce the herbicide resistance evolution rate, allowing time to introduce new herbicide molecules and other innovative strategies.

2.2 Can herbicides be dispensed with in the future?

The work done by Dentzman (2018) reveals the vision and faith of farmers on future dealings with HR weeds. The study suggests that, currently, farmers prefer relying on the hope of the development of new herbicides rather than adapting alternative methods, which include cultural practices. Despite the fact that no new herbicide MOA has been introduced for the last three decades (Davis & Frisvold, 2017), farmers have expressed their continuing expectations for future herbicides (Bonny, 2016; Moss, 2019; Schroeder *et al.*, 2018). Supporting the concluding remarks of the study of Walsh and Powles (2007), the more recent study of Harries *et al.* (2020) has shown that, despite the evidence of herbicide resistance evolution, the use of herbicides will prevail as the primary weed control method in Australia's largest cropping areas. As this implies that there will be continued use of chemical herbicides in the future, it is essential that we must give focused attention to new herbicide strategies with existing materials to control HR weeds (Perotti *et al.*, 2020).

The negative impact of the overuse and over-reliance on herbicides by agriculturalists has been extensively studied, and concerning aspects such as deprivation of biodiversity (Dupont *et al.*, 2018; Dyer, 2018; Reeg *et al.*, 2017; Singh & Wright, 2002), human health issues (Gunier *et al.*, 2017; Sandström *et al.*, 2017) and abiotic environmental pollution (Kremer, 2017; Marcano *et al.*, 2017) have been noted. These findings are consistent across the current range of practices, particularly in western industrialized agricultural countries, where the transformation of natural ecosystems to agricultural lands has become increasingly prominent. Such a situation will promote the use of chemical herbicides, pesticides and nitrogenous fertilizers in order to maintain economic outputs (Myers, 2001). Indeed, the trend of conservation agriculture in most agricultural systems necessitates reliance on herbicide and pesticide use rather than modes of conventional agriculture as the control of pests had previously been facilitated by tillage (Beckie *et al.*, 2004; Beckie *et al.*, 2008; Hobbs, 2007; Knowler & Bradshaw, 2007).

One good example of this situation is the use of the most common non-selective herbicide glyphosate, which has become increasingly popular among farmers because of its low cost, effectiveness on weeds regardless of grass or broadleaf type, ease of use and relatively low impact on the environment and human health (Bullock & Nitsi, 2001; Hammond *et al.*, 2017; Livingston *et al.*, 2015). It is because of these convenient control aspects that the wide continuous and exclusive use of particular herbicides in cropping systems has influenced the weed population shifts toward the evolution of HR weeds. Against this worrying background, we note that there are significant forces demanding continued herbicide use. Thus, it is a critical factor that the awareness of farmers is raised regarding this issue before they implement herbicide-only treatments. It is imperative that we work to assure the sustainable use of herbicides while, at the same time, minimizing their intensive use.

2.3 Integrated weed management (IWM)

The increasing number of HR weeds has raised the attention of both agricultural authorities and the public toward the implementation of integrated weed management (IWM). This strategy is aimed at reducing the possible side effects of the over-use and over-reliance on herbicides. With this approach, possible preventive measures, cultural practices, biological controls and chemical methods can be implemented in combination, encouraging chemical use in weed management to be kept at a minimum (Dentzman, 2018). Although this approach is now gaining increasing interest across the world (Harker & O'donovan, 2013; Perotti *et al.*, 2020), there are nevertheless some factors making farmers reluctant to adopt IWM practices, despite their long-term benefits (Dentzman, 2018; Lamichhane *et al.*, 2017). These barriers include the difficulty of their introduction to the generally large scale of farming enterprises (Dauer *et al.*, 2009), the opposed influence of agribusiness parties (Bonny, 2016), the lack of information on the latest research findings (Mortensen *et al.*, 2012) and the high cost of labour (Egan, 2014).

It has been claimed that a combination of diverse weed management tactics and different crops can be used to achieve a sound economic and environmentally friendly weed management strategy, fulfilling the ultimate goal of IWM, which is assuring the economic sustainability for growers and society with the least effect on the environment (Lamichhane *et al.*, 2017; Perotti *et* *al.*, 2020). We note that cultural practices such as cover crops and crop rotation are being practiced as a part of IWM, where the cover crops are capable of competing with weeds for resources including water, nutrients, light and space (Lamichhane *et al.*, 2017). These will also provide reduced light penetration and increased soil temperature to suppress weeds by acting as mulches. Allelopathic effects against weeds also have been identified in cover crop residues in cropping systems (Kruidhof *et al.*, 2008). We also note that other tactics including intercropping, tillage, mechanical weeding, biological weed control, and, use of competitive crop genotypes can be implemented in accompaniment with chemical herbicides as a part of IWM, thus reducing the overall reliance on herbicides. However, despite the long-term benefits of non-chemical strategies, farmers are reluctant to use them due to prioritised short-term benefits. Also the non-chemical strategies are mostly weather dependent, inconvenient and time consuming, involve an increased cost of labour, have less predictable control levels, suffer from lack of labour skills and specialised equipment (Ehler, 2006; Hurley & Frisvold, 2016; Llewellyn *et al.*, 2004; Moss, 2010; Riar *et al.*, 2013; Wilson *et al.*, 2009)

2.4 Incorporation of herbicide-tolerant crops in IWM

The introduction of HTCs to the cropping industry has now become a major breeding priority, since, as previously noted, farmers can use a particular range of herbicides without threatening their crop's performance (Owen & Zelaya, 2005). A particular example is the introduction of glyphosate-resistant soybean in the US, where soybean cultivation has now dramatically increased due to this alternative simple weed control approach becoming feasible when previously it was difficult and expensive (Gianessi, 2005). This approach has also resulted in reduced marketing prices of herbicides which are commonly used in soybean fields (Gianessi, 2005), but it is now evident that if farmers do not follow the specific instruction when applying herbicides in their fields, the use of HTC can lead to a shift in weed populations where they evolve with herbicide resistance (Lamichhane *et al.*, 2017; Owen & Zelaya, 2005). One pertinent example is the evolution of herbicide-resistant *Oryza sativa* (weedy rice) populations in Italy only five years after the introduction of an IMI-tolerant rice variety (Busconi *et al.*, 2012). A similar incident has also been reported in Greece (Kaloumenos *et al.*, 2013).

Considering such clearly learnt lessons from past, it would appear sensible that the use of HTC as a component of IWM is necessary, as diverse weed management tactics and crop diversification can reduce herbicide resistance evolution and improve biodiversity (Lamichhane *et al.*, 2017).

Further, if HTC can be introduced along with IWM practices, it will be more advantageous in overcoming the evolution of HR weeds, and will also contribute in terms of overall reduced herbicide applications (Kruidhof *et al.*, 2008). As Lamichhane *et al.* (2017) suggest, to make the maximum outcome of this synergism, improved knowledge of weed biology, aspects of management control, externally reviewed stewardship programs, a clear understanding of growers' attitudes, and the implication of public policies are all critical aspects of advanced IWM practices that need to be examined.

When introducing this style of weed control approach, assuring that farmers have a thorough knowledge of HTC stewardship guidelines is mandatory (Moss, 2019). Such mandatory training sessions will provide growers with appropriate recommendations on HTC rotations and herbicide user practices, which they otherwise may have neglected to read on relevant labels and other written material (Lamichhane *et al.*, 2017). Regular monitoring and keeping a good control of management strategies will also help to make the use of HTCs more sustainable (Werth *et al.*, 2008). As an example of this approach, in order to assure the sustainability of Australian glyphosate-tolerant cotton varieties, a detailed and practical framework has been built on the basis of a crop management plan and a grower accreditation course (Werth *et al.*, 2008). In addition to the strict stewardship plans, regular audits, certification processes, sales restrictions and fines for non-compliant growers have been instituted (Lamichhane *et al.*, 2017). Taking all these aspects into account, including HTC as a part of the IWM will ensure reduced pressure on HR weed evolution and also will contribute to the sustainability of the HTC procedures.

2.5 Herbicide MOA combinations

With the understanding of farmers' strong faith in herbicides and their preference for chemical weed control over the non-chemical approaches, it is timely to investigate the nature of possible herbicide application strategies which involve less selection pressure being imposed on herbicide resistance evolution (Harries *et al.*, 2020). It is widely recognised that, with time, repeated use of the same herbicide will cause or catalyse the associated weeds to develop resistance to that particular herbicide group (Knezevic *et al.*, 2009). In this respect, the use of the same herbicide MOA has been listed as the number one risk factor for the evolution of HR weeds (Beckie, 2006). As indicated earlier, as multiple herbicide resistance in weeds becomes a major concern due to over-reliance on available herbicides and there is no rapid introduction of new herbicide MOAs, it is clear that intensive actions must be conducted to make extended use of existing compounds (Powles & Gaines, 2016).

Herbicide tactics to reduce the unintended imposed selection pressure on weeds include herbicide sequences, mixtures, and rotations (Beckie, 2006; Beckie & Harker, 2017). The use of herbicide MOA combinations in one growing season can be done either as a tank mix or as a sequential application (Lanclos *et al.*, 2002) while the herbicide MOA rotations will be between or among the growing seasons (Beckie, 2006; Evans *et al.*, 2016). The use of multiple herbicide MOAs has been recommended and accepted by both herbicide marketing authorities and by farmers' representatives (Owen, 2016), and has now also been recommended by the Weed Science Society of America (Norsworthy *et al.*, 2012). Less literature is available to compare the effectiveness of these methods even though all these techniques are being practiced in delaying herbicide-resistant acquisition by weeds. The success of these approaches has meant that combined herbicide applications have become a strong trend in agriculture as it can lead to an effective alternative weed management strategy while reducing the total use of herbicides and making the approach accessible and more economical for the farmers (Kiran *et al.*, 2010).

Tank mixes are preferred by farmers since it requires only one application for the season, but in terms of herbicide cost and amount, this approach is regarded to be expensive (Evans *et al.*, 2016). Although it is suggested that the herbicide rates can be reduced when applied as tank mixes when compared to using the individual herbicide (Beckie, 2006), the risk to non-target sites and cross-resistance evolution can be increased by low-dosed tank mixes (Gressel, 1995; Lagator *et al.*, 2013). To increase the efficacy of these herbicide mixtures, it is suggested that the herbicide mix should match properties including efficacy, soil residual activity, and the tendency for developed resistance (Beckie & Harker, 2017). It is unlikely that there will be an opportunity for the weeds to evolve simultaneously with multiple resistance to those individual herbicides. Indeed, studies have indicated that delays in herbicide resistance have been achieved more successfully with the use of mixtures than when applied in rotation in situations where the mixed herbicides are equally effective (Beckie & Reboud, 2009; Evans *et al.*, 2016; Powles *et al.*, 1996). This clearly implies that identifying the ideal mix for a particular weed is a key challenge in making a tank mix (Evans *et al.*, 2016). In this respect, the efficacy of particular herbicide tank mixes can be either superior (synergistic) or inferior (antagonistic) compared to the efficacy of a sole application of single herbicide (Baltazar & Smith Jr, 1994; Fish *et al.*, 2015; Fish *et al.*, 2016; Gonzini *et al.*, 1999; Lanclos *et al.*, 2002; Minton *et al.*, 1989). According to the survey of Harries *et al.* (2020), the use of herbicide mixes is now as common as the use of single herbicides in south-west Western Australia. This widespread adaptation of using herbicide mixes by the growers is suggested to be in line with recent recommendations made for herbicide resistance management (Busi & Beckie, 2019; Busi *et al.*, 2020; Evans *et al.*, 2016; Powles & Gaines, 2016).

Depending on the morphology, physiology or the crop growth stage at the time of weed emergence, a single herbicide application may not be adequate for controlling different kinds of weed species (Nath *et al.*, 2018; Tuti *et al.*, 2015). Addressing this concept, the sequential application of herbicide MOA has been reported to be successful in controlling glyphosate-resistant *Ambrosia artemisiifolia* (common ragweed) in Nebraska (Byker *et al.*, 2018). The study by Borger and Hashem (2007) has also shown that a sequential application of two herbicides with different MOA is more effective than using a single herbicide in controlling *Lolium rigidum* (annual ryegrass). However, the study of Gonzini *et al.* (1999) regarding the use of glyphosate as a late post-emergence application following ALS inhibiting herbicides has not shown to improve the control of *Chenopodium album* (common lambsquarters) compared to the tank mix. This suggests that there is an effect related to the plants being larger in size when applying the late post-emergence glyphosate. In contrast, however, *Abutilon theophrasti* (greater velvet leaf) control has improved with the use of glyphosate as a sequential application rather than with a tank mix of ALS-inhibiting herbicides. Therefore, application time and weed growth stage appear to be critical factors to achieve successful weed control when applying the herbicides either as a tank mix or sequentially. In Western Australia, for pre-planting weed control and to delay the onset of herbicide resistance, herbicide MOA rotation has commonly been practiced with glyphosate in conjunction with another non-selective herbicide (Borger & Hashem, 2007). The model produced by Neve *et al.* (2003) has suggested that resistance evolution in *Lolium rigidum* can be delayed by at least 22 years with the rotational application of glyphosate and paraquat. However, it has also suggested that when herbicide MOA rotation is carried out with in-crop selective herbicides on large-scale finite weed populations, there is a probability of acquiring herbicide resistance to both MOAs in a similar time period as when they are applied alone (Diggle *et al.*, 2003). Also, the superior effectiveness of herbicide MOA mixes compared to the rotations have been evident in field-based research, farmer survey questionnaires, and in modelling simulations (Beckie & Reboud, 2009; Diggle *et al.*, 2003; Neve, 2008). Nevertheless, with the sequential application, where both the herbicide MOAs are applied in the same season, it has been shown that herbicide resistant evolution can be delayed up to 30-50 years (Neve *et al.*, 2003). In the study of Diggle *et al.* (2003), it was pointed out that high cost and the ecological implications appeared as the downside of herbicide combination tank mix use. However, unlike in a tank mix, the herbicide usage can be reduced with the sequential application method as it allows a visual estimation of survived weeds which are to be controlled with the second knock. Therefore, it would seem that with the use of the sequential application, the exposure of chemicals to the target weed population and to the environment can be minimized. But as two sprays must be done in one season, the labour and equipment hiring cost is escalated compared to tank mix application.

2.6 Application implications of herbicide MOA combinations in HTC systems

The use of herbicide combinations still carries the risk of crop injury despite being one of the top rating approaches in weed control (Gianessi, 2005). This has made the introduction of herbicide combinations into an HTC environment challenging as it is essential to make sure that all the herbicide MOAs used in the combination are tolerated by the crop. Therefore, the tolerance of the HTC is thus a critical factor to be considered when striving to achieve the maximum efficacy of the herbicide combination as well as the optimum HTC production.

On the other hand, the proactive management of weeds to delay the onset of herbicide resistance has become a very pressing issue. Notwithstanding this urgency, because the cost of adopting the practices to delay herbicide resistance and managing herbicide-resistant weeds are estimated to be nearly the same, farmers are hesitant to change or institute new weed management practices until the weed resistance is evident (Beckie, 2006). This user conflict can also be seen to be a result of their significant concerns on expected short-term financial return, or, in addition, their failure to assess the long-term risks associated with herbicide-resistant weeds (Rotteveel *et al.*, 1997). In this respect, IWM practices are also known to be adopted by farmers only when herbicide resistance is evident in the cropping systems (Beckie, 2006). When cross-resistance and multiple resistance are identified in the cropping system, the number of IWM components is expected to increase (Powles, 1997; Powles *et al.*, 2000). As discussed above, the use of HTC has become a priority in most of the cropping systems around the world. Thus, integrating these herbicide tactics to reduce the selection pressure on weeds to evolve herbicide resistance has become essential.

It has been observed that farmers usually consider the performance and the cost of the herbicide rather than the MOA when selecting herbicides for weed control (Beckie, 2006). Lack of suitable herbicide options to match crop requirements has become a significant obstacle to the practice of herbicide group rotation (Légère *et al.*, 2000). This problem has been partially solved with the introduction of HTC as an improved weed control strategy, which, with time, may alter future herbicide use patterns (Beckie, 2006). This approach permits more herbicide rotation options, allowing lower-risk herbicides to be substituted in place of high-risk herbicides. In that respect, HTC can have an influence in reducing the selection for HR weeds. However, the long-term use of the same herbicide MOA as demanded by HTC will initiate the emergence of new HR weed biotypes (Beckie, 2006). Due to a low frequency of interspecific hybridization and introgressive hybridization, the evolved herbicide resistance in weeds related to the management of HTC systems is considered to be more problematic than herbicide resistance evolved as a result of gene flow (Beckie, 2006; Warwick *et al.*, 1999).

Herbicide application time is another critical factor when introducing an herbicide MOA combination into a HTC system. However, herbicide sequence practices, including double knock-down have been observed to be less affected by the application time compared to a single herbicide application (Borger & Hashem, 2007). Besides, application timing and plant age have shown to be somewhat flexible when a double knockdown herbicide technique is used to control *Lolium rigidum* (Borger & Hashem, 2007). In a sequential application, as the latter herbicide is applied to the surviving weed populations from the first herbicide treatment, the visual estimation of the severity of the weed problem will give an idea on the ideal rate of the latter herbicide application, which will clearly help in reducing the chemical herbicide usage. Although the practice of reduced herbicide rate application has an innate conflict of benefits, it is still widely considered to be effective in controlling weeds, with the advantage of increasing effectiveness ratio of the herbicide and also in increasing the crop sustainability (Holm *et al.*, 2000; Kirkland *et al.*, 2000). These benefits can be related to abiotic factors such as light, relative humidity and temperature prevailing at the time of application, and also the spray volume, droplet size, additives, weed species and weed size (Hall *et al.*, 2000; Holm *et al.*, 2000). Optimal conditions at the time of herbicide application can favour the success of low herbicide rates, making them more effective than the recommended rate applications when the conditions are not optimal.

An increase in growers' satisfaction in weed control has been evident in the area of glyphosate-tolerant corn cultivation where pre-emergent herbicides are applied in a combination or a sequence with glyphosate (Dill *et al.*, 2008). It has been suggested that extension of such weed control programs with cotton and soybeans may be likely to draw increased attention to herbicide MOA combinations to control HR weeds in HTC systems (Dill *et al.*, 2008). For glyphosate-tolerant corn, it is recommended to use a pre-emergent herbicide such as acetochlor or acetochlor plus atrazine together with post-emergent glyphosate (Shaner, 2000). However, while mixtures of these herbicides are expected to delay the selection pressure on weeds, it has been suggested that they may have restricted activity in regard to controlling some weeds such as *Abutilon theophrasti* (L) Medic and *Ipomoea* spp. (Shaner, 2000). These implications are expected to overcome with a better understanding of the herbicide application strategies and the associated HTC in the cropping field.

2.7 Conclusion

Herbicide MOA combinations not only make the weed control more efficient but also reduce the unwanted weed population shifts towards acquiring herbicide resistance. To select the most efficient MOA combination method either as tank mixtures, sequential applications or herbicide rotations in cropping systems, the positive and negative aspects of each approach should be weighed prior to making decisions. Given the benefits of herbicide combinations discussed above, it appears clear that the identification of a particular herbicide MOA combination suitable for a specific herbicide-tolerant crop is critical. Therefore, to make full use of HTC and reduce the demand for the same herbicide MOA, evaluating the crop tolerance to alternative herbicide MOAs will also be advantageous. This identified research gap of HTC tolerance to herbicide MOA combinations and its effectiveness on target weeds will be investigated as a case study in this thesis. Because few studies have been conducted to evaluate the superiority or the inferiority of either of these methods, future research work is recommended to assess the advantages of these strategies to ensure existing herbicide MOAs remain sustainable in the future.

CHAPTER 3 - The effect of *Raphanus raphanistrum* growth stage on the efficacy of ALS-inhibiting herbicides

3.1 Introduction

With the introduction of auxin-analog herbicides in the 1940s, the control of *Raphanus raphanistrum* in cropping systems has mainly relied on herbicide application, which has eventually led to its numerous known herbicide-resistant populations (Walsh, 2004). Acetolactate synthase (ALS)-inhibitors are one of the most commonly used herbicide groups in controlling *R. raphanistrum*. Hence the resistance to ALS-inhibiting herbicides is both widespread and concerning (Hashem *et al.*, 2001a; Yu *et al.*, 2003). To minimize the selection pressure on weeds to evolve resistance, it is mandatory to have a good understanding and a knowledge of herbicide treatments used in cropping systems (Beckie & Harker, 2017; Evans *et al.*, 2016). Knowledge of the factors affecting herbicide efficacy on weeds and their influence on weed control is vital in decision-making for weed management programs (Yamaji *et al.*, 2016). The effectiveness of a herbicide in a plant may vary depending on the environmental factors such as temperature, light, and humidity, together with herbicide properties, plant growth and physiology (Kleinman *et al.*, 2016). Therefore, as the first step in identifying a sequential herbicide MOA combination to control herbicide-resistant *R. raphanistrum*, this chapter will focus on investigating the most effective ALS-inhibiting herbicide and the best weed growth stage for its application.

Previous studies have shown that soil and foliar-applied herbicide toxicity is mainly dependent on soil type, ambient humidity, temperature, irradiance and the weed growth stage at herbicide application (Buhler & Burnside, 1983; Hammerton, 1967; Kleinman *et al.*, 2016; McWhorter *et al.*, 1980; Walker *et al.*, 2012). Among those factors, the weed growth stage is critical in determining the efficacy of herbicide on controlling weeds, as the herbicide uptake and metabolism processes are directly dependent on a plant's growth stage (Metzger *et al.*, 2019; Samunder & Singh, 2004; Soltani *et al.*, 2016). In general, the highest herbicide efficacy is observed when applied at early growth stages as the increased metabolism and hence the fast herbicide degradation in grown plants leads to reduced herbicide efficacies in larger plants (Chauhan & Abugho, 2012; Samunder & Singh, 2004). It has been suggested that, the rate of herbicide use could be reduced up to 75% when applied at the early growth stages of the weed (Defelice *et al.*, 1989; Devlin *et al.*, 1991; Hamill & Zhang, 1997).

The reduced sensitivity of different herbicides with advanced plant growth has been evident in many studies (Barros *et al.*, 2007; Faccini & Puricelli, 2007; Javaid, 2007; Kieloch & Domaradzki, 2011; Kleinman *et al.*, 2016; Soltani *et al.*, 2016). Some of the examples include *Eupatorium capillifolium* (dog fennel) for synthetic auxin inhibitors*, Amaranthus retroflexus* (redroot pigweed)*, Ambrosia artemisiifolia* (annual ragweed)*,* and *Chenopodium album* (lamb's quarters) for glyphosate (Johnson & Norsworthy, 2014; Sellers *et al.*, 2009; Soltani *et al.*, 2016), and *Chenepodium album* and *Anthemis arvensis* (corn chamomile) for tribenuron methyl, iodosulfuron methylsodium + amidosulfuron and metribuzin + amidosulfuron (Kieloch & Domaradzki, 2011). But some plants, such as *Stellaria media* (chickweed) have shown a reduced effect of the growth stage on tribenuron methyl herbicide sensitivity (Kieloch & Domaradzki, 2011). This has also been supported by the study of Metzger *et al.* (2019), where the control of *Ambrosia artemisiifolia*, *Chenopodium album*, *Abutilon theophrasti* (velvetleaf) and *Amaranthus powellii* (powell's amaranth) has not been affected with the post-application timing of tolpyralate + atrazine. According to these studies, the effect of the weed growth stage at the time of herbicide application may vary depending on the herbicide MOA even within the same species. Therefore, not only the weed species and growth stage, but also the herbicide MOA has a great influence on weed control (Kieloch & Domaradzki, 2011).

The most significant differences among the herbicide responses in plants are known to be allied with the herbicide properties, including its relative retention, absorption, and translocation (Hammerton, 1967). Also, the interaction between the plant and the herbicide is both directly and indirectly influenced by the environmental factors which govern the plant growth and its physiology (Caseley & Coupland, 1985; Kleinman *et al.*, 2016; Varanasi *et al.*, 2016; Yamaji *et al.*, 2016). Contact herbicides that are weak in translocation within the plant require a significant coverage of herbicides during the application. Therefore, it is not surprising that the relationship between the plant size and the growth stage is inversely proportional to the herbicide efficacy (Coetzer *et al.*, 2002). This is mainly because insufficient foliar contact of the herbicide cannot control the uncovered plant parts. It is not only with contact herbicides, but also with the systemic herbicides including ALS-inhibitors, that have shown similar interaction between plant growth stage, herbicide coverage and herbicide efficacy (Johnson & Norsworthy, 2014). ALS-inhibiting Imidazolinones are capable of penetrating the leaf cuticle quickly, and once having penetrated the plant, are readily translocated via the xylem and phloem due to its intermediate lipophilicity and moderate to high solubility (Congreve & Cameron, 2018). With this notion, determining the most susceptible growth stage to these herbicides is a critical factor to establish, and this will make their use more economical and sustainable in the future (Klingaman *et al.*, 1992). Therefore, this chapter will evaluate the relationship between the *R. raphanistrum* growth stage and the ALS-inhibiting herbicide application time, using two *R. raphanistrum* biotypes: resistant and susceptible to identify the best herbicide treatment for its control.

3.2 Methodology

3.2.1 Seed collection

Raphanus raphanistrum seeds of two biotypes; ALS-inhibiting herbicide-resistant and herbicide susceptible, collected from South Australia were purchased from Plant Science Consulting P/L, South Australia. They were stored in labeled, dark, air-tight glass bottles at room temperature in the seed ecology laboratory at Federation University, Australia, until used. The resistant seeds were confirmed for their resistance to triasulfuron, imazamox + imazapyr, and 2,4D showing a survival percentage of 80, 50, and 30% respectively at 28 days after application (DAA) when sprayed at the 3-4 leaf stage.

3.2.2 Experimental design

The experiment was conducted using a completely randomised design in a temperature-controlled glasshouse at the Mount Helen Campus of Federation University Australia in May to December 2018. The day/ night temperature in the glasshouse was maintained at 22/18 ºC, and the humidity was maintained at 60-70%.

Plastic pots (19 cm height and 18 cm diameter) were filled with 3kg of soil from Horsham lentil fields which had no history of herbicide use for more than two years. Soils were silty clay loam and the composition was determined using the laser particle size analysis method, and showed pH 6.9 (H_2O), 6% sand, 69% silt, and 25% clay (Hunt & Gilkes, 1992). Five seeds of each *R. raphanistrum* biotype, resistant and susceptible, were sown at a depth of 1 cm and were thinned down to two plants per pot, once the seedlings were established. Plants were sprayed with two ALS-inhibiting herbicides (imazamox + imazapyr and imazethapyr) at a constant herbicide rate ; imazamox + imazapyr 24.75 g.a.i/ha (g/ha, hereafter) + 11.25 g/ha and imazethapyr 70 g/ha at the 2-4 leaf stage (2-4L), the 6 leaf stage (6L), the 8 leaf stage (8L) and the flowering stage using a trolley sprayer. The spray pressure was set at 200kPa, and mini drift air-inclusion nozzles were used with a spray angle of 110ºC, maintaining a height of 50 cm above the pot rim when spraying.

Controls were treated in an identical fashion to the experimental plants, excepting for the herbicide treatment. Plants were watered daily to eliminate any water stress. The seedling survival was determined 28 days after herbicide application (DAA) to express percentage survival with the survival criterion being at least one new green leaf emerging in the plant post the herbicide spray. Percentage visual herbicide damage was recorded at 28 DAA with a criterion of 0% resembling no herbicide damage and 100% for non-survived plants. At the plant's maturity, the plant height and the number of pods/plant were recorded. Finally, all the plants were harvested, and the aboveground plant parts were put in paper bags and dried to a constant weight in an oven at 70 ºC for 72 hours to discern the aboveground dry weight.

3.3 Statistical Analyses

The experiment was conducted with five pots (five replicates) with two plants in each totalling 10 plants for each treatment (n=10). All data were analysed with the IBM SPSS statistics 25.Ink statistical software release 18. Data were analysed using the general linear model (GLM). According to the Shapiro-Wilks test, non-normal residues were treated with common transformations, but the normality or equal variance assumptions were not greatly improved. Proving the robustness of raw data, the ANOVA outputs obtained from transformed and untransformed data were seen to be similar. Because ANOVA tests are reasonably robust to the slight departures from normality and equal variance (Hahns-Vaughn, 2017), all the analyses were conducted on the original data. For all pairwise comparisons, Tukey's Honesty test was conducted to determine the incidence of significant differences based on the mean responses to different herbicide treatments at a 0.05 confidence level.

3.4 Results

The results obtained for percentage survival, percentage visual herbicide damage, plant height, number of pods per plant, and aboveground plant dry weight are shown in figure 3.1.

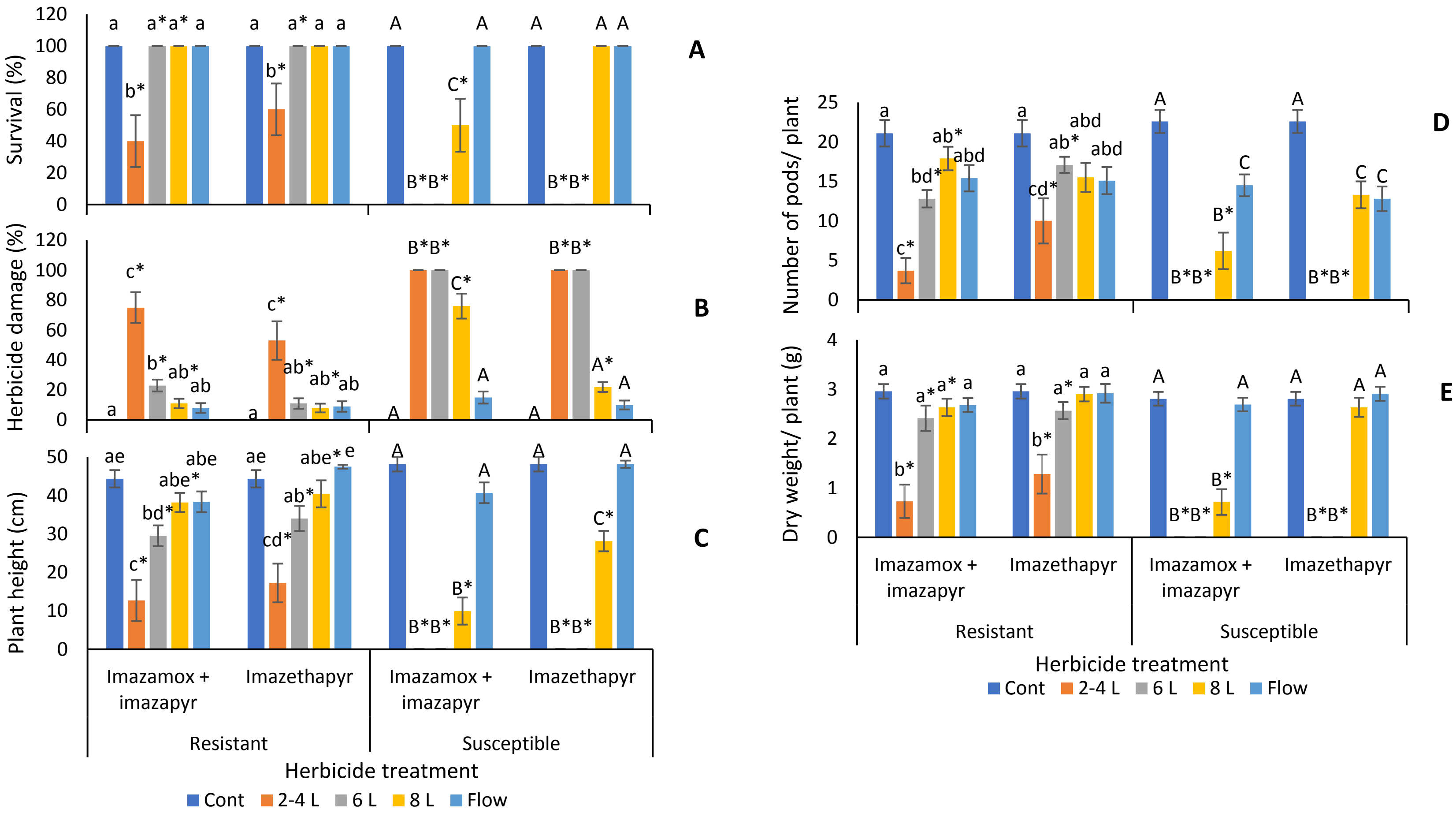


Figure 3.1. A. Survival (%) **B.** Herbicide damage (%) at 28 days after application **C.** Plant height **D.** Number of pods/plant **E.** Aboveground plant dry weight at harvest for herbicides Imazamox + imazapyr and imazethapyr applied at 2-4L, 6L, 8L, and flowering growth stages to herbicide-resistant and susceptible *R. raphanistrum* biotypes. The lines at the top of the bars represent the +/- standard errors. Bars with different letters of the same case indicate significant differences across treatments within the biotype and the asterisks (*) denote the treatments with significant differences between the biotypes.

3.4.1 Percentage survival

Percentage survival of *R. raphanistrum* plants at 28 DAA showed a significant interaction effect of herbicide treatment by biotype ($p<0.05$). In treatments with imazamox + imazapyr, the interaction effect was found to be significant in all the treatments other than the flowering stage. In contrast, the imazethapyr treatments showed a significant interaction effect only in 2-4 leaf and 6 leaf stage treatments (Figure 3.1.A). In the resistant biotype, the treatment effect was significantly different from control only in the treatments applied at 2-4 leaf stage, but in the susceptible biotype, the treatment effect was significant in all the treatments other than the imazamox + imazapyr flowering, imazethapyr 8 leaf, and flowering compared to the control. Regardless of the biotype and the herbicide, flowering stage treatments resulted in 100% survival. The 2-4 leaf and 6 leaf stage treatments of susceptible plants, irrespective of the herbicide, showed a 0% survival at 28 DAA. In almost all the treatments, the resistant biotype showed a higher percentage survival compared to the susceptible biotype, and the percentage survival was higher in imazethapyr treatments regardless of the biotype. Overall, the delayed herbicide treatments at later growth stages showed increased number of plant survival.

3.4.2 Percentage visual herbicide damage

The interaction effect of herbicide treatment by biotype was significant in percentage visual herbicide damage at 28 DAA ($p<0.05$). The interaction was significant in almost all the treatments other than the two flowering treatments and control (Figure 3.1.B). In the resistant biotype, the herbicide damage was significantly different from controls only in imazamox + imazapyr 2-4 leaf and 6 leaf, and imazethapyr 2-4 leaf treatment. In contrast, all the treatments other than imazamox + imazapyr flowering, imazethapyr 8 leaf and flowering, the susceptible biotype showed a significant difference from controls. Increased herbicide damage at early growth stage treatments was prominent in both biotypes, and the imazamox + imazethapyr treatments showed increased herbicide damage compared to the treatments of imazethapyr (Figures 3.2 and 3.3).

3.4.3 Plant height

A significant interaction effect of herbicide treatment by biotype was found in the parameter of plant height at maturity ($p<0.05$). The interaction effect was evident to be significant in all most all the growth stage treatments except the two flowering stage treatments ($p>0.05$). Stunted plant growth was clearly observed in early growth stage treatments but not when applied at flowering stage (Figure 3.1.C). In the resistant biotype, imazamox + imazapyr 2-4 leaf and 6 leaf stage treatments showed significant stunting compared to the controls but only at 2-4 leaf treatment with imazethapyr. In contrast, this significance was evident in all most all the growth stage treatments other than the two flowering stage treatments in susceptible biotype.

3.4.4 Number of pods

The number of pods per plant was significantly affected by the interaction effect of herbicide treatment by biotype (p<0.05). The interaction effect was significant in all the treatments other than the two flowering stage treatments and the imazethapyr 8 leaf treatment (p>0.05) (Figure 3.1.D). In the resistant biotype, the treatments of imazamox + imazapyr 2-4 leaf and 6 leaf, and imazethapyr 2-4 leaf showed a significant difference in the number of pods per plant from the control. In contrast, in the susceptible biotype, all the treatments of imazamox + imazapyr and imazethapyr were significantly different from the number of pods in the control. Overall, the number of pods did not show a specific trend along with the increasing growth stage of the herbicide treatment.

3.4.5 Aboveground plant dry weight

The interaction effect of herbicide treatment by biotype was significant in the aboveground plant dry weight (p<0.05). Among the treatments involved in imazamox + imazapyr, a significant interaction effect was not evident only in the flowering stage treatment (Figure 3.1.E). In the treatments with imazethapyr, the 8 leaf, and flowering stage were both found to be non-significant. Within the resistant biotype, the plant dry weight was significantly different only in the two treatments of the 2-4 leaf stage compared to the control. In contrast, other than the treatments of imazamox + imazapyr flowering, imazethapyr 8 leaf and flowering treatments, all the other treatments in the susceptible biotype were significantly different from the control.

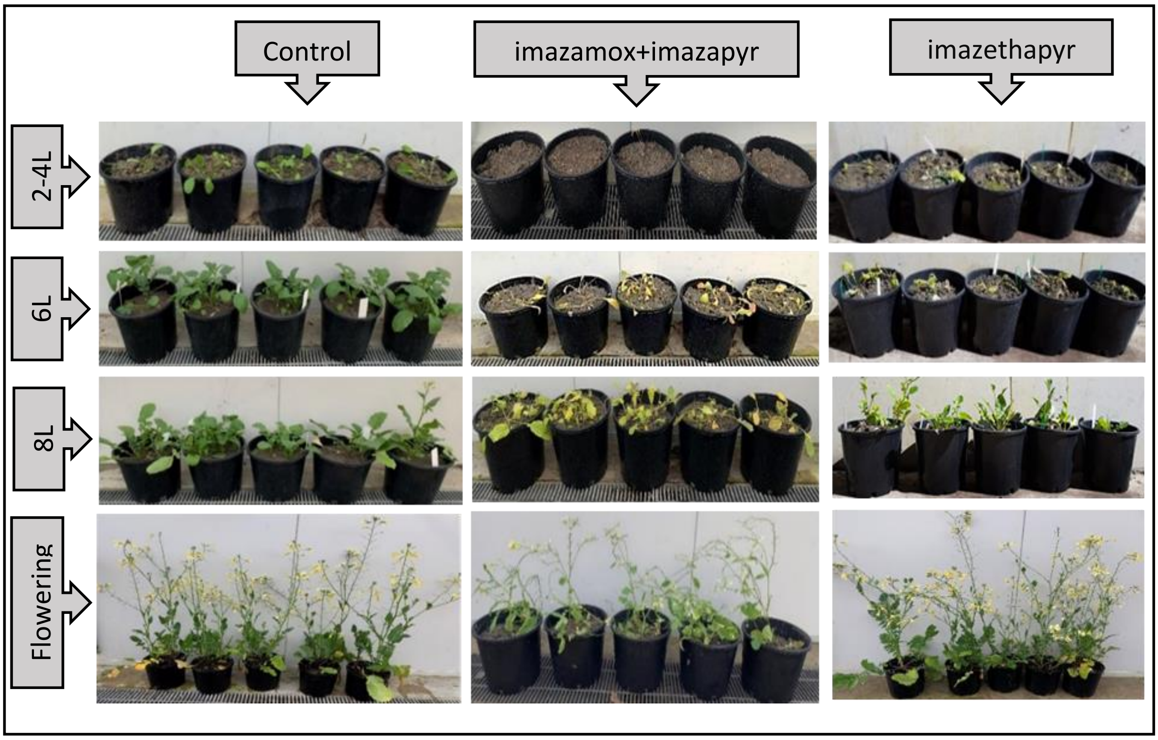


Figure 3.2. Visual herbicide damage in the susceptible *R. raphanistrum* biotype for the herbicides Imazamox + imazapyr and imazethapyr applied at 2-4L, 6L, 8L and flowering growth stages

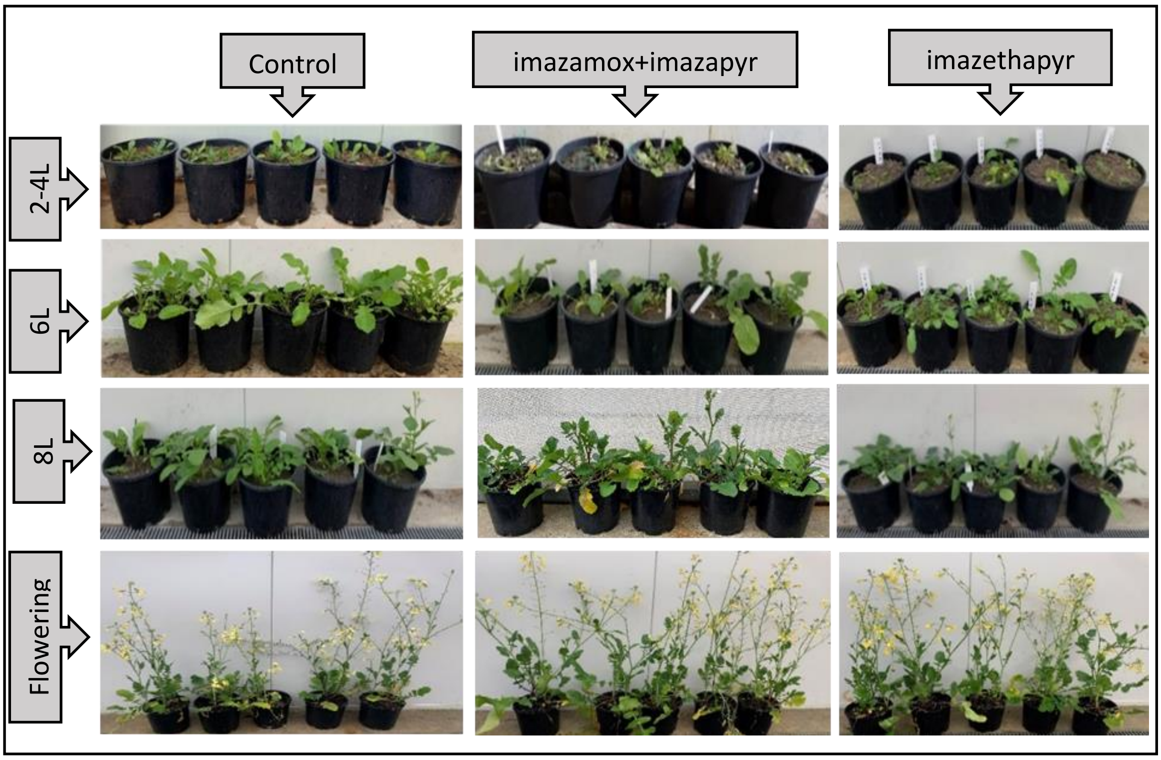


Figure 3.3. Visual herbicide damage in the resistant *R. raphanistrum* biotype for the herbicides Imazamox + imazapyr and imazethapyr applied at 2-4L, 6L, 8L, and flowering growth stages.

3.5 Discussion

According to the results of this study, the two biotypes showed a significant difference in their responses towards the applied herbicide treatments. The resistant biotype with the pre-confirmed known 50% survival for imazamox + imazapyr 2-4 leaf treatment resulted in a reduced percentage survival of 40% in the current study. This observation of difference may be a result of altered growing conditions in the two trials (Cheng & Ni, 2013) where the plants were transferred back to the glasshouse after the herbicide application in the current study, but the plants were left under natural conditions in the pre-confirming study at Plant Science Consulting P/L, South Australia. The resistant biotype showed a percentage survival >20% at the 2-4 leaf stage to both of these ALS-inhibiting imidazolinone herbicides that we applied, suggesting resistance in *R. raphanistrum* to both herbicides. The increased percentage survival in the treatment of imazethapyr 2-4 leaf stage (60%) compared to that of imazamox + imazapyr (40%) proved the increased sensitivity in resistant *R. raphanistrum* plants to imazamox + imazapyr. This was evident in all the evaluated parameters and was prominent, especially in the susceptible biotype when treated at early growth stages.

Supporting the fact of effective weed control at the early growth stages of the weed (Barros *et al.*, 2007; Chauhan & Abugho, 2012; Dennis *et al.*, 2016; Faccini & Puricelli, 2007), *R. raphanistrum* management proved to be effective in the treatments applied at the 2-4 leaf stage compared to later-stage treatments in the current study. In the resistant biotype, plant mortality was observed only in the earliest growth stage, (the 2-4 leaf stage). The susceptible biotype recorded 0% survival even at the 6 leaf stage regardless of the herbicide. Stunted plant growth and dry weight reduction was evident in early growth stage treatments but was not prominent in 8 leaf and flowering treatments in the resistant biotype. The reduced efficacy of the herbicides with increasing plant size can be associated with the increased herbicide metabolism in large plants (Chauhan & Abugho, 2012; Singh & Singh, 2004), reduced herbicide spray coverage, or physiological changes associated with plant maturity and bolting (VanGessel *et al.*, 2009). Despite being resistant to both herbicides, the resistant biotype showed an increased sensitivity to imazamox + imazapyr compared to imazethapyr, suggesting its potential to be included in a herbicide combination as a sequential component. Overall, the treatments accompanying early growth stages proved to be effective in controlling *R. raphanistrum* compared to the later growth stages such as the 8 leaf and flowering stage.

Imazamox + imazapyr and imazethapyr belong to the Imidazolinone family of ALS-inhibiting herbicides, which lethally decrease the protein synthesis in plants by inhibiting the action of the ALS enzyme (Tranel & Wright, 2002). Imazapyr and imazamox, are mainly absorbed from the foliage and then translocate through the plant phloem and xylem (Shaner & Mallipudi, 1991). These rapidly taken up chemicals start acting on plant inhibition within 24 hours after application, whereas visual plant damage is known to appear only one week after the application (Vencill, 2002). Imazethapyr also acts similarly, absorbing through leaves and roots with a rapid translocation to the meristem via xylem and phloem (Plaza *et al.*, 2006). In our study, the different results of these two herbicides on *R. raphanistrum* can be due to the nature and number of active ingredients and their efficacy on the biotypes tested. Imazamox + imazapyr consists of two active ingredients, whereas imazethapyr has only one active ingredient in its formulation. Imazethapyr is known to be more efficient in controlling weeds when applied as soon as possible after the weeds emerge, that being typically around 10 days after the emergence (Grey *et al.*, 1995; Wilcut *et al.*, 1991). The differences among the metabolism of imidazolinone herbicides can also be another reason for the differential behaviour of the two *R. raphanistrum* biotypes upon the two herbicide treatments (Kuk *et al.*, 2008; Shaner & Mallipudi, 1991).

Along with the recorded increased number of herbicide-resistant *R. raphanistrum* populations, its cross-resistance to ALS-inhibiting herbicides has also been documented widely, including Australia, Brazil and South Africa (Costa & Rizzardi, 2014; Hashem *et al.*, 2001a; Pandolfo *et al.*, 2016; Smit & Cairns, 2001; Yu *et al.*, 2012). Supporting the finding of resistant biotype cross-resistance to both imidazolinone herbicides in the current study, the study of Manley *et al.* (1998) has noted that the ALS-inhibiting resistant biotypes are often cross-resistant to the other herbicides within the family but vary in cross-resistance to the herbicides from other families within the MOA. Also, the resistance to one herbicide compound in a chemical group of ALS-inhibiting herbicide does not necessarily prove its resistance to other compounds of the same chemical group (Tranel & Wright, 2002). The study of Pandolfo *et al.* (2016) has shown the broad ALS-inhibiting herbicide resistance present in *Raphanus sativus* (Feral radish), in Argentina. In their study, highlighting the intensity of the cross-resistance in *R. sativus*, some populations have shown the resistance to all 10 tested ALS-inhibiting active ingredients across five families of the herbicide group. Supporting the cross-resistance in weeds within the ALS-inhibiting imidazolinone family, in the study of Kuk *et al.* (2008), *Oryza sativa* (red rice) has shown an increased resistance of at least 6-fold to imazapyr in imazethapyr resistant accessions. The study has also shown that the mechanism of resistance could be the involvement of an altered herbicide binding site. The reduced target site sensitivity conferred by a mutated gene of the ALS gene has been documented in many studies (Tranel & Wright, 2002; Yu & Powles, 2014). Among the 26 amino acid substitutions conferring ALS-inhibiting herbicide resistance, Ala 122, Pro 197, Asp 376, and Trp 574 have been identified in *R. raphanistrum* biotypes. Non-target site resistance to ALS-inhibiting herbicides has also been evident in resistant weed biotypes but is less common especially in dicotyledonous weed species (Tan & Medd, 2002; Yu *et al.*, 2012; Yu & Powles, 2014).

The reproductive success of the survived members of the resistant *R. raphanistrum* population has been observed in the current study, with the survived resistant plants producing pods, although with a comparatively low number compared to the controls. The fecundity or the number of viable seeds produced by a single plant has not been studied in the current study. In this respect, in the study of Taylor *et al.* (2015), the application of ALS-inhibiting herbicide, Midas® at early growth stages of cereal crop when the *R. raphanistrum* plants were small has resulted in a 93.7% mortality rate, but this was referred to as "unacceptable level of control" as the survived plants could set viable seeds despite being stunted. The previous study also suggests the survival of six *R. raphanistrum* plants/ m^2 at crop maturity, is capable of adding 700 fresh seeds per square metre into the soil seed bank. Reduced *R. raphanistrum* seed production, when treated with flumetsulam at the early vegetative growth stages and early flowering is evident in the study of Madafiglio (2002). This study indicated that the percentage reduction in seed production reduces with the increasing growth stage in both vegetative and reproductive stages. Of importance is the comment that *R. raphanistrum* plants treated with a selective herbicide at the early bud stage to mid flowering is expected to reduce seed set by 100% (Madafiglio, 2002). Hence, future work needs to be conducted to evaluate the seed production and seed viability to understand the fitness of resistant plants to thrive and multiply in subsequent generations. The resistant plants with the ability to produce viable seeds, suggest their propensity to increase in number in the succeeding generations foreshadowing the risk of them becoming dominant in a cropping system in the future (Heap, 2014). This can be further accelerated with the continuous use of the same herbicide MOA, especially high-risk herbicide MOAs including ALS-inhibitors. With this notion, identifying a herbicide MOA combination to treat herbicide-resistant *R. raphanistrum* is decisive. Therefore, with the results obtained in the current experiment, in terms of the weed, *R. raphanistrum*, the most effective herbicide treatment is imazamox + imazapyr 2-4 leaf stage treatment to incorporate as one component of the herbicide MOA combination. With these results in mind, the next step will be the assessment of crop flexibility at different growth stages for these ALS-inhibiting herbicides to identify the application window to assure crop safety. This will be investigated in Chapter Four.

CHAPTER 4 - Tolerance of faba bean cultivars; *PBA Bendoc* and *PBA Samira* to ALS- inhibiting herbicides

4.1 Introduction

For maintenance of a successful agricultural system, particularly pulse production on an economic scale, effective weed management is critical. In Australia, the use of herbicides is currently the primary method for controlling weeds in broadacre intensive farming systems. *Raphanus raphanistrum*, *Brassica rapa*, *Sonchus oleraceus*, *Lactuca serriola, Lepidium draba, Oxalis pes-caprae, Lolium temulentum, Lolium rigidum,* and *Bromus sp.* are some of the significant weeds associated with pulses (GRDC, 2017; Roberts *et al.*, 2020). For pulses, there is a limited range of herbicide options, particularly for broadleaf weeds, which poses a significant management problem (Morishita, 2017; Smitchger *et al.*, 2012). Low safety margins between control of target weeds and the economical production of pulse crops advised for many registered herbicides, presents a further difficulty for commercial level pulse cultivation (McMurray *et al.*, 2016). Also, pulses have inherent poor competitiveness with weeds, increasing the reliance on effective herbicide use.

Among limited herbicide options, pre-emergent herbicides registered for use with faba beans include trifluralin, pendimethalin, tri-allate, cyanazine, simazine, terbuthylazine, and diuron, whilst post-sowing pre-emergent herbicide options which are currently registered are simazine, metribuzin, and imazethapyr. For control of grass weeds, the group A herbicides acetyl coenzyme A carboxylase inhibitors, are registered for in-crop application. However, for controlling in-crop broadleaf weeds in faba bean crops, only one herbicide, imazamox is registered but this has a small safety margin and can result in crop injury (GRDC, 2017). Overall, the lack of cost-effective and safe post-emergent herbicide options have made weed control, especially of broadleaf weeds, challenging for faba bean production. Therefore, to improve crop safety and to increase herbicide weed management options, identification, and development of herbicide-tolerant cultivars of commercial crops, including faba bean, has become a major breeding priority (Lamichhane *et al.*, 2017). In this regard, a new imidazolinone tolerant faba bean cultivar *PBA Bendoc* was released in Australia in 2018 (Seednet, 2020). *PBA Bendoc* was the first faba bean cultivar with a proven high tolerance to group B ALS-inhibiting imidazolinones. The introduction of this new cultivar permits a post-emergent herbicide treatment with Intercept® (imazamox & imazapyr) (Nufarm, 2020) up to the six node stage in faba bean (PBA, 2018; Seednet, 2020). Whilst allowing in-crop broadleaf weed management options, *PBA Bendoc* also assures the crop safety when grown in soils with herbicide residues, including sulfonylureas (PBA, 2018).

ALS-inhibiting herbicides act by preventing the biosynthesis of branched-chain amino acids such as valine, leucine, and isoleucine, in growing plants (Duggleby *et al.*, 2008; Ray, 1984). This group of herbicides is also categorised with the highest risk of having plants developing resistance (Beckie & Tardif, 2012; Heap, 2017). One major factor making ALS inhibitor-resistance more pronounced over other resistant types is its diversity within the group, with over 55 active compounds in five chemical classes, which is twice as many as any other herbicide group (Heap, 2014). Alterations in the ALS enzyme can make ALS inhibitor-resistant in most cases since the vulnerability of ALS inhibitors is due to the ability of the ALS enzyme to go through many mutations but still continue to be functional (Heap, 2014). Despite its "high-risk" status, due to their excellent crop safety records, low application rates, high herbicidal activity, low mammalian toxicity and reduced chemical load to the environment (Scarabel *et al.*, 2015), ALS-inhibiting herbicides are currently being widely used for controlling cereal and legume crop weeds. This cluster of herbicides incorporates five families of chemical groups; sulfonylureas, imidazolinones, triazolopyrimidines, pyrimidinyl thiobenzoates, and sulfonyl-aminocarbonyl-triazolinones (Rey-Caballero *et al.*, 2017). However, of major concern is that, in recent years, an increased level of herbicide resistance in many weeds has become a significant issue (Evans *et al.*, 2016; Saari *et al.*, 1994). Many common weeds found in crop production areas, including weeds of particular interest such as *Raphanus raphanistrum*, have developed significant resistance toward some of these herbicides (Beckie & Tardif, 2012; Duhoux *et al.*, 2015; Hashem *et al.*, 2001a; M. J. Owen *et al.*, 2015; Owen *et al.*, 2007; Walsh *et al.*, 2007). Therefore, it is becoming evident that, in order to achieve timely and sustainable control of weeds, growth stage of both crop and weed species at the time of herbicide application is critical.

The growth stage at which the crop is treated with herbicide can be critical in terms of crop injury, crop physiology and the resultant crop yield (Jefferies *et al.*, 2016; Martin *et al.*, 1990; Robinson *et al.*, 2015). Generally, it is essential to effectively control weeds during the establishment and early growth phase of the crop to minimise effects from competition (Tursun *et al.*, 2016). However, in winter cropping seasons in southern Australia the residual activity of many herbicides only lasts 6-8 weeks into the season and late germinating weeds can then cause later season crop competition, grain quality issues at harvest and ongoing weed burden in subsequent crops in the rotation. With this imidazolinone-tolerant cultivar of faba bean, it is important to identify the most tolerant growth stages to these herbicides as this will permit a flexible and safe herbicide application regime depending on the level of weed infestation. Therefore, the aim of this chapter is to assess the response of the herbicide-tolerant faba bean cultivar, *PBA Bendoc* in comparison with a conventional cultivar *PBA Samira* to the ALS-inhibiting herbicides; Intercept® (Imazamox + imazapyr) and imazethapyr, at different growth stages.

4.2 Methodology

Two field trials were conducted in the Wimmera region of western Victoria, near the regional city of Horsham (Figure 4.1) in 2018 and 2019 (2018 trial site: 36.42.928” S 142.06.703” E; 2019 trial site: 36°43'43.6"S 142°09'31.5"E). Both trials were conducted in farmer’s paddocks which had been used for long-term cropping rotations, including a range of cereal and legume crops.

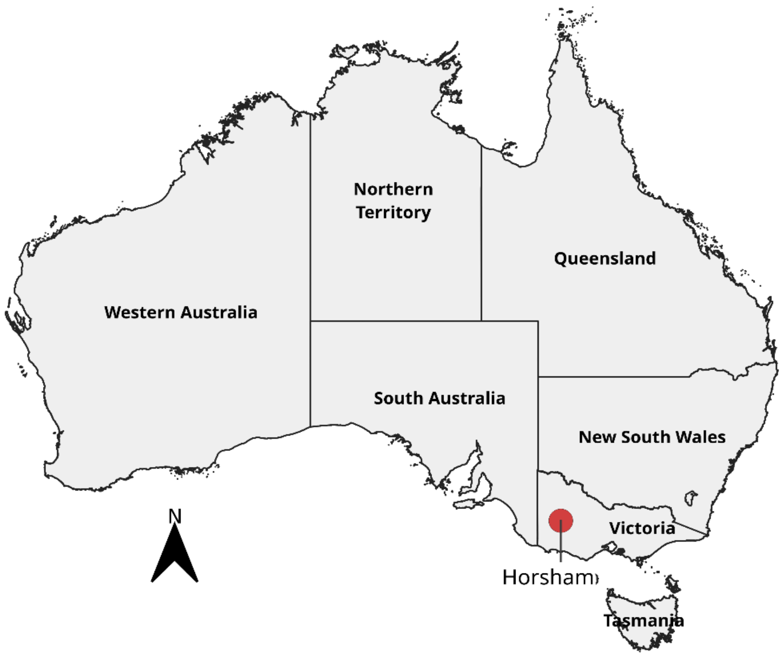


Figure 4.1. Map of Australia showing the location of study site (Horsham is showed with the Red Dot).

The small plot field trials compared an herbicide tolerant faba cultivar (*PBA Bendoc*⸸) with a commercially grown intolerant cultivar (*PBA Samira*). Eight herbicide treatments were applied: two herbicides, ‘imazamox + imazapyr’ compared with ‘imazethapyr’ and four application timings related to crop growth, (i) post-sowing and pre-emergent (PSPE), (ii) 4 node (4N), (iii) 8 node (8N), and (iv) 100% flowering (flow), and were all compared with an control. The experiments were designed as a split-plot with four replicates in 2018 and three replicates in 2019. Herbicide treatment was the main block and faba bean cultivar as the subplot.

Field trials were sown on 21 May in 2018 and 14 May in 2019, between rows into standing stubble of wheat via a small plot seeder with narrow lucerne points and press wheels to imitate a no till cropping system. Seed was sown at a target density of 20 plants/m^2 and a depth of 8 cm, with 80 kg/ha fertilizer (Monoammonium phosphate: 9.2% nitrogen, 20.2% phosphorus, 0% potassium, 2.7% sulfur and 2.5% zinc). To ensure adequate nodulation, rhizobia (*Rhizobium leguminosarum* bv. Vicae; Group E/F) as a peat granule (TagTeam®) was sown with seed (5kg/ha). Plots were 8 m long with four rows at 36 cm row space. To minimise weeds during the experiments, plots were sprayed pre- sowing with glyphosate (90 g/ha), propyzamide (500 g/ha), trifluralin (480 g/ha) and simazine (720 g/ha). Insects and fungal diseases were controlled by the application of suitable pesticides and fungicides at relevant stages of crop growth.

The herbicide products were applied to plots at each of the timings at their recommended application rates (imazamox + imazapyr - 24.75 g /ha + 11.25 g ai/ha and imazethapyr - 70 g/ha) in 100 L/ha of water using a Silvan electric backpack sprayer (200 kPa pressure, flat fan Hardi mini drift 015 nozzles) and boom.

4.3 Measurements and Analysis

Weather was monitored weekly for temperature and rainfall data as recorded by the weather bureau station at Horsham aerodrome (36.67° S 142.17° E). Graphs were then plotted with monthly average rainfall and temperature to compare the two experimental years with the average long-term data.

Soils were sampled at sowing to a depth of 100 cm, to determine soil moisture and its physical and chemical characteristics. Collected soil samples were sent to the Soil and Plant Analysis Laboratory, WA, for a comprehensive analysis of Nitrate Nitrogen (NO_3-N), Ammonium Nitrogen (NH_4-N),Phosphorous (P), Potassium (K), Sulphur (S), Copper (Cu), Iron (Fe), Manganese (Mn), Zinc (Zn), Boron (B), Exchangeable cations (Al, Ca, Mg, K, Na), soil electrical conductivity (EC), pH, bulk density (BD), organic carbon (OC) and moisture (water), using standard methods (Table 1 and Table 2).

Herbicide damage was assessed 28 days after the herbicide application (DAA) giving a visual percentage score of 0 (no herbicide damage) to 100 (complete plant death). Stunted growth, leaf blackening, leaf chlorosis, leaf cupping, and, ultimately, plant death, were the symptoms accounted for when assessing ALS-inhibiting herbicide damage. At maturity, the density of weeds in a plot was assessed visually regardless of the species (0 - no weeds present to 100 - complete weed infestation). The weed score data for 2019 trial is not presented as 100% weed control was achieved in all treatment plots. Faba bean plant height from the soil surface to growing tip was measured using a one-metre measuring tape. Above ground dry biomass was recorded by cutting all plants off at ground level in a 1 m section across all rows from each plot and drying in an oven at 70°C for three days. Grain yield was recorded by harvesting each plot with a small plot harvester. Harvest index was calculated as grain yield: biomass ratio.

Data were analysed using a balanced linear mixed model univariate method (IBM SPSS statistics 25.Ink statistical software). Two-way ANOVA was used to investigate the simple main effects to explore the interaction effect. According to the Shapiro-Wilks test, non-normal residuals were treated with common transformations, but the normality or equal variance assumptions were not greatly improved. Proving the robustness of raw data, the ANOVA outputs obtained from transformed and untransformed data were very similar. Given that ANOVA tests are reasonably robust with regard to slight departures from normality and equal variance (Hahns-Vaughn, 2017), all the analyses were conducted on the original data.

Data from the two trials were compared before analysis, and due to the significant difference observed between the two trials, data were analysed separately for the two years.

4.4 Study site

4.4.1 Soil and climate conditions

In both trial sites, the soils were alkaline Vertisols (Table 4.1 and 4.2). Exchangeable cations: Ca^{2+}, Mg^{2+}, K^+ and Na^+ were comparatively higher in the 2018 trial site but other parameters were closely similar. This area has a Mediterranean-like climate with warm/hot dry summers and cooler wetter winters. According to the data recorded by the weather bureau station at Horsham aerodrome (36.67° S 142.17° E), the typical Horsham climate consists of an annual rainfall: 364 mm; growing season rainfall: 275 mm; mean annual maximum temperature: 22.2°C; and mean annual minimum temperature 7.3°C (Figures 4.2 and 4.3).

Table 4.1. Soil profile analysis for the 2018 trial site at different depths.

Depth	NH_4-N	NO_3-N	P	K	S	Cu	Fe	Mn	Zn	B	EC
(cm)					(mg/kg)						(dS/m)
0-10	9.5	17.2	15.0	939	20.0	1.1	33.0	19.5	0.9	2.9	0.3
10-20	4.6	4.2	7.0	713	11.0	1.2	40.0	5.1	0.3	3.5	0.2
20-40	3.9	2.4	6.0	610	10.0	1.2	45.0	4.8	0.2	4.3	0.3
40-60	2.7	2.8	5.0	619	13.0	1.4	45.0	3.0	0.2	10.9	0.5
60-100	2.5	3.4	6.0	654	30.0	1.5	42.0	3.5	0.3	20.9	0.8

Depth	Al	Ca	Mg	K	Na	pH		BD	OC	Water	
(cm)	Exc (meq/100g)					($CaCl_2$)	(H_2O)	g/cm^3	%	gravimetric (%)	Total (mm)
0-10	0.0	78.0	14.0	5.5	2.8	7.6	8.1	1.3	1.1	18.0	23.0
10-20	0.0	80.0	14.0	3.8	2.5	7.7	8.2	1.2	0.7	23.0	28.0
20-40	0.0	68.0	20.0	3.4	7.9	7.8	8.6	1.3	0.6	27.0	73.0
40-60	0.0	54.0	26.0	3.4	16.7	8.0	8.9	1.3	0.4	27.0	71.0
60-100	0.0	48.0	27.0	3.4	22.1	8.3	9.0	1.1	0.4	30.0	133.0

Note : The abbreviations given in the table represent: the basic soil elements; EC – Electrical conductivity; BD – Bulk density; and OC – Organic carbon.

Table 4.2. Soil profile analysis for the 2019 trial site at different depths.

Depth	NH_4-N	NO_3-N	P	K	S	Cu	Fe	Mn	Zn	B	EC
(cm)					(mg/kg)						(dS/m)
0-10	14.0	13.0	35.0	688	29.2	1.7	46.6	18.9	1.2	4.7	0.3
10-20	11.0	12.0	18.0	491	29.9	1.8	49.5	16.1	0.7	7.5	0.4
20-40	8.0	8.0	12.0	450	17.1	2.0	49.1	11.7	0.5	13.3	0.3
40-60	5.0	6.0	7.0	456	88.0	1.9	51.8	7.3	0.2	18.0	0.6
60-100	4.0	4.0	7.0	490	176.8	2.0	48.3	7.4	0.3	14.7	0.7

Depth	Al	Ca	Mg	K	Na	pH		BD	OC	Water	
(cm)	Exc (meq/100g)					($CaCl_2$)	(H_2O)	g/cm^3	%	gravimetric (%)	Total (mm)
0-10	0.1	28.5	5.7	1.8	1.6	7.1	7.7	1.1	1.6	16.1	17.2
10-20	0.1	26.7	6.4	1.4	2.6	7.3	8.0	1.2	1.1	22.7	27.0
20-40	0.0	22.6	8.1	1.2	4.6	7.5	8.5	1.1		23.7	52.5
40-60	0.1	20.2	8.8	1.3	7.8	7.7	8.6	1.2		25.7	58.8
60-100	0.1	18.8	9.1	1.3	9.2	7.8	8.7	1.2		27.0	127.0

Note : The abbreviations given in the table represent: the basic soil elements; EC – Electrical conductivity; BD – Bulk density; and OC – Organic carbon.

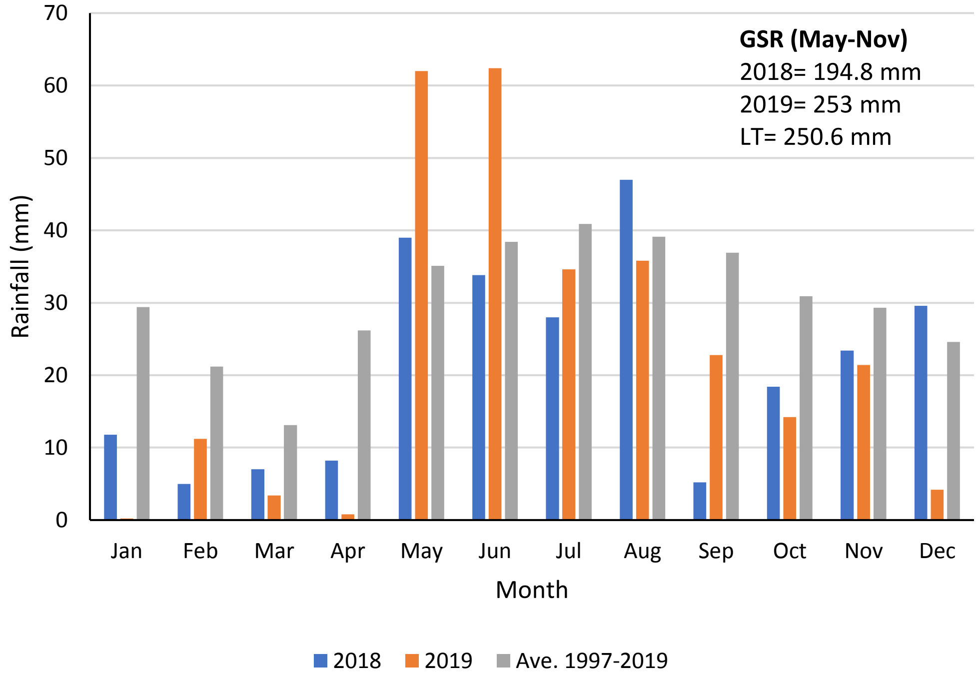


Figure 4.2. Monthly rainfall in 2018 and 2019 at Horsham trial site compared with long-term averages.

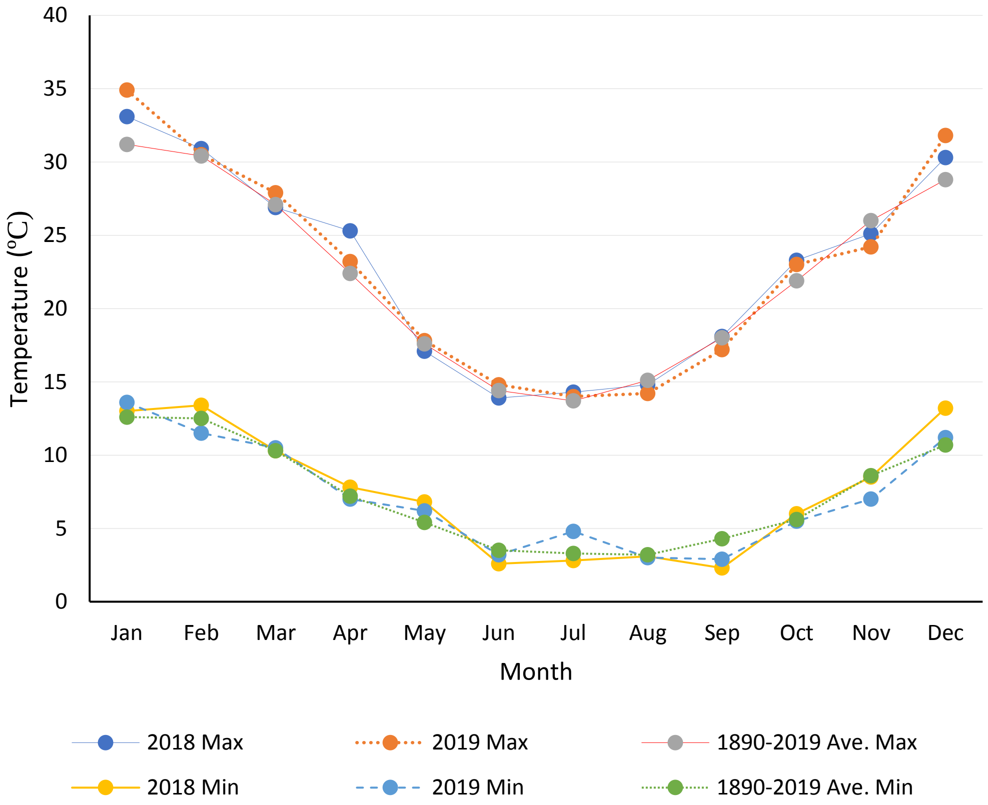


Figure 4.3. Average monthly maximum and minimum temperatures in 2018 and 2019 at Horsham trial site compared with long-term averages.

4.5 Results

4.5.1 Climate

In the 2018 growing season (May-Nov), rainfall was significantly less than the long-term average (Figure 4.2). The monthly rainfall from January to April was also below average, which meant that the trial was sown into dry soil during early May. The first rainfall event to stimulate establishment occurred seven days after sowing; with average rainfall recorded for the rest of the month. Rainfall for June to August was consistent with the long-term average, but September and October, the critical months for reproductive growth, were extremely dry. Minimum temperatures during the growing season were generally lower than the long-term average minimum, and higher than the long-term average maximum. In particular, it was extremely cold during September with low minimum temperatures, while in October high temperatures were apparent. In addition to the average cold temperatures, there were several frosts that affected the pod set.

Similar to 2018, rainfall was below average from January to April. However, above average rainfall in May and June was followed by average or slightly below average falls from August to October. This assured an above average growing season rainfall compared to the long-term rainfall during that period. Therefore in 2019, faba bean seeds were sown into moist soil beds followed by a rainfall event three days after sowing. Several frost events occurred during spring, especially in September where the minimum temperature dropped significantly below the average. They started rising there after compared to the long-term average. The maximum temperature that was mostly below or near average throughout the growing season helped in maintaining mild temperatures but with an exception in October which was slightly high.

4.5.2 Plant establishment, growth, herbicide damage and weeds

In 2018 faba bean establishment and early crop growth was affected due to the drought conditions and they were also hampered due to residual herbicide effects of group B and I herbicide levels observed in the paddock. In both years, plant establishment was not affected by the herbicide treatments, but plants were visibly stunted in all treatment plots in 2018 (Figure 4.10 and 4.11), including the controls due to weather conditions. Weed growth was evident in plots as a result of poor crop competition and reduced herbicide efficacy affected by the drought. The severely dry months of September and October further affected the crop, leading to a reduced yield in 2018. Herbicide damage was not clear among the treatments or between the cultivars as the efficacy of the herbicides were severely affected by limited soil moisture.

In contrast, the mild temperatures along with adequate rainfall, ensured excellent faba bean seedling establishment and early crop growth in the 2019 trial. Above average rainfall occurred in May and June, and along with the near average rainfall in July and August this provided the optimum moisture conditions to assure well-grown faba bean plants (Figure 4.12 and 4.13). No weeds were observed in the plots as the herbicide efficacy and crop competition were influenced by the adequate moisture and mild temperature conditions which overcame weed growth. Therefore in 2019, well established and grown plants were observed throughout the season compared to 2018.

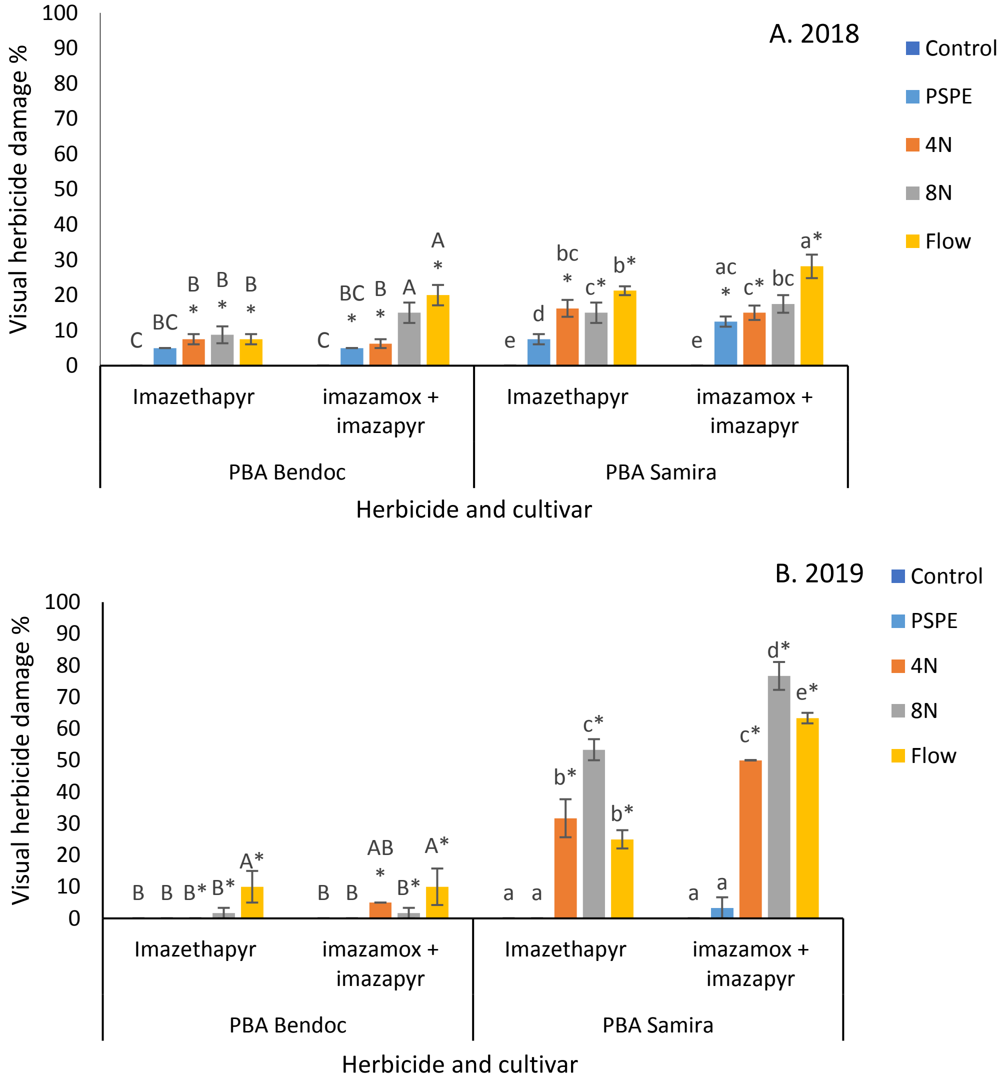


Figure 4.4. Herbicide damage score recorded 28 days after application of the herbicides 'imazamox + imazapyr (24.75 g ai/ha + 11.25 g ai/ha)' and 'imazethapyr (70 g ai/ha' applied at Post-sowing pre-emergent (PSPE), 4 node (4N), 8 node (8N), and flowering (flow) growth stages to a herbicide tolerant faba bean, *PBA*

Bendoc in comparison with the intolerant cultivar, *PBA Samira* at Horsham in **A.** 2018 and **B.** 2019. The lines at the top of the bars represent the standard errors. Bars with different letters of the same case indicate significant differences across treatments within the cultivar and the asterisks (*) denote the treatments with significant differences between the cultivars.

In 2018, overall herbicide damage from the applications of treatments was generally low, with slight symptoms of stunting, chlorosis and necrosis. There was a significant two-way interaction ($P < 0.05$) between herbicide treatment and cultivar (Figure 4.4.A). In the tolerant cultivar, *PBA Bendoc*, low herbicide damage scores were observed with application of imazethapyr; no significant differences were observed between the timings. Imazamox + imazapyr, caused a similar level of symptoms to imazethapyr at the PSPE and 4N application timings, but significantly higher damage scores were observed with 8N and flowering applications. In the conventional cultivar, *PBA Samira*, herbicide damage scores were significantly higher than *PBA Bendoc* for all comparable treatments. For imazethapyr, damage scores were lowest when applied PSPE and highest when applied at flowering. The 4N and 8N applications were equivalent and significantly higher than PSPE and 8N application was significantly lower than flowering. Application of 'imazamox + imazapyr' at PSPE and flowering stages, resulted in slightly higher damage scores than imazethapyr, but were similar at the 4N and 8N stages.

In 2019, the herbicide damage was much more prominent compared to 2018, resulting in stunted plants with severe leaf chlorosis and necrosis in *PBA Samira*. The interaction effect of the herbicide treatment by the cultivar on herbicide damage at 28 DAA was significant ($p<0.05$). Regardless of the treatment, *PBA Samira* showed increased herbicide damage compared to *PBA Bendoc* except at the PSPE stage (Figure 4.4.B). In *PBA Bendoc*, all treatments were equivalent except at the flowering application which was significantly higher in both herbicide treatments. In *PBA Samira*, all the imazamox + imazapyr applications showed increased herbicide damage compared to the applications of imazethapyr. This increase was significant in 4N, 8N and flowering treatments but not with PSPE treatment. In both the cultivars, the least herbicide damage was observed in the PSPE treatments and highest in the 8N and flowering stages respectively, for *PBA Samira* and *PBA Bendoc*. *PBA Samira* showed an increased sensitivity to the herbicide imazamox + imazethapyr whereas *PBA Bendoc* was equally tolerant to both the herbicides.

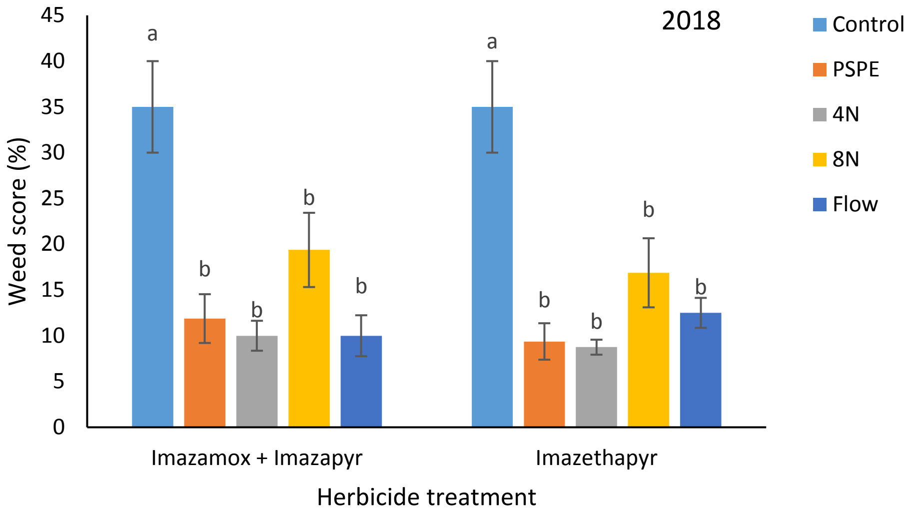


Figure 4.5. Weed scores recorded at the maturity in faba bean treated with the herbicides 'imazamox + imazapyr (24.75 g ai/ha + 11.25 g ai/ha)' and 'imazethapyr (70 g ai/ha)' at Post-sowing pre-emergent (PSPE), 4 node (4N), 8 node (8N), and flowering (flow) growth stages at Horsham in 2018. The lines at the top of the bars represent the standard errors. Bars with different letters of the same case indicate significant differences across treatments.

In 2018, the highest weed score was observed in the control treatments due to poor in-crop weed control. The analysis showed that the interaction effect of treatment by cultivar was not significant in the weed score results (p>0.05), yet it showed a significant herbicide treatment effect (p=0.001). Therefore, the results were averaged between cultivars and presented in Figure 4.5. Overall, the control was significantly different from the rest of the treatments where the weed scores were comparatively less. The highest weed score was recorded in control (35) across both cultivars followed by the 8N growth stage regardless of the herbicide (19 and 16 for imazamox + imazapyr and imazethapyr respectively). No weeds were recorded in the 2019 trial.

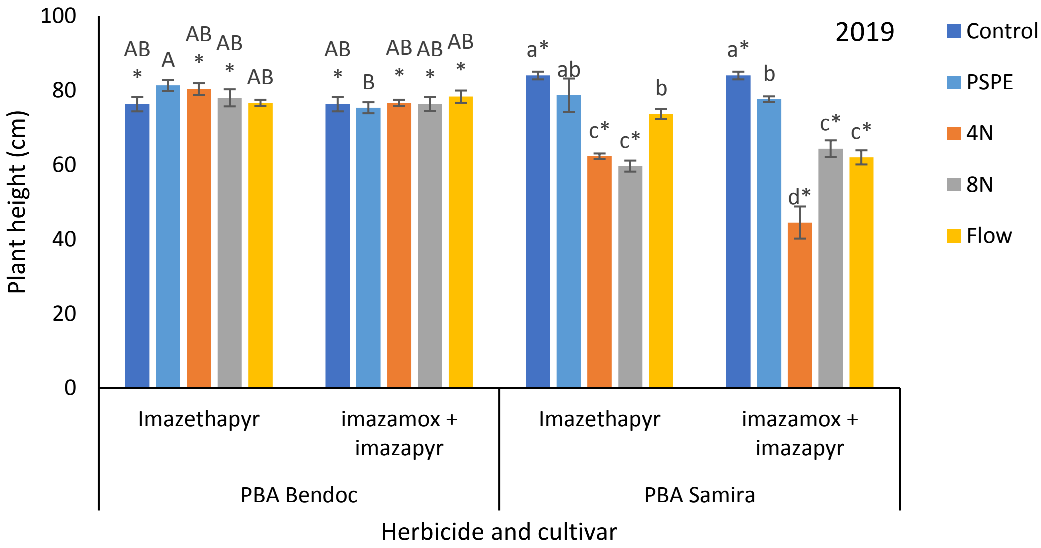


Figure 4.6. Plant height of herbicide tolerant faba bean, *PBA Bendoc* in comparison with the intolerant cultivar, *PBA Samira* to the herbicides Imazamox + imazapyr and imazethapyr applied at Post-sowing pre-emergent (PSPE), 4 node (4N), 8 node (8N), and flowering (f flow) growth stages at Horsham in 2019. The lines at the top of the bars represent the standard errors. Bars with different letters of the same case indicate significant differences across treatments within the cultivar and the asterisks (*) denote the treatments with significant differences between the cultivars.

In 2018, due to the dry conditions, overall plant growth was stunted with heights less than 50 cm. There was no significant difference between cultivars or herbicide treatments or in their interaction effect (p>0.05) and hence not presented in a figure. The average plant height in 2018 was recorded as 43.8 cm (± 1.4)

In contrast, in 2019 trial, growth was more vigorous resulting in taller plants and the effects of herbicide treatments were evident. The two-way interaction, treatment by cultivar was also significant(p<0.05) (Figure 4.6). This significance in the interaction was not evident in the treatments of imazamox + imazapyr PSPE (p=0.413), imazethapyr PSPE (p=0.351), and imazethapyr flow (p=0.296). In the *PBA Bendoc* cultivar, none of the treatments showed a significant difference (p>0.05) compared to the control in plant height (average plant height of 76.3 cm). In contrast, in *PBA Samira*, all treatments except the PSPE treatment of imazethapyr (p=0.069) showed a significant reduction in plant height compared to the control treatment (p<0.05). The stunting was much more prominent in 4N and 8N treatments and especially in the 4N treatment of imazamox + imazapyr.

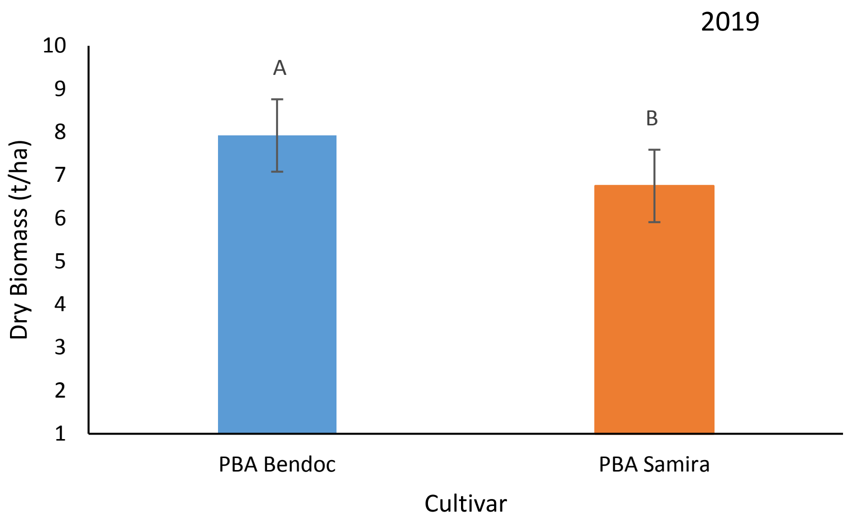


Figure 4.7. Above-ground dry biomass at maturity of herbicide tolerant faba bean, *PBA Bendoc*, in comparison with the intolerant cultivar, *PBA Samira* in2019. The lines at the top of the bars represent the standard errors. Bars with different letters of the same case indicate significant differences across the cultivar.

Overall, the above-ground dry biomass was much lower in the 2018 trial compared to the 2019 trial, due to poorly grown stunted plants resulting from the drought conditions which occurred during seedling establishment. In 2018, no significant difference was observed in the interaction effect of treatment by cultivar (p=0.431). There was also no significant difference among any of the herbicide treatments (P=0.758) or between the cultivars (P=0.641), hence not provided in a figure. Overall, the above ground biomass in 2018 was averaged 2.5 t/ha, across all treatments.

In 2019, above-ground biomass was variable and ranged from 3.8 To 10.4 t/ha. However, the results showed that the average above-ground biomass at maturity was not significantly affected by the interaction of treatment by cultivar (p=0.100) or in the treatment factor (p=0.061), but the cultivar effect was found to be significant (p=0.002) (Figure 4.7). Overall, biomass was higher in the *PBA Bendoc* with an average of 7.92 t/ha compared to *PBA Samira* which was 6.75 t/ha.

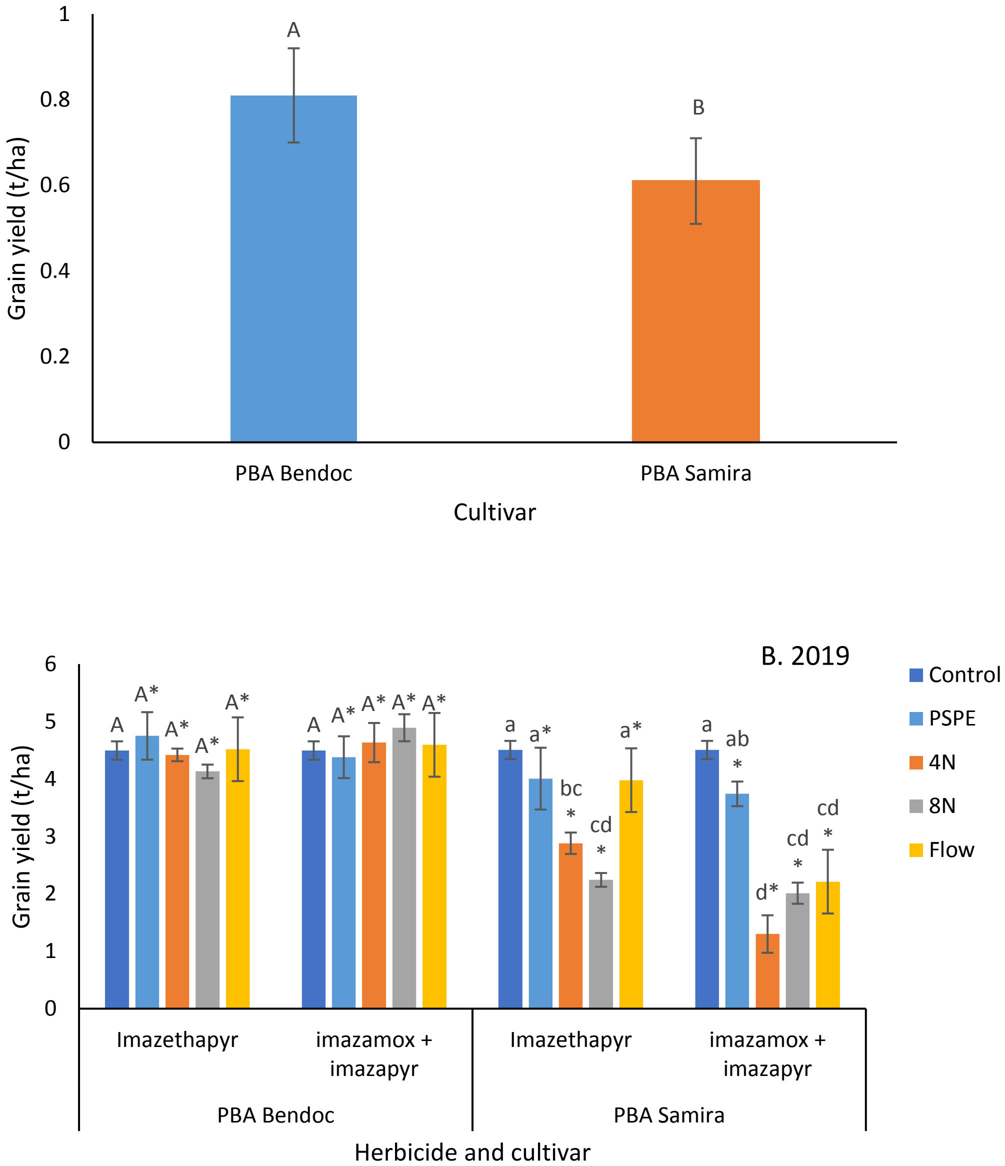
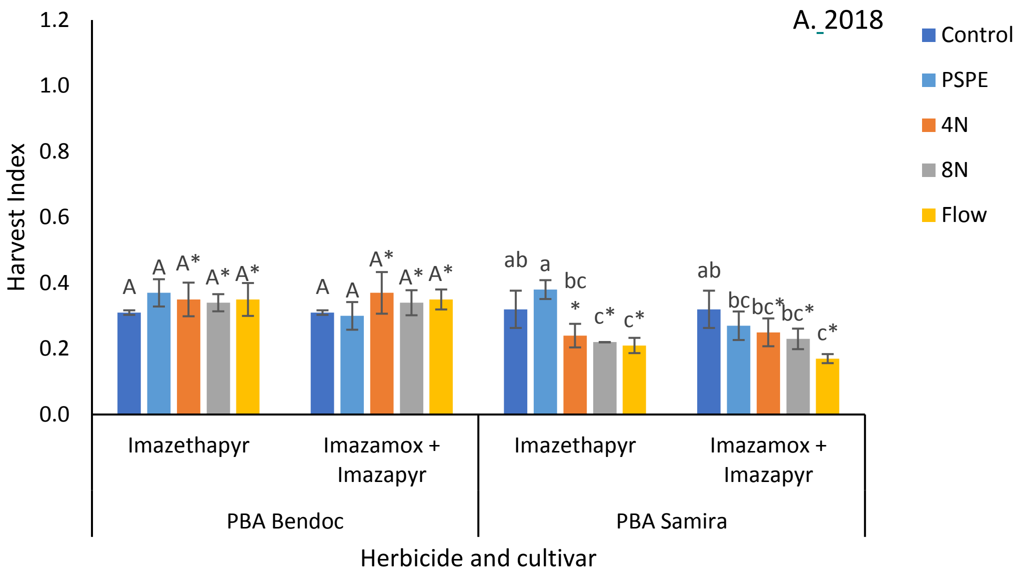


Figure 4.8. Grain yield of herbicide tolerant faba bean, *PBA Bendoc* in comparison with the intolerant cultivar, *PBA Samira* to the herbicides Imazamox + imazapyr and imazethapyr applied at Post-sowing pre-emergent (PSPE), 4 node (4N), 8 node (8N), and flowering (flow) growth stages at Horsham in **A.** 2018 and **B.** 2019. The lines at the top of the bars represent the standard errors. Bars with different letters of the same case indicate significant differences across treatments within the cultivar and the asterisks (*) denote the treatments with significant differences between the cultivars.

Due to prevailing dry and frosty conditions in 2018, grain yields were less than 0.9 t/ha in all treatments. The interaction effect of the treatment by cultivar was not significant (p=0.068) but a significant difference was observed in the cultivar (p<0.05) but not with the treatment (p=0.261). Therefore, Figure 4.8.A represents the grain yields for the two cultivars averaged across the treatments. The averaged grain yield was higher in PBA Bendoc (0.81 t/ha) compared to that of PBA Samira (0.61 t/ha) in 2018.

The 2019 grain yield was significantly higher than the 2018 results with an average grain yield in controls around 4.5 t/ha. The two-way interaction effect of the treatment by cultivar was significant in the grain yield of faba bean (p<0.05). This was significant in all the treatments other than the control. No significant difference was observed among any of the treatments in *PBA Bendoc*. But in *PBA Samira*, all the treatments other than PSPE treatments and imazethapyr flowering treatment, showed a yield reduction greater than 50% compared to the control regardless of the herbicide. This proved the improved tolerance in *PBA Bendoc* to both the herbicides (Figure 4.8.B). The lowest and the highest grain yields in *PBA Samira* were observed in the treatment of imazamox + imazapyr 4N and control, which averaged 1.3 t/ha and 4.5 t/ha respectively.



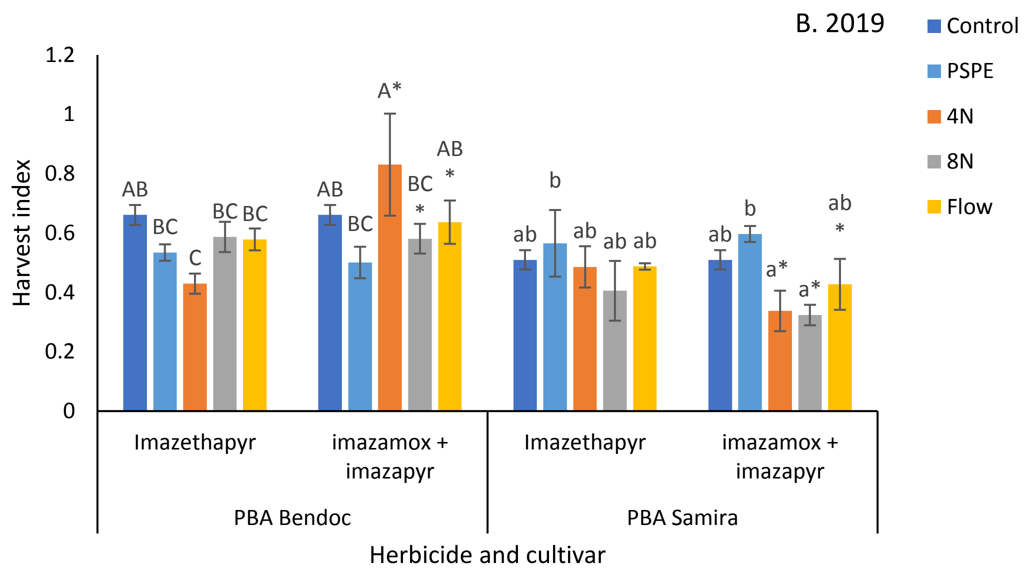


Figure 4.9. Harvest index of herbicide tolerant faba bean, *PBA Bendoc* in comparison with the intolerant cultivar, *PBA Samira* to the herbicides Imazamox + imazapyr and imazethapyr applied at Post-sowing pre-emergent (PSPE), 4 node (4N), 8 node (8N), and flowering (flow) growth stages at Horsham in **A.** 2018 and **B.** 2019. The lines at the top of the bars represent the standard errors. Bars with different letters of the same case indicate significant differences across treatments within the cultivar and the asterisks (*) denote the treatments with significant differences between the cultivars.

As a result of the drought conditions, the resulting reduced grain yields and dry biomasses in the 2018 trial led to a significant reduction in harvest index (the ratio between the grain yield and the biomass). The two-way interaction of treatment by cultivar was significant (P=0.041) in the 2018 trial. Also, the cultivar effect showed a significant effect (p<0.05) on the harvest index but not the herbicide treatment (p=0.167) (Figure 4.9.A). In *PBA Bendoc*, there was no significant difference in harvest index between treatments with an average of 0.34 recorded. In *PBA Samira,* a significant drop was observed along with the advancing growth stage. All the treatments at 4N, 8N and flowering were found to be significantly different between the cultivars regardless of the herbicide (p<0.05). In both cultivars, the highest harvest index was observed in the treatment of imazethapyr PSPE (0.37) which implies a better control of weeds at early stages of the crop growth resulting in an increased yield: biomass ratio.

In the 2019 trial, the harvest index was significantly improved due to increased yields and dry biomass values obtained in that year. However, the harvest index was significantly affected by the cultivar (p<0.05) and interaction effect of treatment by the cultivar (p=0.006) but not the herbicide treatment (p=0.500). In *PBA Bendoc*, all the treatments showed a small harvest index compared to the control other than the imazamox + imazapyr 4N treatment. The significance was evident only in the treatment of imazethapyr 4N which was the lowest harvest index in *PBA Bendoc*. In *PBA Samira*, other than the two PSPE treatments all the other treatments were comparatively low compared to controls, but these differences were not enough to be statistically significant. The cultivar effect was found to be significant in the treatments of all imazamox + imazapyr treatments other than the imazamox + imazapyr PSPE treatment (p=0.347). In contrast, the cultivar effect was not significant in any of the imazethapyr treatments. In most of the treatments, *PBA Bendoc* showed an increased harvest index compared to the *PBA Samira* and this was more prominent in the treatments of imazamox + imazapyr (Figure 4.9.B). This implies the increased sensitivity in *PBA Samira* to imazamox + imazapyr treatments compared to the imazethapyr.

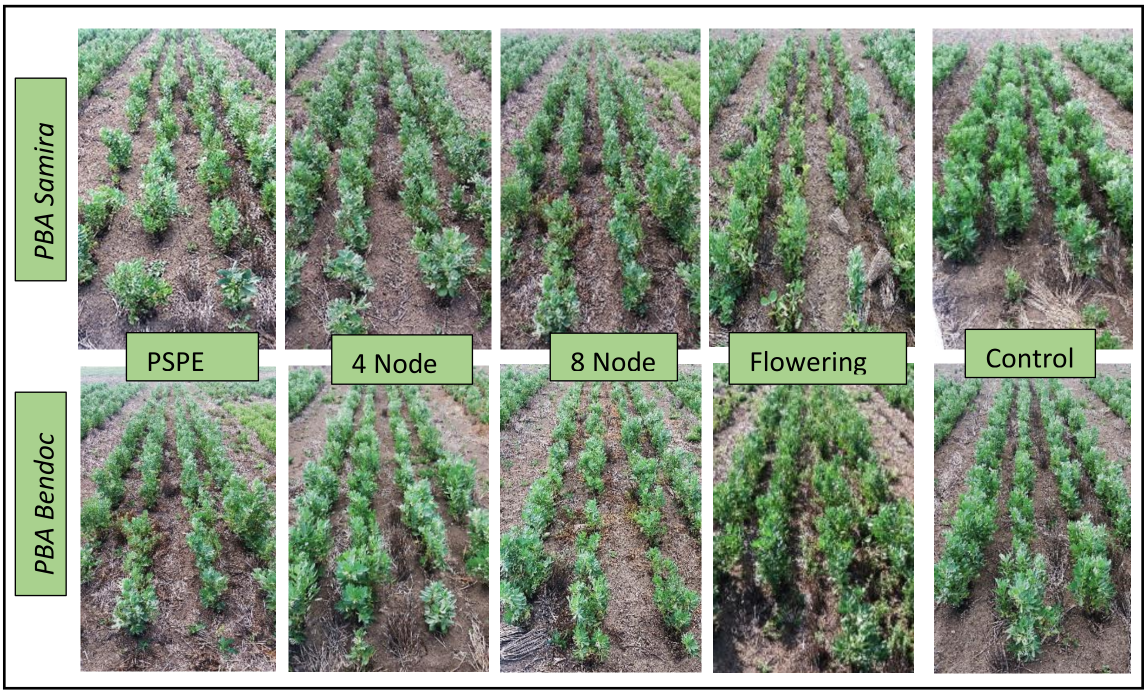


Figure 4.10. The herbicide damage 28 days after application in herbicide tolerant faba bean, *PBA Bendoc* in comparison with the intolerant cultivar, *PBA Samira* to the herbicide Imazamox + imazapyr applied at PSPS, 4N, 8N, and flowering growth stages in the 2018 trial.

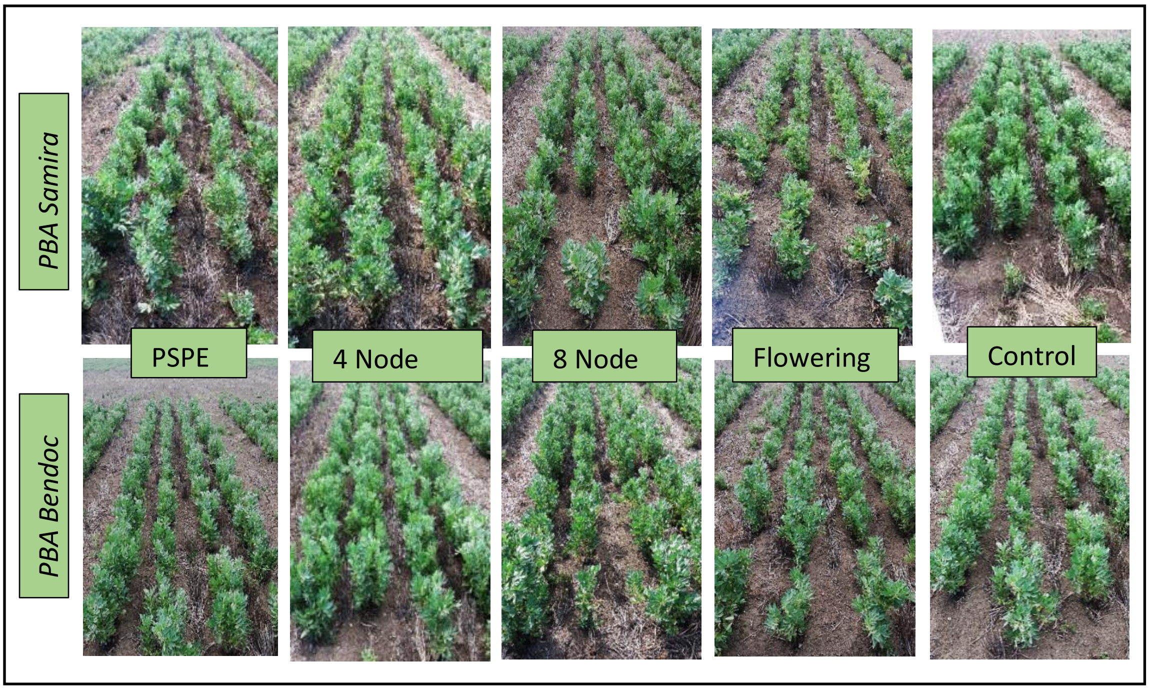


Figure 4.11. The herbicide damage 28 days after application in herbicide tolerant faba bean, *PBA Bendoc* in comparison with the intolerant cultivar, *PBA Samira* to the herbicide imazethapyr applied at PSPS, 4N, 8N, and flowering growth stages in the 2018 trial.

Figure 4.12. The herbicide damage 28 days after application in herbicide tolerant faba bean, *PBA Bendoc* in comparison with the intolerant cultivar, *PBA Samira* to the herbicide Imazamox + imazapyr applied at PSPS, 4N, 8N, and flowering growth stages in the 2019 trial.

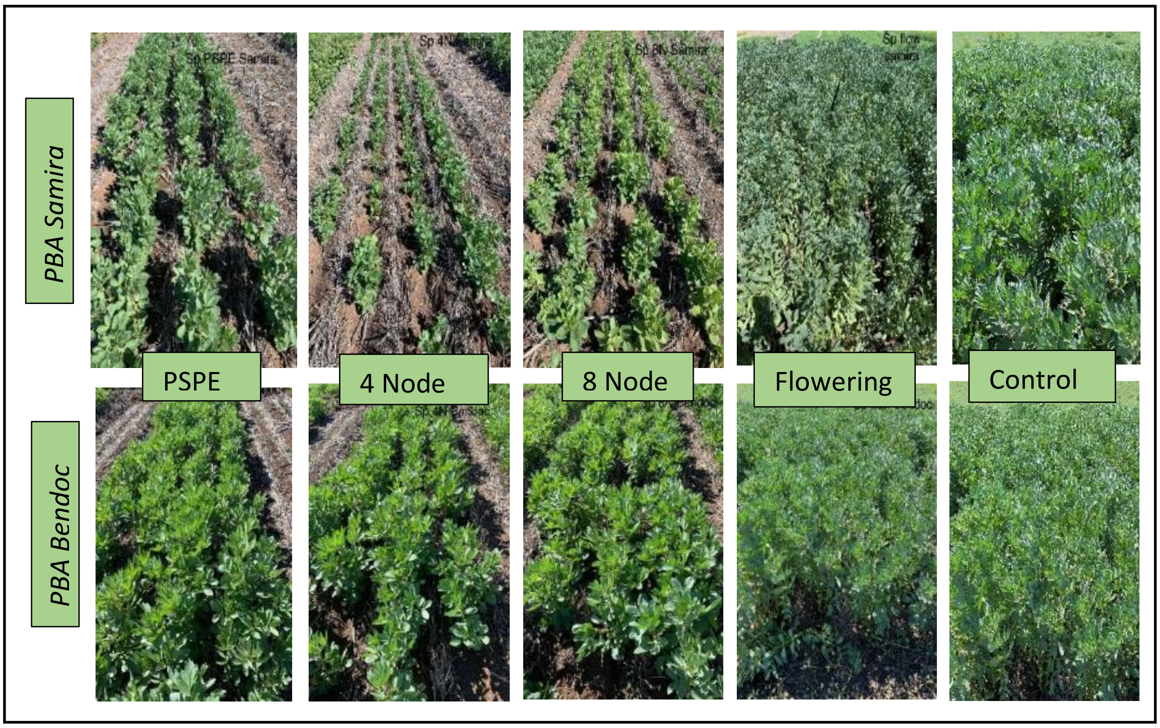


Figure 4.13. The herbicide damage 28 days after application in herbicide tolerant faba bean, *PBA Bendoc* in comparison with the intolerant cultivar, *PBA Samira* to the herbicide imazethapyr applied at PSPS, 4N, 8N, and flowering growth stages in the 2019 trial.

4.6 Discussion

According to the results obtained, the herbicide-tolerant cultivar, *PBA Bendoc* was tolerant to both herbicides, imazamox + imazapyr and imazethapyr, applied at all growth stages while the conventional cultivar, *PBA Samira*, suffered significant crop damage and yield loss. *PBA Bendoc* did show a slight increase in herbicide damage when treated at the flowering stage but it did not affect grain yield. These findings are consistent with previous research (Mao *et al.*, 2019) supporting current herbicide registrations recommendations of applying intercept® (imazamox + imazapyr) up to the six node growth stage (Seednet, 2020).

In these trials, it was noted that *PBA Samira*, despite significant crop damage, was often able to recover and produce grain yield. This shows the naturally occurring slight inherent imidazolinone tolerance in faba bean. As a result, Raptor ® (imazamox 700 gai/kg), an imidazolinone herbicide, has been registered for use as an in-crop weed control option for conventional faba bean cultivars despite causing some crop injury (GRDC, 2017). The inherent tolerance in beans is supported by Bukun et al. (2012), which demonstrated increased imazamox metabolism in beans compared to lentils, resulting in a limited herbicide translocation to the target site. The herbicide metabolising mechanisms in plants convert lethal active ingredients in herbicide to less-toxic compounds making the plant tolerant to a particular herbicide. This defence mechanism comprises four main phases; conversion, conjugation, secondary conversion and transportation, and finally, the metabolite deposition (Yuan *et al.*, 2007). Therefore the active ingredients are prevented from reaching their site of action. Homo-Glutathion, which is present in leguminous crops, including faba beans, conjugates with the metabolites of phase one, making them more soluble in water to transport them for deposition (McGonigle *et al.*, 1998). The study of Mao *et al.* (2019) also has explored the chemical mutagenesis potential of different faba bean biotypes in developing herbicide-tolerant faba bean cultivars. Enhancing such inherent traits in legumes, HTCs such as *PBA Bendoc*, are developed to deliver more value to the agricultural industry. Of particular interest to our study, the Cytochrome P450 monooxygenases (P450s) enzymes, which are involved in initial phase conversion of this process is known to have a significant effect on metabolising ALS-inhibiting herbicides, especially sulfonylureas and some imidazolinone herbicides (de Carvalho *et al.*, 2009). Based on this suggestion, the reduced phytotoxicity observed in tolerant *PBA Bendoc* plants in our current study may be a result of the enhanced inherent herbicide tolerance of beans to imidazolinone products compared to the sensitive conventional cultivar (de Oliveira Jr & Inoue, 2011).

In the current study, increased herbicide crop damage was observed with the increasing growth stage of the herbicide application. The efficacy of foliar-applied herbicides varies depending on herbicide deposition and retention (Brunskill, 1956; Gossen *et al.*, 2008), plant species, morphology, orientation, and age of the leaves (Anderson, 1987; Tu *et al.*, 1986). Supporting the increased herbicide damage when applied at later growth stages of the crop, the study of Wall (1997) has shown that the herbicide damage in canola was severe when treated with thifensulfuron:tribenu at six-leaf stage when the plants were almost at the reproductive and bolting stage. The study of Jensen (1993) has shown the increased herbicide damage and yield reduction in peas when herbicides are applied at later stages compared to the early growth stages. This was suggested to be a result of quick plant recovery associated with young plants resulting in increased yields. In their study, the highest crop tolerance was observed when the plants have just emerged. The vertical leaf arrangement in young plants suggests retaining less herbicides compared to more horizontally oriented larger plants leading to less herbicide damage (Anderson, 1987; Jensen, 1993). Therefore, early weed control is crucial in terms of both crop safety and weed susceptibility as the herbicides can no longer be effective on some weeds, once grown beyond a particular growth stage. This can ultimately lead farmers to apply high herbicides rates, which may result in crop damage. Also, the extended periods of weed infestation can cause substantial yield losses to the farmers (Johnson *et al.*, 2004).

The increased herbicide damage at later growth stages suggests that it is best to apply herbicides before the faba beans reach the flowering stage. This finding also supports the early weed management in faba bean cropping systems to reduce any yield losses. Given that faba bean is a poor weed competitor and highly sensitive to both broadleaf and grass weeds, the performance of faba beans is not only affected by the weed species and their densities but also the exposure period to the weed infestation (Agegnehu & Fessehaie, 2006). Weeds compete with crops for light, space, nutrients and water, and, if neglected, can cause adverse effects on crop yield quality and quantity (Halford *et al.*, 2001; Kavaliauskaitė & Bobinas, 2006). Therefore, implementing adequate and timely weed management strategies is crucial in faba bean cropping systems to prevent any unnecessary yield losses due to weeds (Wakweya & Dargie, 2017). The critical weed control period varies depending on crop species, infesting weed, and also the climate conditions in the cropping area (Mortimer, 1984; Rajcan & Swanton, 2001). The critical weed control period is defined as the duration in which the crop must be kept weed-free to avoid yield losses greater than 5% (Hall *et al.*, 1992). According to the study of Kavurmaci *et al.* (2010), the critical weed control period for faba bean growth under typical growing conditions in South Turkey, is 45 days after emergence (DAE) and the critical weed competition period should begin from 30 DAE. The study also highlighted that the yield losses could be expected to increase significantly under moisture stressed conditions and high weed densities. Another study conducted in Italy also proved the importance of early weed management in faba bean with a critical period determined to be 28-33 DAE in their studies (Frenda *et al.*, 2013). Adding more to these findings, the study of Tawaha and Turk (2001) has also shown that increased yields of faba bean could be obtained when the weeds are managed between 25-75 days after sowing in cropping fields. Therefore, the reduced herbicide damage when treated at early faba bean growth stages in our study provide evidence to support the importance of early weed management that fall within this critical weed control period leading to less herbicide damage and good overall performance of the crop.

As described in the results, the field data arising from the two trials undertaken in different years varied greatly in almost all the parameters evaluated. The intensity of drought conditions that prevailed during the seedling establishment phase of the 2018 trial has been the reason for this difference. In contrast, in 2019, monthly rainfall at the time of seedling establishment, May/ June/ July, was much higher and hence allowed an excellent seedling establishment and plant growth at the early stages leading to vigorous, well-grown plants throughout the season in 2019. Soil moisture and drought stress also played a vital role in efficacy of both soil and foliar-applied herbicides (Bagavathiannan *et al.*, 2017). In terms of foliar-applied herbicides, the herbicide efficacy can be reduced under water-stressed conditions as the drought conditions can cause stomatal closure, thickened cuticles, and thereby a reduced herbicide uptake into the plant (Oyarzabal, 1991; Steptoe *et al.*, 2006) and hence, less herbicide damage. In the study of Olson *et al.* (2000) the increased sulfonylurea damage in spring wheat was evident under saturated soil moisture conditions compared to the soils with one-third of saturation. Reduced herbicide crop damage under moisture stress conditions has also been apparent in the study of Dickson *et al.* (1990), where the *Avena sativa* did not show any herbicide damage within one month after the application of fluazifop or glyphosate under severe drought conditions. Changes in the rainfall can have adverse effects on imidazolinone efficacy on the plants (Malefyt *et al.*, 1991) as they are systemic herbicides which are uptaken via both roots and foliage and actively translocated in the xylem and phloem to the actively growing parts of the plant. In soil-applied herbicides, its solubility, activation, plant uptake, and herbicide efficacy may vary depending on the soil moisture levels (Dickson *et al.*, 1990; Muzik, 1976). The soil moisture stress increases the adsorption of herbicides to the soil particles, making them unavailable for plant uptake via roots (Dao & Lavy, 1978). Crop root growth and structure are known to be genetically inherent, which is specific to the crop species. Yet, the local edaphic factors, including soil characteristics and water stress, may have an influence on its architecture (Smit & Groenwold, 2005). Despite the inherence of shallow roots in faba bean, soil moisture stress can lead to increased profound root growth or an increased root: shoot biomass ratio (Husain *et al.*, 1990; Reid, 1990). While the crop is adapting to the drought with such morpho-physiological changes, the ALS-inhibiting herbicides that are relatively mobile in soil under moist rainy conditions (Congreve & Cameron, 2018), tend to retain in the top-soils with restricted movement due to lack of soil moisture. On the other hand, with adequate small rainfall events, herbicide leaching incorporates the herbicides throughout the upper layers of soil (Bzour *et al.*, 2019), making them available to be uptaken by the typically shallow faba bean roots . This phenomenon may have resulted in the observed increased herbicide efficacy for the 2019 plants grown under adequate soil moisture conditions compared to the faba bean plants in 2018 dry growing season.

As discussed above, the faba bean seedling establishment and overall plant growth and vigour were significantly affected by the drought conditions that prevailed during the 2018 trial. Whilst faba bean is known as a semi-arid crop, it is nevertheless sensitive to the moisture stress conditions (Loss *et al.*, 1997) and the different growth and fecundity responses may vary depending upon the growth stage of the plants during the time that the soil moisture limitation was imposed (Ye *et al.*, 2018; Zeleke & Nendel, 2019). The observation of reduced herbicide efficacy in 2018 compared to the 2019 trial where water stress was not evident, is also supported by literature from previous studies (Burke *et al.*, 1985; Skelton *et al.*, 2016; Weller *et al.*, 2019). It has been reported that the overall plant physiological processes can be affected by the induced stress conditions by both biotic and abiotic factors during their growth period, leading to a restricted growth (Patterson, 1995; Teixeira *et al.*, 2013; Wheeler *et al.*, 2000). These studies have shown the extent of negative impacts on crops due to the extreme abiotic conditions such as unfavorable temperatures (Peng *et al.*, 2004; Prasad *et al.*, 2006; Wheeler *et al.*, 2000) and moisture stress (Manickavelu *et al.*, 2006; Maseko *et al.*, 2019; Wijewardana *et al.*, 2019). In addition to the extreme abiotic conditions, the herbicides applied to the cropping system can also add extra stress on crop plants (Bagavathiannan *et al.*, 2017). Even though the HTCs can metabolise some herbicides, they are still vulnerable to insects and disease attacks, particularly since their physiologies are being altered temporarily (Bradley *et al.*, 2002; Duke *et al.*, 2006). These effects on crops can become seriously adverse in situations where stress conditions appear simultaneously in a cropping system (Bagavathiannan *et al.*, 2017).

Supporting all these studies, in our 2018 trial, the drought conditions prevailing at the time of seedling establishment, reproductive stages, and the herbicide application, lead to reduced herbicide damage and yield in faba bean plants compared to the 2019 trial where the rain was abundant. Stunted plant growth can also be regarded as a combined effect of herbicides and the extreme abiotic conditions during the growing season. Considering all the results obtained from the current study, the best application timing for both the herbicides can be suggested as the PSPE stage, which resulted in the least herbicide damage, regardless of the faba bean cultivar. The use of pre-emergent herbicides helps in minimising the crop damage and yield loss due to crop-weed competition at early crop growth stages and therefore play a significant role in Australian cropping areas. However, the post-emergent herbicides are also an important aspect in herbicide weed control strategies to deal with the late emergent weeds and to achieve good weed management throughout the growing season. In the current study, *PBA Bendoc* cultivar proved its increased herbicide-tolerance at all growth stages conferring no yield loss in response to both herbicides. This finding makes *PBA Bendoc* an excellent herbicide-tolerant crop as it permits both pre-emergent and post-emergent application without threatening crop safety. This also allows other pre-emergent herbicides to be incorporated in the *PBA Bendoc* cropping system, assisting in diversifying weed management strategies. Therefore, the results of the current study will be of great importance to the faba bean growers and agricultural industries in making decisions on in-crop weed control in faba bean cropping fields.

In our step-wise approach of introducing a herbicide MOA combination to control *R. raphanistrum* in faba bean cropping systems, the importance of weed growth stage at herbicide application and the efficacy of these ALS-inhibiting herbicides on *R. raphanistrum* was previously evaluated in Chapter Three. Crop tolerance towards those herbicides was then assessed in this chapter with *PBA Bendoc* and *PBA Samira* faba bean cultivars to assure crop safety before recommending to incorporate as an in-crop application. This identified broad ALS-inhibiting herbicide tolerance in *PBA Bendoc* with the proven safe in-crop application, incorporation of another herbicide MOA into faba bean cropping system appears feasible. Therefore, with the collective results obtained from these chapters, the next chapter will evaluate the faba bean crop tolerance to PSII-inhibiting metribuzin, to incorporate in the potential herbicide MOA combination to make these herbicides and HTC more sustainable for the future.

CHAPTER 5 - PS II- inhibiting metribuzin tolerance in *PBA Bendoc* and *PBA Samira* faba bean cultivars

5.1 Introduction

The herbicide-tolerant crop flexibility to ALS-inhibiting herbicides at different growth stages was investigated in Chapter Four. As discussed in previous chapters, herbicide-tolerant crops offer obvious opportunities to control weeds, but reliance on one herbicide MOA has proven to lead to increased herbicide resistance of several problematic agricultural weeds (Beckie, 2006; Lamichhane *et al.*, 2017; Owen & Zelaya, 2005). As a consequence of this side-effect, it is recommended that integrated weed management strategies be used to reduce the imposed selection pressure, which may involve the introduction of an alternative herbicide MOA (Sammons *et al.*, 2007). Therefore, this chapter investigates the flexibility of *PBA Bendoc* and *PBA Samira* faba bean cultivars towards different rates of PSII-inhibiting metribuzin. The findings will help to identify the suitability of metribuzin to be incorporated as a second herbicide MOA in faba bean crops.

Indeed, the use of herbicide MOA combinations has been shown to reduce the selection pressure on weeds, thus decreasing the probability of weed population shifts (Beckie & Harker, 2017; Powles & Gaines, 2016; Stoltenberg, 2002). Yet, before introducing a herbicide MOA combination to a cropping system, it is prudent to evaluate the efficacy of the herbicides in relation to the infesting weeds and to determine the tolerance of the crop to the proposed herbicide MOAs. Failure to take this step may cause significant crop yield losses because of poor weed control and possible herbicide damage to the crop (Kurre *et al.*, 2017). As specifically mentioned in the previous chapter, the herbicide-tolerant *PBA Bendoc* faba bean cultivar has been developed with conventional breeding methods to acquire tolerance to ALS-inhibiting imidazolinone herbicides. Our particular interest is to introduce the PS II-inhibiting metribuzin herbicide as an additional herbicide MOA, which is currently not registered for in-crop application for faba bean cultivation. The label recommendation for metribuzin use in faba bean cropping systems is as a post-sowing pre-emergent application, with the recommended rate being between 180 g/ha to 380 g/ha, depending on the soil clay content. It is noted that the use of low herbicide rates in weed management has become a common approach, as it provides residual control in conservation tillage systems (Owen, 2016). But at the same time, it is recognised that the use of lower herbicide doses than recommended on the label, can lead to enhanced evolution of herbicide resistance in weeds (Délye *et al.*, 2013; Heap & Duke, 2018; Owen, 2016). Therefore, it is critical to identify the most effective herbicide rate in terms of both weed control and crop safety. This refers to the carefully measured and applied dosages to a cultivated broad acre to control a weed infestation without challenging the introduced crop resistance. In addition, by carefully monitoring the herbicide dose, the possibility of building up resistance through mutation within a weed species is avoided (Bonny, 2016). Already having the knowledge of imidazolinone herbicide-tolerant traits in the *PBA Bendoc* faba bean cultivar, this investigation attempts to evaluate its PSII-inhibiting metribuzin sensitivity as an in-crop application and compare it with the alternate *PBA Samira* cultivar.

Among all herbicide MOAs, more than half of the existing herbicides comprise chemicals that interrupt the photosynthetic pathway (Heap, 2014), leading to plant death. Such compounds present in the photosystem II (PS II) inhibiting Group C herbicides, share a common effect of blocking the PSII Hill reaction (Lu *et al.*, 2019). PSII inhibitors comprise nine sub-class categories; amides, benzothiadiazinones, nitriles, phenylcarbamates, pyridazinones, triazines, triazinones, uracils, and ureas (Croplife Australia, 2017). Within the PSII grouping, at the plastoquinone (PQ) binding site on the D1 protein, these compounds compete with PQ. By the inhibition of the PSII electron transport mechanism, it is found that the carbon reduction cycle and the consequent production of NADPH and ATP, are all prevented. This leads to the decline of carbohydrate levels within the plant and an increase in associated oxidative stresses (Powles & Yu, 2010).

Metribuzin, or (4-amino-6-t-butyl-3-methylthio)-1,2,4-triazine-5(4H)-one), is an asymmetric triazinone herbicide with a lifespan judged to be low-to-moderate in the environment, and shows an intermediate solubility in water (1165 mg/l at 20^{o}C) which confers high mobility in soil (Guimarães *et al.*, 2018). The continuous use of such chemicals on a large scale for genetically diverse weed populations, has led to the evolution of PSII-inhibiting herbicide-resistant weeds (Powles & Yu, 2010), and many incidents of this phenomenon have been reported during the last few decades (Burnet *et al.*, 1991; Hashem *et al.*, 2001b; Nabipour *et al.*, 2017; Sheets, 1970; Walsh, 2004). Therefore, to discourage the use of the any kind of same herbicide MOA in herbicide-tolerant cropping systems and to make the use of these herbicides and HTCs more sustainable, our focus is to find out the tolerance of this herbicide-tolerant *PBA* faba bean cultivar to metribuzin. This information will provide leads to identify a herbicide combination strategy where both ALS-inhibiting and PSII-inhibiting herbicides can be incorporated within this cropping system.

5.2 Methodology

The experiment was conducted at Federation University, Australia, in 2018 and 2019, in a completely randomised design with five replicate pots for each treatment with three plants in each adding up to 15 plants per treatment (n=15). The day/ night temperature in the glasshouse was maintained at 22/18 ºC, and the humidity was maintained at 60-70%. Faba bean seeds were treated with a potent inoculant for nitrogen fixation and increased yield, by applying a slurry inoculation procedure using group F Nodulaid®N/T prior to planting. Three seeds of each Faba bean genotype, *PBA Samira* and *PBA Bendoc*, were planted at a depth of 2 cm in plastic pots (19 cm height and 18 cm diameter). The silty clay loam soils collected from fertile Horsham lentil fields were used in the experiment. The soil composition was determined by the laser particle size analysis method as pH of 6.9 (in H_2O), and a composition of 6% sand, 69% silt and 25% clay (Hunt & Gilkes, 1992). Of importance was that the soil had no history of herbicide use for more than two years. Plants were sprayed at the four-node stage using a trolley sprayer, rates representing a wide range of fractional herbicide applications of the recommended field rates of metribuzin. These multiples were 0x, 1/8x (26.25 g/ha), 1/4x (52.5 g/ha), 1/2x (105 g/ha), 1x (210 g/ha - recommended PSPE rate) and 2x (420 g/ha). The spray pressure was set at 200 kPa and minidrift air-inclusion nozzles with a spray angle of 110° and 50 cm distance between the nozzles were used in the boom fitted at the height of 50 cm above the pot rim. Controls were maintained without any herbicide treatment.

Plants were watered daily to eliminate any water stress, and the percentage survival was determined 28 days after herbicide application (DAA) with the criterion of at least one green leaf in the plant. Percentage visual herbicide damage was recorded weekly until 28 DAA, with a visual estimation scale of 0-100% where 0 represents no herbicide damage and 100% being complete death of the plant. At maturity, plant height, number of leaves, number of side shoots, and number of pods per plant, were all recorded. All the plants were then harvested and dried to a constant weight in an oven at 70^{o}C for 72 hours for the estimation of above-ground dry weight.

5.3 Statistical analyses

Data were analysed using IBM SPSS Statistics 25.Ink statistical package. Respecting the non-normality of the original data, all the data were transformed using common transformations, but the normality of the transformed data or the equal variance assumptions were not significantly improved. Therefore, all the analyses were performed on the original data as ANOVA tests are reasonably robust to slight departures from normality (Hahns-Vaughn, 2017). A two-way ANOVA method was used to determine the overall effect of different herbicide dosages on the two faba bean cultivars. For all pairwise comparisons, Tukey's Honesty test was conducted to assess the incidence of significant differences based on the mean responses to different herbicide treatments at a 0.05 confidence level.

5.4 Results

The repeated experiments did not show any significant difference. Hence the data from the two trials were combined before analysis (n=30 per treatment). At the lower rates of metribuzin, 26.25, 52.5, and 105 g/ha, the treated plants survived to maturity, and evidenced only slight departures compared to the control treatments. Conversely, at the higher rates, 210 and 420 g/ha, plants could not survive until maturity. The values were averaged over the cultivars and presented when the effect of cultivar was not significant (Figures 5.2).

5.4.1 Percentage visual herbicide damage

Table 5.1. Percentage herbicide damage in *PBA Bendoc* and *PBA Samira* measured at seven-day intervals until 28 days after application. The numbers within brackets show the standard error of the mean.

Rate (g/ha)	**7 DAA (%)**		**14 DAA (%)**		**21 DAA (%)**		**28 DAA (%)**	
	PBA Bendoc	*PBA Samira*	*PBA Bendoc*	*PBA Samira*	*PBA Bendoc*	*PBA Samira*	*PBA Bendoc*	*PBA Samira*
26.25	0.5 (± 0.3)	1.5 (± 0.4)	1.5 (± 0.4)	2.8 (± 0.5)	2.8 (± 0.5)	3.7 (± 0.4)	3.5 (± 0.5)	3.7 (± 0.4)
52.5	3.8 (± 0.4)	5.2 (± 0.4)	7.7 (± 0.6)	7.7 (± 0.5)	9.7 (± 0.5)	8.0 (± 0.5)	13.8 (± 1.1)	10.2 (± 1.0)
105	20.7 (± 1.3)	7.2 (± 0.5)	29.0 (± 1.1)	19.5 (± 0.6)	35.3 (± 1.0)	47.2 (± 1.1)	66.3 (± 3.5)	69.0 (± 3.2)
210	37.5 (± 1.1)	34.3 (± 1.1)	82.5 (± 1.2)	61.7 (± 1.2)	99.7 (± 0.4)	84.7 (± 1.1)	99.7 (± 0.3)	100 (± 0.0)
420	41.7 (± 0.8)	37.3 (± 1.0)	81.5 (± 1.0)	78.7 (± 0.6)	100 (± 0.0)	99.0 (± 0.7)	100 (± 0.0)	100 (± 0.0)

The percentage herbicide damage showed a significant rate by cultivar interaction (p<0.05). At the early stages of measurement (until 21 DAA), the lower rates of herbicide application (26.25 g/ha and 52.5 g/ha) did not show any significance (p>0.05) between the cultivars, but at the higher rates (105, 210 and 420 g/ha) the *PBA Bendoc* cultivar initially appeared to be more sensitive to

Metribuzin ($p<0.05$) (table 5.1). However, at 28 DAA, there was no significant effect of the cultivar at any application rate ($p>0.05$). For both of the cultivars, the application rate of 26.25 g/ha and the control treatment did not show any significant difference, indicating that this was the best rate with the least herbicide damage to the crop ($p>0.05$).

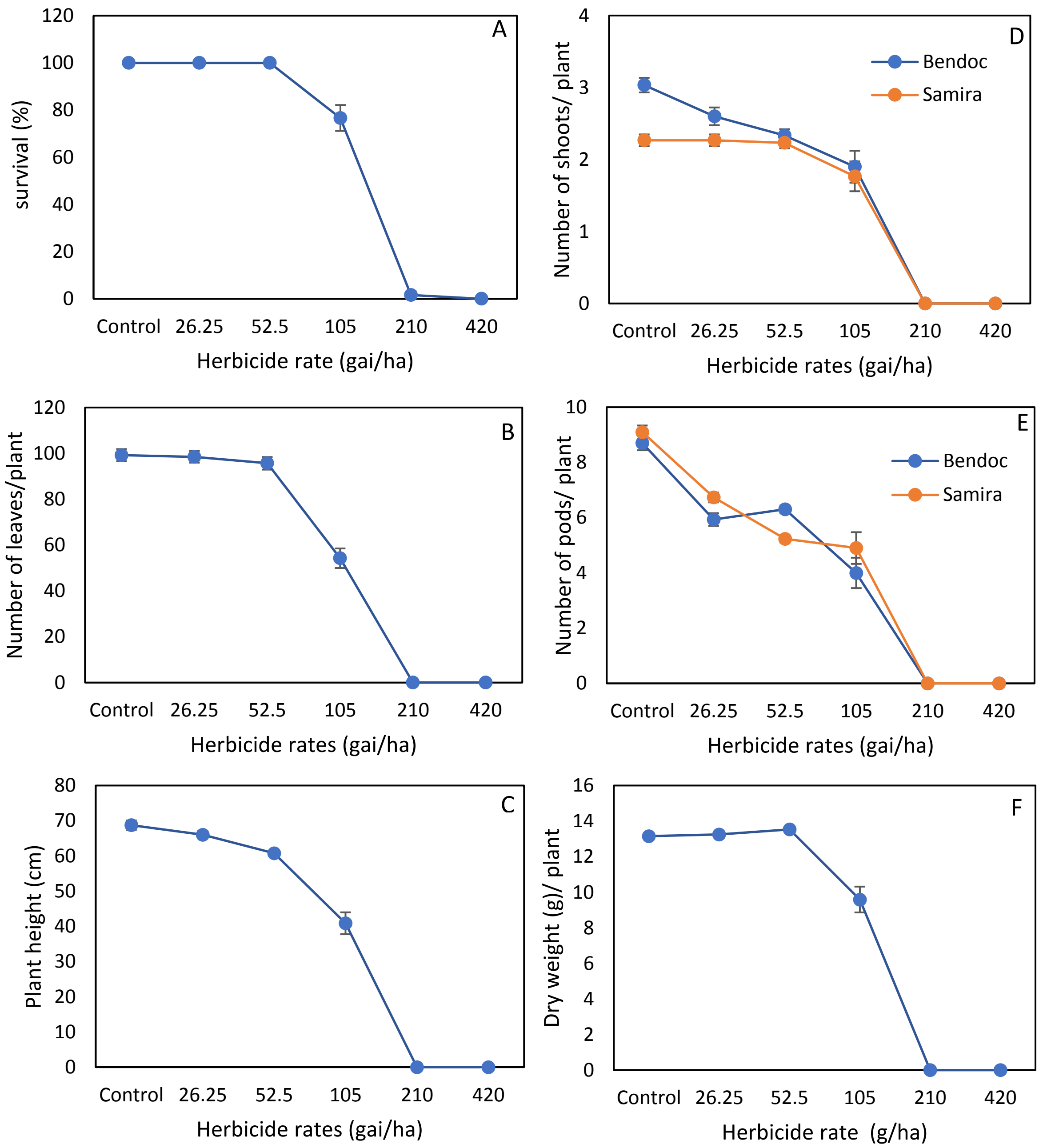


Figure 5.1. A. Percentage survival at 28 days after application, **B.** Number of leaves/ plant, **C.** Plant height, **D.** Number of shoots/plant, **E.** Number of pods/plant **F.** Above-ground plant dry weight in *PBA Bendoc* and *PBA samira* cultivars for different metribuzin rates. The values were averaged over the cultivars and presented when the cultivar was not significant. Vertical bars represent the standard error of the mean.

5.4.2 Percentage survival

It was found that the interaction effect of cultivar by rate was not significant in determining the percentage survival of faba bean plants (*p*=0.995). The cultivar effect was also not significant (*p*=0.774) but the herbicide application rate indicated a difference (*p*<0.05). Therefore, the percentage survival values were averaged over the cultivars and presented in the graph (Figure 5.1.A) (n=60). The rates of 105, 210, and 420 g/ha showed a significant difference compared to the control (*p*<0.05) but not the lower rates; 26.25 and 52.5 g/ha. Therefore, in terms of percentage survival, both cultivars responded similarly, and the two low rates, 26.25 and 52.5 g/ha assured a 100% survival at 28 DAA.

5.4.3 Number of leaves per plant

The number of leaves at harvest did not show a significant difference in the interaction effect of cultivar by herbicide rate (*p*=0.861). The difference was also not significant when comparing the effect on the cultivars (*p*=0.092) but the herbicide rates indicated a difference (*p*<0.05). Therefore, the averaged values for both the cultivars were pooled before analyses (Figure 5.1.B) (n=60). The number of leaves in the control was not significantly different compared to the two lower rates; 26.25 and 52.5 g/ha (*p*>0.05), but was significantly different to the higher herbicide rates (*p*<0.05). Based on these results, it appears that both cultivars are comparatively tolerant of the two lower levels of herbicide.

5.4.4 Plant height

The cultivar by rate interaction was not significant when measured by plant heights (*p*=0.696). The results also showed that the plant height differences were not significant between the cultivars (*p*=0.434) but were different between the applied herbicide rates (*p*<0.05). Therefore, the plant heights were averaged across the cultivars and showed in the graph (Figure 5.1.C) (n=60). Other than the treatment of 26.25 g/ha rate (*p*>0.05), all the other rates showed a significant difference compared to the control (*p*<0.05). Consequently, in terms of plant height, the lowest application rate 26.25 g/ha, which did not show any plant stunting, was identified as the best herbicide application rate for both faba bean cultivars.

5.4.5 Number of side shoots per plant

The interaction effect of cultivar by herbicide rate was significant in the number of side shoots per plant (p=0.004) (Figure 5.1.D). This interaction was evident in the lowest herbicide rate; 26.25 g/ha (p=0.033) and in the control (p<0.05) with a higher number of shoots in *PBA Bendoc*. The highest number of side shoots were observed in the untreated *PBA Bendoc* control (3), and the least was in the *PBA Samira* 105 g/ha herbicide treatment (1.7) when considering the surviving plants. In *PBA Bendoc*, only the 26.25 g/ha treatment was similar to the control but in *PBA Samira* both the low rates, 26.25 and 52.5 g/ha were similar. Overall, the *PBA Bendoc* cultivar showed a higher number of side shoots regardless of the treatment, but interestingly, compared to the controls, the *PBA Samira* cultivar was less affected by applied herbicide rates.

5.4.6 Number of pods per plant

The interaction between the cultivar and the applied herbicide rate was significant in terms of the number of pods per plant (p=0.002) (Figure 5.1.E). This significance was observed in the low herbicide rate treatments; 26.25. 52.5, 105 g/ha (p<0.05) but not in the higher rates; 210, 420 g/ha and the controls (p>0.05). *PBA Bendoc* showed a lower number of pods in all the treatments other than the 52.5 g/ha treatment. Overall, in both the cultivars, the control was significantly different from all the other herbicide treatments (p<0.05), implying that the treatments have reduced the number of pods produced per plant in both *PBA Bendoc* and *PBA Samira* cultivars.

5.4.7 Plant dry weight

The above-ground plant dry weights showed no significant difference between the two cultivars of faba bean (p=0.246), or for the interaction of herbicide rate by cultivar (p=0.972). However, a significant difference was observed between the applied herbicide rates (p<0.05). Hence, the values of the dry weight for two cultivars are averaged together and analyzed (Figure 5.1.F) (n=60). The above-ground plant dry weight of the control was not significantly different for the two lowest applied herbicide rates; 26.25 and 52.5 g/ha (p>0.05) but were significantly different from the higher herbicide application rates (p<0.05). Overall, the two faba bean cultivars showed a similar tolerance towards all the applied herbicide rates and 26.25, and 52.5 g/ha rates appeared to be safe as their dry weights were similar to the controls.

Figure 5.2. Comparison of herbicide damage at 28 DAA in two faba bean cultivars for different rates of metribuzin.

5.5 Discussion

This experiment was conducted to evaluate the level of PSII-inhibiting herbicide (metribuzin) tolerance in two faba bean cultivars to determine if its introduction as a component of the herbicide MOA combination might be efficacious. According to the analysis of the results, low herbicide rates (26.25 and 52.5 g/ha) of metribuzin did not appear to affect either cultivar of faba bean plants when compared to the higher application rates tested (105, 210 and 420 g/ha) for almost all of the parameters evaluated. As is well known, the most common herbicide damage observed from the use of metribuzin on faba bean plants is leaf necrosis and stunted growth (Bertholet and Clark, 1985). However, this stunting of plants was not significant in the tests with the lowest herbicide rate of 26.25 g/ha. Herbicide rates of 210 and 420 g/ha showed more than 50% herbicide damage within the first two weeks of application. Overall, both cultivars showed a similar tolerance towards the lower herbicide rates applied, with the *PBA Samira* strain proving to be less affected in terms of the number of side shoots. Both cultivars were significantly affected with the application of herbicide rates higher than 52.5 g/ha, suggesting that it is not safe to recommend these rates in-crop for these faba bean cultivars.

In pulses, metribuzin is commonly used as a post-sowing pre-emergent (PSPE) herbicide but with occasional use as a post-emergent, which can result in crop damage (Brand, 2012). A similar study has been conducted by Mao *et al.* (2015), with four different faba bean cultivars screened for their metribuzin tolerance in hydroponics, followed by a field validation study. In this study, the faba bean lines, *AF3109* and *Nura* have shown an increased tolerance to metribuzin compared to the other two cultivars, 1952/1 and Farah when applied as post-emergent at five node stage. Supporting the results of the current study, herbicide damage has shown an increase with the increasing herbicide rates, culminating in total plant death at high rates (540, 720 g/ha) in 1952/1 and Farah faba bean cultivars. This study has also shown that a yield reduction can also be associated with the increasing herbicide rates depending on the tolerance of the faba bean cultivar. Around 200 different faba bean accessions screened for metribuzin has resulted in 95% similar or less resistance results as *Nura*. Among the rest of the 5% with improved tolerance, two lines exhibited higher tolerance even compared to AF3109 (Mao *et al.*, 2015). The study of Maalouf *et al.* (2016) in Lebanon and Morocco, also screened 140 accessions of faba bean for metribuzin tolerance when applied as post-emergent at a rate of 210 g/ha. Among the tested accessions, 62 lines have shown tolerance to metribuzin treatment. These studies not only help to screen the tolerant line for future breeding programs but also for the objective of our current study, to introduce alternative herbicide MOAs into those cropping systems.

A similar study has been conducted with glyphosate-tolerant cotton, which introduced different herbicide MOAs to discourage the sole reliance on glyphosate in the cropping system (Iqbal *et al.*, 2019). In this cotton-related work, the post sowing pre-emergent (PSPE) application of pendimethalin showed improved weed control and an increased yield compared to the solitary post-application of glyphosate (Iqbal *et al.*, 2019). This also suggests that leaving one herbicide MOA in a herbicide combination as a PSPE application will not only assure the crop safety but also will help to combat the early growing weeds in the cropping system, especially in poor weed competitors such as pulses. Therefore, in situations where the same herbicide MOA is demanded, HT cropping systems that integrate diverse herbicide strategies, including pre-plant herbicide application, can work to reduce the selection pressure on weeds to evolve with herbicide resistance.

In a cropping system, the ultimate expectation of a farmer is to achieve a good, high quality yield. Though we have not investigated the yield in our current study, we nevertheless could observe a significant reduction in the number of pods compared to the controls in both the cultivars, even with the lowest herbicide rate applied. This may imply a reduced yield in faba bean with the metribuzin in-crop application, which is undoubtedly not appealing for the farmers. Considering all the results obtained from this experiment, it can be concluded that the metribuzin sensitivity in both faba bean cultivars is comparatively similar, and the least herbicide rate tested, which was 26.25 g/ha, had the least effect on the plants when compared with the controls. The primary concern, according to these results, was the significant number of pods per plant reduction in both the cultivars, even at the lowest herbicide rate applied. Therefore, further studies, especially field trials, should be conducted to evaluate the above parameters in real field conditions before introducing PS II-inhibiting metribuzin as a component in a herbicide combination ready to apply as an in-crop in faba bean cropping system. A particular focus should be given on grain yield, as a reduction of the number of pods was evident in current pot trials.

Given that the ALS-inhibiting chemical group is in high-risk herbicide category, its continuous use is inevitably leading to a rapid evolution of herbicide resistance in weeds (Tranel & Wright, 2002; Whitcomb, 1999). This is also evident in the international survey of Heap (2019), which showed a global chronological increase in herbicide-resistant weeds to ALS-inhibiting herbicides over the last few decades. Consequently, any attempt to discourage the solitary use of a herbicide belonging to this chemical group will add to its sustainability as an effective herbicide for the future. Moreover, the development of a herbicide-tolerant crop, regardless of it being genetically modified or conventionally bred, is an asset that comes with a considerable cost, time, and effort (Devine, 2005; Green, 2018). It also brings improved yet straightforward weed control strategies, such as reduced labour and machinery costs, improved quality and quantity of yield, and flexibility in herbicide application timing, etc. (Sankula *et al.*, 2005). Therefore, to make the most out of HTCs and to make this approach sustainable for the future, it is essential to avoid misuse of the herbicide MOA for which it is tolerant. With this concern, any attempt to reduce or to discourage the continuous application of imidazolinone herbicides in the imidazolinone tolerant faba bean line will be advantageous on its long-term acceptance as a valuable asset.

Based on the above results, we can only recommend metribuzin to be applied as a PSPE (label recommendation 210 g/ha) in *PBA Bendoc* and *PBA Samira* cropping fields due to the observed significant pod reduction when applied in-crop even at the lowest herbicide rate. However, though metribuzin is recommended for use as PSPE in faba bean, its activity has been shown to be seasonal and variable (Bertholet & Clark, 1985; Lemerle & Hinkley, 1991; Mao *et al.*, 2015). Therefore, the next chapter will further investigate the effect of PSPE application of metribuzin as a component of herbicide combination on *PBA Bendoc* and *PBA Samira* faba bean cultivars.

CHAPTER 6 – Effect of sequential application of ALS-inhibiting and PSII-inhibiting herbicides on *PBA Bendoc* and ALS-inhibiting herbicide-resistant *Raphanus raphanistrum*

6.1 Introduction

As discussed in previous chapters of this thesis, the introduction of herbicide-tolerant crops (HTCs) is one of the significant reasons for herbicide-resistance evolution in weeds during the last few decades. The introduction of glyphosate-tolerant crops and sole reliance on glyphosate in cropping systems, which lead to a gradual increase of glyphosate-resistant weeds, is one such example where a HTC was done wrong (Green, 2018; Sammons *et al.*, 2007; Shaner, 2000). As it was difficult to develop glyphosate resistance in crops, the thinking or the prediction that glyphosate resistance evolution in weeds in nature would be difficult was implanted at the introduction of glyphosate-tolerant crops (Bradshaw *et al.*, 1997). With such learned lessons from past misuses of herbicides, it is suggested that using the herbicide mode-of-action (MOA) tolerant by the HTC in combination with other herbicide MOAs will make both the HTC and the existing herbicides sustainable for the future (Green & Owen, 2010). Therefore, the objective of this chapter is to identify the best herbicide combination strategy to control herbicide-resistant *Raphanus raphanistrum* while assuring the HTC safety.

The herbicide combination strategies include herbicide MOA rotations, sequential applications, and tank mixes; depending on the application time of herbicide MOAs involved. Herbicide MOA rotation and sequential application are also known as herbicide "cycling" which employ the two herbicide MOA applications in subsequent growing seasons and the same growing season respectively while the tank mixes involve a simultaneous application of herbicide MOAs (Beckie, 2006; Evans *et al.*, 2016). Herbicide rotation is recognised as the most common herbicide resistance management strategy in Australia (Beckie & Reboud, 2009; Peerzada *et al.*, 2019; Shaner *et al.*, 1999). Despite the efficacy of such combinations in controlling weeds while reducing the herbicide resistance evolution, selecting the herbicides and herbicide MOAs without imposing any crop damage and yield loss has become challenging in HTC systems. The study of Harker *et al.* (2000) has investigated HT canola crop injury and yields with the application of standard herbicides (recommended for conventional canola) and designated herbicides. The tank mix of two standard herbicide MOAs has shown no crop injury but a yield reduction compared to the designated herbicide treatments. In contrast, in the study of Iqbal *et al.* (2019) the introduction of an alternative herbicide MOA in glyphosate-tolerant cotton as a pre-emergent herbicide has proven to improve the seed cotton yield compared to the post sole application of glyphosate. Therefore, before introducing any alternative herbicide MOA combinations to the HTCs, it is a must to evaluate the crop performance and weed control for the suggested herbicide combination.

In our previous experiment in Chapter Five of this thesis, we identified the best herbicide rate and time for PS II-inhibiting herbicide metribuzin (Met) application in *PBA Bendoc* to be as the label recommendation (Metribuzin 210 g/ha PSPE application). Improved tolerance in *PBA Bendoc* to ALS-inhibiting herbicides; imazamox + imazapyr, and imazethapyr at different growth stages were investigated in Chapter Four. Weed herbicide sensitivity was also investigated in Chapter Three, where the most sensitive growth stage of *R. raphanistrum* was recognized to be the 2-4 leaf stage for the highly effective ALS-inhibiting herbicide; imazamox + imazapyr regardless of the biotype. Bringing all these findings together, this chapter will discuss the effect of sequential applications of metribuzin and imazamox + imazapyr on *PBA Bendoc* and herbicide-resistant *R. raphanistrum*. Therefore, the objective of this chapter is to evaluate the efficacy of these herbicide MOA combinations in terms of crop sensitivity and herbicide-resistant *R. raphanistrum* control. The findings of this experiment will be of great value for herbicide application decision making to discourage the continued use of the same herbicide MOA. Further, this will also help to identify the best herbicide strategy to control herbicide-resistant *R. raphanistrum* while exploring the flexibility of *PBA Bendoc* for these herbicide MOA combinations to assure crop safety.

6.2 Methodology

The experiment was conducted in a completely randomised design in a temperature-controlled glasshouse at Mount Helen Campus of Federation University Australia in 2019. The day/night temperature and humidity in the glasshouse were maintained at 22/18^{o}C and 60-70%, respectively.

Five seeds of *R. raphanistrum* resistant biotype and Faba bean *PBA Bendoc* cultivar were planted at a depth of 1 cm and 2 cm respectively in plastic pots (19 cm height and 18 cm diameter). Once the seedlings were established, plants were thinned down to two *R. raphanistrum* and three faba bean plants per pot. The silty clay loam soil (Hunt & Gilkes, 1992) used for this experiment was collected from Horsham lentil fields with a composition of pH 6.9 (H_2O), 6% sand, 69% silt, and

25% clay; with no history of herbicides for more than two years. The recommended herbicide rates were used for both ALS-inhibiting imazamox + imazapyr (24.75 g/ha + 11.25 g/ha); and PS II inhibiting metribuzin 210 g/ha. The spray pressure was set at 200 kPa, and mini drift air inclusion nozzles with a spray angle of 110° were used in the boom holding at the height of 50 cm above the pot rim. Each treatment consisted of five replicates. Controls were maintained without any herbicide treatment (rate 0). The experiment consisted of six treatments separately for *PBA Bendoc* and *R. raphanistrum*; Control, Metribuzin PSPE, Metribuzin PSPE followed by imazamox + imazapyr at four different growth stages (Table 6.1).

Plants were watered daily to eliminate any water stress, and the seedling survival was determined 28 days after herbicide application (DAA) with the criterion of one green leaf in the plant. Percentage herbicide damage was recorded weekly until 28 DAA. At maturity, plant height, number of leaves, leaf area, and number of pods/plant were recorded. To measure the leaf area, the leaves were gently removed manually, and the area was measured using a Planimeter (Paton Electronic Planimeter developed in conjunction with CSIRO. Serial number 711-14-531/21). All the plants were then harvested and dried in an oven at 70°C for 72 hours to discern the above-ground dry biomass.

Table 6.1. The herbicide treatments at different growth stages for *PBA Bendoc* and *Raphanus raphanistrum*.

Treatment	**Herbicide and growth stage applied**		
	Metribuzin (Met)	imazamox + imazapyr (Int)	
		PBA Bendoc growth stage	*R. raphanistrum* growth stage
1 (control)	-	-	
2	PSPE	-	-
3	PSPE	PSPE	2-4 leaf stage (4L)
4	PSPE	3-4 node (4N)	6 leaf stage (6L)
5	PSPE	6-8 node (8N)	8 leaf stage (8L)
6	PSPE	Flowering (Flow)	Flowering (Flow)

Note: The abbreviations used in the table are post-sowing pre-emergent as PSPE; metribuzin as Met; imazamox + imazapyr as Int.

6.3 Data Analysis

Univariate and repeated measure methods in the General linear model (GLM) were conducted using the IBM SPSS statistics 25.Ink statistical software release 18; to analyse all the data. With the results of the Shapiro-Wilks test, non-normal residues were treated with common transformations to obtain a set of normal data, but the normality or equal variance assumptions were not greatly improved. The ANOVA outputs obtained from transformed and untransformed data produced similar results. With the fact that ANOVA tests are reasonably robust to the slight departures of normality and equal variance (Hahns-Vaughn, 2017), all the analyses were conducted on the original data. For Post-hoc multiple comparisons, Tukey's honest significant difference (HSD) was performed.

6.4 Results

6.4.1 *Raphanus raphanistrum*

A 100% control of *R. raphanistrum* was achieved in all the treatments with PSPE metribuzin 210 g/ha, and hence the data is not shown. No plants survived for a second herbicide application (Figure 6.3).

6.4.2 Faba bean

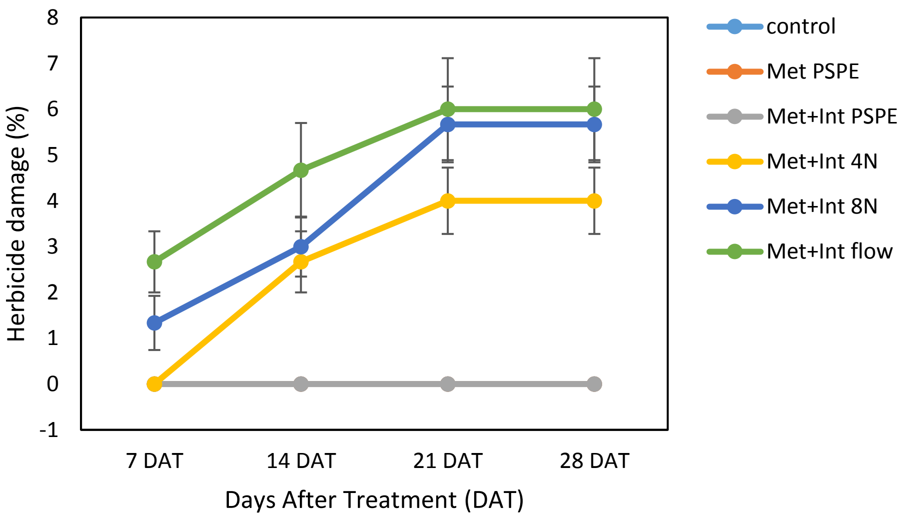


Figure 6.1. Percentage visual herbicide damage in faba bean plants at 7-day intervals after the herbicide treatments. Vertical bars represent the standard error (+/-) of the mean.

The effect of the herbicide treatment was significant in percentage visual herbicide damage ($p<0.05$) (Figure 6.1 and 6.4). At the beginning (7 DAA), control was significantly different only from the treatment of metribuzin 210 g/ha PSPE + imazamox 24.75 g/ha + imazapyr 11.25 g/ha flowering ($p<0.05$). But beyond 14 DAA, treatments other than Metribuzin 210 g/ha PSPE, and imazamox 24.75 g/ha + imazapyr 11.25 g/ha PSPE + Metribuzin 210 g/ha PSPE, all the other treatments showed a significant difference from the control ($p<0.05$). The least herbicide damage was observed in the herbicide treatments where both the herbicides were kept as PSPE and in the PSPE metribuzin treatment (0%) and the highest was in the imazamox 24.75 g/ha + imazapyr 11.25 g/ha flowering treatment (6%).

The effect of herbicide treatment was not significant when comparing the percentage survival at 28 DAA as all the plants in all the herbicide treatments survived with a percentage of 100% ($p>0.05$) (Figure 6.2.A). Therefore, all the herbicide treatments were similarly tolerant by the *PBA Bendoc* faba bean plants while being equally similar to the controls in terms of percentage survival at 28 DAA. The parameters, above-ground plant dry weight, plant height, number of leaves, leaf area and number of pods in faba bean plants were also not significantly affected ($p>0.05$) by the herbicide treatment. This proves the *PBA Bendoc* cultivar's improved tolerance to all the herbicide treatments evaluated in this experiment. The graphs are provided in figure 6.2.

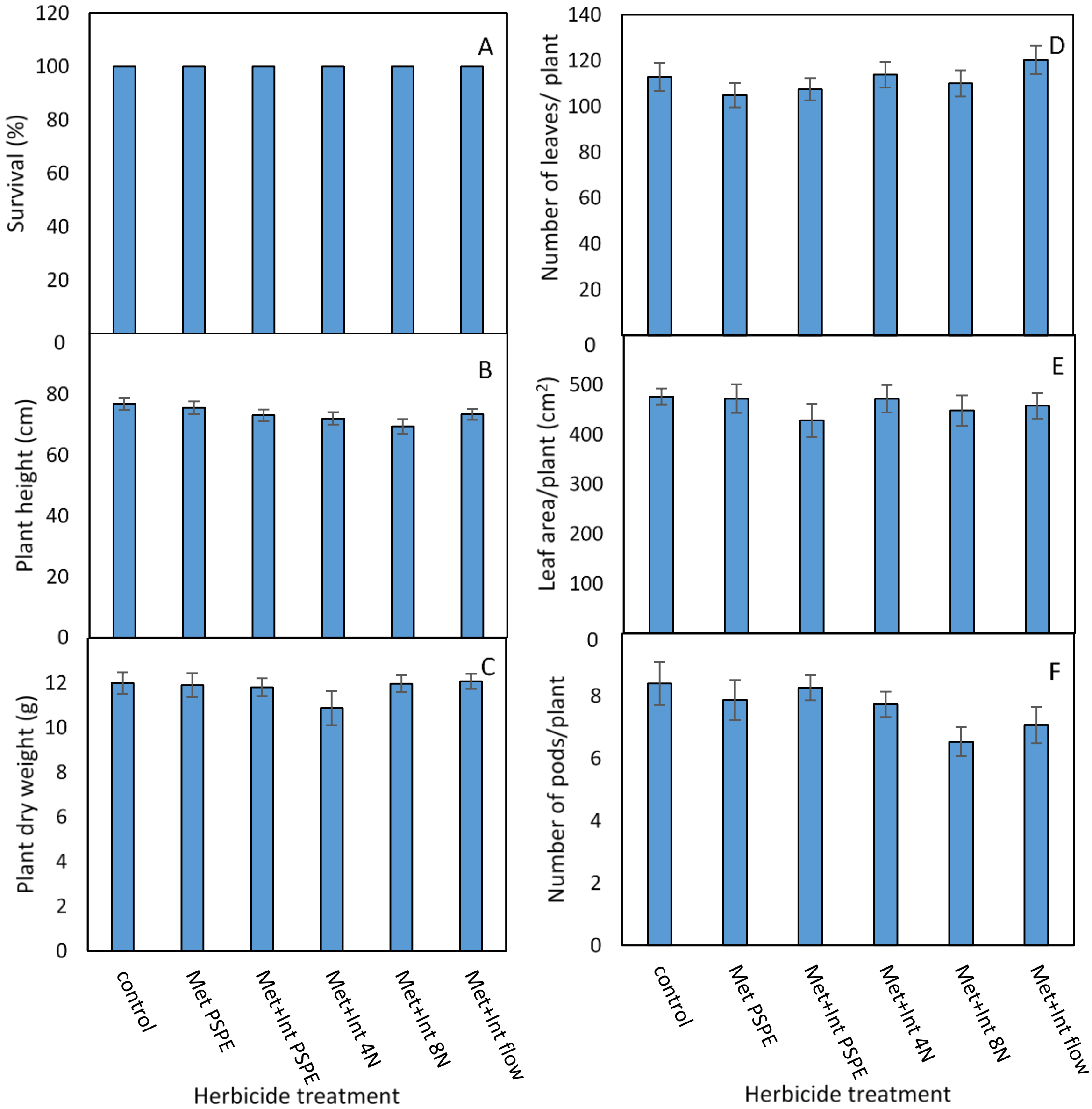


Figure 6.2. A. Percentage plant survival at 28 DAA, **B.** Plant height, **C.** Above-ground plant dry weight, **D.** Number of leaves per plant, **E.** Leaf area per plant and **F.** Number of pods per plant on Faba bean plants at harvest for different herbicide treatments. Vertical bars represent the standard error (+/-) of the mean.

Figure 6.3. The visual herbicide damage in *R. raphanistrum* representing all the herbicide treatments compared to control.

Figure 6.4. The visual herbicide damage in *PBA Bendoc* for the herbicide treatments compared to the control

6.5 Discussion

In this study, the application of metribuzin 210 g/ha at the PSPE stage proved to be the best treatment to control *R. raphanistrum*. This herbicide treatment assured a 100% control of *R. raphanistrum,* which does not encourage a second herbicide in the cropping system unless any seedlings escape. With regards to the crop, metribuzin 210 g/ha PSPE treatment showed an excellent tolerance in *PBA Bendoc* for all the parameters evaluated, with a similar performance compared to the controls. This finding strengthens the claim of metribuzin 210 g/ha PSPE application for controlling *R. raphanistrum* while assuring the *PBA Bendoc* crop safety.. This finding will be beneficial to discourage the sole application of ALS inhibiting herbicides in the *PBA Bendoc* faba bean cropping area as it provides information on another promising herbicide MOA (PS II inhibiting metribuzin). Therefore, PS II inhibiting metribuzin can be recommended as a PSPE treatment in this cropping field to reduce the selection pressure on weeds for ALS-inhibiting herbicides without threatening the crop safety. The use of pre-emergence herbicides leading to a situation where no further herbicide treatments are required has also been supported in the study of Ellis and Griffin (2002). Also, it has suggested that the reduced herbicide resistance in weeds is promising when another herbicide MOA is used as PSPE treatment in HT cropping systems (Ellis & Griffin, 2002).

In *PBA Bendoc*, the effect of herbicide treatment was not significant in any of the parameters evaluated other than percentage visual damage ($p<0.05$). The percentage visual damage was not significantly different from the control when both the herbicides were kept as PSPE. Considering the other parameters, *PBA Bendoc* proved to be equally tolerant to all the herbicide treatments tested as there was no significant difference among the treatments and the control. But to assure the least herbicide damage to the crop, herbicide treatment of imazamox 24.75 g/ha + imazapyr 11.25 g/ha PSPE + metribuzin 210 g/ha PSPE or the sole application of metribuzin 210 g/ha PSPE can be recommended as the best herbicide treatments. Yet, as all the herbicide treatments resulted with a percentage visual damage less than 7%, the other herbicide treatments can also be regarded as safe to use in *PBA Bendoc* faba bean cropping systems if required.

In the real field conditions, these identified herbicide treatments are ideal if the weed; *R. raphanistrum* is also at the PSPE stage, which will assure its 100% control. But in chapter three, we have proven that a 60% control of ALS-inhibiting herbicide-resistant *R. raphanistrum* could be achieved when imazamox 24.75 g/ha + imazapyr 11.25 g/ha is applied at 2-4 leaf stage. Since *PBA*

Bendoc proved to be quite flexible with almost all the herbicide treatments, rather than using both herbicides together as PSPE, the application of PSPE metribuzin 210 g/ha alone will leave another herbicide option to incorporate at a later stage if required. Therefore, if *R. raphanistrum* seedlings escape the initial herbicide treatment of PSPE metribuzin 210 g/ha application, small seedlings of *R. raphanistrum* up to the 4 leaf stage, should be controlled with the second application of imazamox 24.75 g/ha + imazapyr 11.25 g/ha. This strategy could not be tested as there were no escaped seedlings from the Metribuzin 210 g/ha PSPE treatment. Hence future work needs to be done to investigate the effect of imazamox 24.75 g/ha + imazapyr 11.25 g/ha on *R. raphanistrum* plants that survive applications of PSPE metribuzin 210 g/ha.

Therefore, this study suggests two herbicide strategies that can be used in *PBA Bendoc* cropping systems depending on the *R. raphanistrum* seedling survival upon the initial herbicide application. With this notion, the first strategy is, in a situation where a 100% control of *R. raphanistrum* is achieved with the PSPE metribuzin 210 g/ha sole application. It demonstrates that no second herbicide MOA is required for control of *R. raphanistrum*. Therefore, in terms of sustainable use of both herbicide MOAs in *PBA Bendoc* cropping systems in the long term, herbicide MOA rotation can be recommended as one herbicide strategy to implement. Further studies are required to investigate other potential herbicide MOAs to use as herbicide rotations in this cropping field. The second strategy is the application of imazamox 24.75 g/ha + imazapyr 11.25 g/ha at 2-4 leaf stage if the *R. raphanistrum* seedling survival is evident after the initial treatment of PSPE metribuzin 210 g/ha. This approach makes the strategy a sequential application that will involve future research work, as mentioned before. The crop, *PBA Bendoc* growth stage at the application of imazamox 24.75 g/ha + imazapyr 11.25 g/ha is not a pressing issue as the results of our current study proved its flexibility for the in-crop application of this herbicide.

Highlighting the need for such herbicide resistance control tactics in today's agriculture, the study of Costa and Rizzardi (2014), has shown that resistant *R. raphanistrum* plants were insensitive to the ALS-inhibiting herbicide metsulfuron-methyl until twice the dose of commercial purpose. With this regard, any step taken to avoid continuous use of the same herbicide MOA is vital in reducing the rate of herbicide resistance evolution in weeds. Even though herbicide MOA rotation is the most commonly practiced herbicide strategy to reduce selection pressure, it is known to be less effective in reducing the non-target site resistance in weeds (Beckie & Harker, 2017; Beckie & Reboud, 2009). It is also recommended to use low-risk herbicide MOAs back-to-back with high-risk herbicide MOAs (eg. ACCase and ALS inhibitors) when implementing the herbicide rotation cycles (Beckie & Harker, 2017). With this notion, future work needs to be done to identify a potential low-risk herbicide MOA to incorporate in the herbicide MOA rotation cycle along with moderate risk metribuzin.

The sequential application of herbicides of different MOAs has been adopted as a result of reduced or zero till systems where the second tactic of the sequential strategy was initially supposed to be an unrelated technique to the initial herbicide knockdown (Davidson *et al.*, 2019). Supporting the current study's recommendation, the effectiveness of sequential applications of two different herbicide MOAs has been reported around the world (Davidson *et al.*, 2019; Stewart *et al.*, 2011). The increased potential to slow down glyphosate resistance has been evident with the sequential application of different herbicide MOA (Davidson *et al.*, 2019). In the study of Borger and Hashem (2007), good control of *L. rigidium* was achieved by the application of glyphosate followed by paraquat at different growth stages and has proven to be more effective than their single treatment. The same herbicide combination treatment has shown a 100% control of *Echinochloa colona* plants at different growth stages ranging from early to late tillering. This has also been supported by the study of Davidson *et al.* (2019), where the sequential application of isoxaflutole followed by paraquat achieved a 100% control of *E. colona* while all the other tested sequential applications also stood out to give a high level of control when compared to the single herbicide applications. The sequential application of group I herbicides followed by paraquat with a 7-day interval between the applications has also proved to be effective in controlling *Conyza bonariensis* (L.) Cronq. compared to their single herbicide applications (Werth *et al.*, 2010). The sequential herbicide application method has proven to be effective on both broadleaf and grass weeds and to control herbicide-resistant weed populations (Davidson *et al.*, 2019). This also imposes an assuage selection pressure for the selective in crop herbicides as its effectiveness can result in reduced numbers of herbicide-resistant weeds (McGillion & Storrie, 2006).

Therefore, according to the results obtained in the current experiments and considering the real field conditions where the weeds can be at any growth stage, it is recommendable not to apply both the herbicides simultaneously as PSPE. As discussed above, with the proven efficacy of metribuzin on *R. raphanistrum*, sole application of metribuzin 210 g/ha at PSPE can be recommended as the best strategy leaving the option of imazamox 24.75 g/ha + imazapyr 11.25 g/ha as a potential second herbicide MOA to control escaped *R. raphanistrum* seedlings. The time between the two herbicide applications can also vary depending upon the weed species, weed growth stage, and the herbicide MOAs involved (McGillion & Storrie, 2006). Supporting the herbicide strategies of different MOAs recommendations from other studies, findings of the current study will be a highlight as it provides information on both weed control and crop safety. Therefore, to discourage the use of the same herbicide MOA; ALS-inhibiting herbicide in the *PBA Bendoc* cropping system, these findings will be advantageous as we could identify another promising herbicide MOA; PS II-inhibiting metribuzin to control the troublesome ALS-inhibiting herbicide-resistant *R. raphanistrum* while assuring the faba bean *PBA Bendoc* crop safety.

CHAPTER 7 – Differential germination success of resistant and susceptible biotypes of *Raphanus raphanistrum* to ALS-inhibiting herbicides

7.1 Introduction

As discussed in the previous chapters, *Raphanus raphanistrum* has become a problematic weed all over the world, especially in temperate regions including Europe, North America, South America, Australia and New Zealand (Parsons & Cuthbertson, 2001). Its success as a widespread weed is attributed to its characteristics of prolonged seed longevity in soil, the large number of seeds produced by a single *R. raphanistrum* plant, competitiveness due to rapid seedling establishment and its fast growth rate (Cheam, 1986; Walsh *et al.*, 2007). Reduction in grain crop yield due to the crop-weed competition has been evident with *R. raphanistrum* populations present in major crops including faba bean, canola and wheat (Blackshaw *et al.*, 2002; Code & Donaldson, 1996; Eslami *et al.*, 2006; GRDC, 2013). According to Llewellyn *et al.* (2016), *R. raphanistrum* is Australia's most problematic broad leaf weed, covering an area of 5,091,752 ha, and causing a yield and revenue loss of 192,321 tonnes and $A53 million respectively for Australian grain growers. Consequently, herbicide resistance in *R. raphanistrum* has now become the major challenge for cropping systems in Australia (M. J. Owen *et al.*, 2015). It has been clearly identified that *R. raphanistrum* is resistant to the herbicide modes of action of ALS-inhibitors, PS II inhibitors, synthetic auxins, carotenoid biosynthesis inhibitors, and EPSP synthase inhibitors (Heap, 2019). Of these chemicals, ALS-inhibitors, especially subgroups sulfonylurea and imidazolinones, are most commonly used to control *R. raphanistrum,* hence resistance is more evident towards these herbicides in cropping systems (Boutsalis & Powles, 1995; Hashem *et al.*, 2001a; Hashem & Dhammu, 2002; Pandolfo *et al.*, 2013; Walsh *et al.*, 2001; Yu *et al.*, 2003).

Herbicide resistance in a weed species arises from continued natural selection of the initial few resistant plants in a population, leading to virtually complete resistance of the population in future generations due to alterations in genetic and phenotypic characteristics (Heap, 2014; Maxwell *et al.*, 1990). To implement herbicide-resistance prevention and assist weed management tactics, the evaluation of seed germination and seedling emergence in resistant and susceptible biotypes of this species is essential (Gill *et al.*, 1996; Tang *et al.*, 2015; Wu *et al.*, 2016). Studies to this point have indicated that interaction between internal (biotic) and external (abiotic) factors contribute to aggressive seed germination tactics, which lead to its successful establishment in ecological niches (Tang *et al.*, 2015).

Internal factors which affect seed germination include seed maturity, seed vigour, dormancy mechanisms and genetic composition of the seed (Wu *et al.*, 2016). Directly influencing most of these factors are the enzymatic action and other physiological changes which occur while acquiring herbicide resistance, and these mechanisms within resistant biotypes may significantly alter the seed germination and ecological fitness of the seeds (Cechin *et al.*, 2017; Eberlein *et al.*, 1999). The soil seed bank persistence of herbicide-resistant biotypes is also determined by the characteristics of their seeds (Ghersa & Martınez-Ghersa, 2000), noting that the seed 'fitness' differences of susceptible and resistant biotype seeds come from their different invading ability in natural habitats (Torres-García *et al.*, 2015). As a result of these changes, the seed germination characteristics, the fitness of the plant to compete, and the aggressive nature of the invasiveness of the emerged seedlings, may also vary (Wu *et al.*, 2016). It has also been noted that the fitness costs between resistant and susceptible biotypes can vary when grown in herbicide-free ecological niches, an aspect which needs to be carefully investigated (Délye *et al.*, 2013; Vila-Aiub *et al.*, 2015). In a similar manner, studies have shown that the Acetyl CoA carboxylase inhibiting herbicide resistance in grasses comes with a serious fitness cost compared to susceptible seeds, in terms of reduced seed vigour, longevity and germination (Gundel *et al.*, 2008; Vila-Aiub *et al.*, 2005a; Vila-Aiub *et al.*, 2005b). Therefore, to fully understand the systematic basis for weed management, it is essential to study the germination success of herbicide resistant and susceptible seeds, particularly under different abiotic conditions.

Light, temperature, burial depth, pH and water availability are some of the external factors affecting the germination and emergence of weed seeds (Chachalis & Reddy, 2000; Chauhan *et al.*, 2006d; Koger *et al.*, 2004). Among these variables, other than moisture, temperature and light are widely regarded as the most critical environmental factors to regulate seed germination (Ebrahimi & Eslami, 2012), but it has been noted that temperature responses can be varied among and within species as the genetic composition of the plant alters (Debeaujon *et al.*, 2000; Van Assche *et al.*, 2002). Further, moisture stress imposed by salt levels and osmotic potential in the growth medium can delay seed germination as it restricts water and iron intake of the seed (Norsworthy & Oliveira, 2006). The complexity of this issue is illustrated by the observation that whilst pH can affect the seed germination rates, some weeds are tolerant to a wide range of pH levels, and in addition, for some weeds, high iron concentrations can be toxic (Chejara *et al.*, 2008;

Ebrahimi & Eslami, 2012; Mahmood *et al.*, 2016). The depth of seed burial is another determining factor in seed germination and seedling emergence as it limits light penetration and alters ambient germination temperatures (Shaw *et al.*, 1991).

A comparison between the herbicide resistant and susceptible seeds of *R. raphanistrum* has not been previously conducted, and hence this study will lead to new findings to address the complexity of *R. raphanistrum* soil seed bank management. Consequently, the objective of this study is to evaluate and compare the germination success of ALS-inhibiting herbicide-resistant *R. raphanistrum* and its sensitive biotype to different abiotic factors: temperature, photoperiod, pH, osmotic stress, salinity stress, and burial depth.

7.2 Materials and methods

7.2.1 Seed collection and processing

Raphanus raphanistrum seeds of the two biotypes, ALS-inhibiting herbicide resistant and susceptible, grown under same edaphic conditions in South East South Australia were purchased in May 2019, from Plant Science Consulting, South Australia. The resistant seeds confirmed their resistance to Triasulfuron with 80% survival and Imazamox + Imazapyr with 50% survival at the 3-4 leaf stage in the pre-confirmed studies carried out by Plant Science Consulting, South Australia. Seeds were stored until required in labelled, dark, air-tight glass bottles at room temperature (19ºC) in the seed ecology laboratory at Federation University, Australia, Mt Helen, Australia. Before the germination trials, seeds were surface-sterilized using 1% w/v sodium hypochlorite for 5 min and washed with sterile reverse-osmosis (RO) water.

7.2.2 General seed germination protocol

Five experiments were conducted to assess the germinability of herbicide resistant and susceptible *R. raphanistrum* seeds. Each experiment consisted of three replicates of 20 seeds for each population and were arranged in a completely randomized design. The experiments were repeated to give a total of six replicates for each biotype in each treatment.
Germination experiments were conducted during July-September 2019 at the seed ecology laboratory, Federation University Australia, Mt Helen, Australia. The surface sterilised seeds were evenly placed in a 9 cm diameter Petri dish lined with Whatman No 10 filter paper. Filter papers were moistened with 9 ml of RO water or the relevant treatment solution (see below) to provide

adequate moisture for the seeds to germinate. Petri dishes were sealed with Parafilm to ensure moisture retention. To mimic the 24 h dark conditions, Petri dishes were wrapped with double-layered aluminium foil and green light was used while taking the observations to avoid any interference with light. Petri dishes were then incubated in seed germination cabinets (Thermoline Scientific and Humidity cabinet, TRISLH-495-1SD, Vol. 240, Australia) equipped with cool-white fluorescent lamps to provide a photosynthetic photon-flux of 40 µmol $m^{-2}s^{-1}$. Observations were made daily for six weeks and the seeds were regarded as germinated when the radicle was approximately 2 mm long and cotyledons had emerged (Ferrari & Parera, 2015). Non-germinated seeds were tested for their viability using 2,3,5-triphenyltetrazolium chloride (TTC) (Saatkamp *et al.*, 2011; Van Waes & Debergh, 1986). Based on the findings of the temperature and photoperiod experiment, the identified optimal temperature and photoperiod; 25ºC/15ºC alternating 12-hourly day/night temperature under a 24 h dark condition, was used in subsequent experiments.

7.2.3 Effect of temperature and photoperiod on seed germination

Seeds of the two biotypes were exposed to two photoperiod regimes, 12 h light/12 h dark and 24 h dark, under four temperature regimes in incubators set at 17ºC/7ºC, 25ºC /15ºC, 30ºC /20ºC and 35ºC/25ºC (alternating 12-hourly day/night temperature). The temperature regimes were selected to include the temperature variations in Victoria during the normal germination periods of *R. raphanistrum* (autumn and winter), and the hotter summer extremities.

7.2.4 Effect of pH

A range of buffer solutions (pH values of 4 to 10) to examine the effect of pH on seed germination was prepared according to Chachalis and Reddy (2000). For control comparisons, distilled water was used with a pH of 6.2. Buffers were prepared using 2 mM solutions of potassium hydrogen phthalate (pH 4), MES (2-(N-morpholino) ethanesulfonic acid) (pH 5 and 6), HEPES (N-2-hydroxyethyl) piperazine-N0-(2-ethanesulfonic acid)) (pH 8) and Tricine (N-Tris (hydroxymethyl) methyl glycine) (pH 9 and 10). The specific pH values were obtained by adjusting with 1 M HCl or NaOH. *R. raphanistrum* seeds were placed in the Petri dishes lined with relevantly moistened filter papers and were incubated.

7.2.5 Effect of osmotic stress

Effect of osmotic stress on seed germination was tested in aqueous polyethylene glycol (PEG) solutions with an average molecular weight of 8000 (SigmaAldrich, St. Louis, MO, USA). PEG was dissolved in sterilised distilled water to obtain different concentrations of osmotic potential solutions adjusted to the incubation temperature (0, -0.1, -0.2, -0.4, -0.6, -0.8, and -1.0 MPa) (Michel, 1983). Seeds were placed in Petri dishes lined with filter papers moistened with respective PEG solutions and were incubated in the standard way.

7.2.6 Effect of salinity stress

To evaluate the effect of salinity on seed germination of *R. raphanistrum*, a range of sodium chloride (NaCl) concentrations (0, 25, 50, 75, 100, 150, 200, and 250 mM) were prepared by dissolving NaCl (Mallinckrodt Baker, Phillipsburg, NJ, USA) in sterile distilled water. The concentrations were selected to simulate the salinity conditions in typical Australian soils (Chauhan *et al.*, 2006b). The seeds were placed in Petri dishes lined with filter papers moistened with different concentrations of NaCl and were incubated.

7.2.7 Effect of seed burial depth

For the seed burial depth experiment, silty clay loam soil was collected from Horsham (S 36.42.928; E 142.06.703), Victoria in 2018 which had a pH of 6.9 (in H_2O) and a composition of 6% sand, 69% silt and 25% clay (Hunt & Gilkes, 1992). To check the effect of seed burial depth, seeds were buried under different depths (soil surface, 1, 2, 4, 6, 8 cm) of soil. The punnets were first layered with sterilised soil and twenty seeds were placed on soil surface in each punnet (10 cm x 6 cm x 6 cm). Then the seeds were covered with more sterilised soil to achieve the desired depths. A constant water supply was facilitated by placing the punnets in a large butcher's tray filled with water and then the trays were incubated in the germination cabinets. A seed/seedling was considered germinated/emerged when the cotyledons were visible.

7.3 Statistical analysis

All the data were subjected to analysis of variance (ANOVA), using IBM SPSS Statistics version 25. The effect of temperature and photoperiod on seed germination of *R. raphanistrum* biotypes was evaluated using a three-way ANOVA with main effects of temperature, photoperiod and biotype. The optimal growing conditions identified during these experiments were used for subsequent experiments. The data from other experiments were each subjected to a two-way ANOVA with the various factors of pH/osmotic stress/burial and biotype. For each of the ANOVAs conducted, significant ($p<0.05$) main effects were further explored with Tukey's Honestly significant difference (HSD) tests. Significant interactions ($p<0.05$) were further explored with simple main effects analyses with Bonferroni adjustments.

For each parameter and replicate, the final germination percentage (FG%) was calculated as *FG% = (SG/NS) x 100* where SG and NS are the final number of seeds germinated and the total number of seeds per replicate, respectively. Mean germination percentages (GP%) were then calculated with the FG% calculated for each treatment allowing them to be expressed in bar graphs. Following the calculation in Coolbear *et al.* (1984), the time taken for 50% germination or emergence (T50/E50) was calculated using the following formula:

$$T50\ or\ E50\ = ti\ \frac{(\frac{N}{2} - ni)(tj - ti)}{(nj - ni)}$$

where N represents the total number of seeds germinated or emerged and ni and nj the cumulative number of seeds germinated by adjacent counts at times ti (day) and tj (day), respectively. This implies that ni < N/2 < nj.

The mean germination or the emergence time (MGT/MET) expressing the rate of seed germination or the seedling elongation, was calculated with the following formula described in Ellis and Roberts (1981):

$$MGT\ or\ MET = \frac{\sum Dn}{\sum n}$$

where n represents number of seeds germinated on day D and Dn the number of days taken since the beginning of the experiment.

The formula given by the AOSA and SCST (1993) was used to calculate the germination index or the emergence index (GI/EI) to measure the percentage and rate of germination of *R. raphanistrum* seeds:

$$GI\ or\ EI = \frac{No\ of\ germinated\ or\ emerged\ seedlings}{Days\ of\ final\ count} + \cdots\cdots + \frac{No\ of\ germinated\ or\ emerged\ seedlings}{Days\ of\ final\ count}$$

7.4 Results

7.4.1 Effect of temperature and photoperiod

The results obtained for temperature and photoperiod germination percentage data are shown in figure 7.1 and all the other data are presented in table 7.1 and table 7.2.

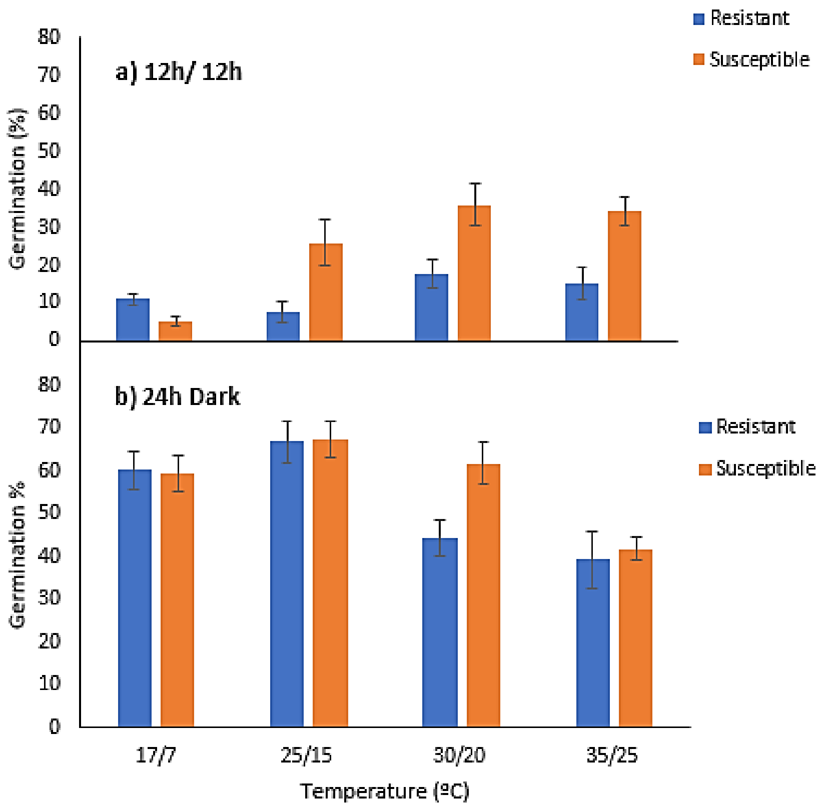


Figure 7.1. Effect of **(a)** temperature and 12h light/dark photoperiod on germination of herbicide resistant and susceptible *R. raphanistrum* seeds, and **(b)** temperature and 24h dark photoperiod on germination of herbicide resistant and susceptible *R. raphanistrum* seeds. Vertical bars represent standard error of the means.

The results showed that the germination percentage (GP) was significantly affected by the interaction between the biotype x temperature (*p*=0.009) but not with biotype x photoperiod interaction (*p*=0.089) or the three-way interaction of biotype x photoperiod x temperature (*p*=0.166). The significance of the interaction between photoperiod x temperature (*p*<0.05) suggests that the seed germination of *R. raphanistrum* under higher temperatures is less favoured by 24 h dark conditions regardless of the biotype. In contrast, under 12 h light/ 12 h dark photoperiod, both biotypes showed an increase in GP (Figure 1). The significant biotype x temperature interaction indicated that the susceptible seeds had higher germination rates in all temperatures except 17ºC/7ºC where there was no difference between the biotypes (Figure 1 and Table 1). In both biotypes, the GP was significantly different (*p*<0.05) between the two photoperiods, with a higher GP under dark conditions regardless of the temperature. The highest GP was observed under the 25ºC/15ºC temperature range under 24 h dark conditions in both resistant (66.6%) and susceptible (67.5%) biotypes. It was reduced to 10.8% and 5%, for the resistant and susceptible populations respectively under the 17ºC/7ºC and 12 h light condition which represented the lowest germination in both biotypes. The highest time to start germination (TSG) was observed under the lowest temperature regime: 17ºC/7ºC for both biotypes (~4 days). The germination index (GI) representing the germination speed and the percentage emergence was significantly different between the biotypes (*p*=0.036) and was evident in the 25ºC/15ºC temperature regime (*p*=0.005). The mean germination time (MGT) was similar in both biotypes with no significant difference (*p*>0.05). Therefore, according to the results obtained, the condition of 25ºC/15ºC complete dark (24 h) was seen to be the optimum conditions for *R. raphanistrum* seed germination and hence was chosen for the rest of the experiments.

Table 7.1. Effect of temperature and photoperiod on seed germination of resistant and susceptible biotypes of *Raphanus raphanistrum*.

Treatments		GP/SE		GI or EI		MGT/MET		T50/E50		TSG/TSE	
	Temp °C	Res.	Sus.	Res.	Sus.	Res.	Sus.	Res.	Sus.	Res.	Sus.
12 h Light/12h Dark	35/25	15.0 a*	34.2 ab*	1.0 ab	2.3 ac	5.9 a	6.0 a	5.7 ab	4.2 a	3.0 a	1.7 b
	30/20	17.5 a*	35.8 b*	1.1 ab	1.4 c	4.3 a	5.8 a	3.7 ab	4.7 a	3.0 a	3.7 ac
	25/15	7.5 a*	25.8 b*	0.4 b*	1.5 a*	3.8 a	5.9 a	2.7 b	4.4 a	2.8 a	2.0 bc
	17/7	10.8 a	5.0 a	0.3 a	0.1 b	9.1 b	6.2 a	7.7 a	5.8 a	6.0 b	6.2 a
24h Dark	35/25	39.2 a*	41.7 ab*	4.0 ab	3.9 ac	2.9 a	4.0 a	1.7 ab	1.9 a	1.7 a	1.5 b
	30/20	44.2 a*	61.7 b*	4.1 ab	4.1 c	4.3 a	3.3 a	2.1 ab	2.7 a	1.5 a	2.5 ac
	25/15	66.7 a*	67.5 b*	5.6 b*	6.9 a*	4.6 a	2.9 a	1.9 b	1.7 a	1.5 a	1.5 bc
	17/7	60.0 a	59.2 a	3.1 a	2.8 b	4.9 b	6.1 a	3.4 a	4.1 a	2.8 b	2.8 a
Significance (*p* values)	Bio* temp	0.009		0.093		0.675		0.677		0.193	
	Bio* Photo	0.089		0.293		0.790		0.887		0.321	
	Temp* Photo	0.000		0.000		0.672		0.146		0.006	
	Bio*Temp*Photo	0.166		1.648		0.010		0.168		0.838	
	Bio	0.000		0.036		0.918		0.811		0.818	
	Temp	0.006		0.000		0.002		0.002		0.000	
	Photo	0.000		0.000		0.000		0.000		0.000	

Note: In the Table, Photo = photoperiod; Temp = temperature; Res. = resistant biotype; Sus. = Susceptible biotype; GP/SE = germination percentage/ seedling emergence percentage; GI/EI = germination/ emergence index; MGT/MET = mean germination/emergence time; T50/E50 = time taken for 50% germination/ emergence; TSG/TSE = time to start germination/emergence. Asterisks (*) indicate differences of biotypes across the rows and different letters down the column represent differences in the temperature for each measure.

Table 7.2. Effect of different levels of pH, osmotic potential, NaCl concentration and burial depth on seed germination of resistant and susceptible biotypes of *Raphanus raphanistrum*.

Treatments		GP/SE		GI or EI		MGT/MET		T50/E50		TSG/TSE	
		Average		Res.	Sus.	Res.	Sus.	Res.	Sus.	Res	Sus
pH	4	56.6b		4.1 ab	4.8 cb	4.1 a	3.3 de	2.6 a	1.7 bd	1.3 bd*	2.0 a*
	5	57.1ab		4.8 ab*	9.5 a*	4.2 a	2.9 de	2.6 a*	0.7 d*	1.0 d	1.0 b
	6	62.1ab		4.9 ab*	3.7 d*	4.7 a*	7.0 a*	3.3 a	3.2 a	1.0 d*	2.0 a*
	6.2	65.4ab		4.3 ab*	5.3 b*	4.1 a	3.8 ce	2.75 a	1.8 bd	1.7 bc	2.0 a
	7	64.6ab		4.1 ab*	9.0 a*	5.4 a*	2.6 e*	3.6 ab*	1.0 d*	1.3 bd	1.0 b
	8	67.1a		5.0 a	4.3 bcd	5.3 a	5.6 ab	3.0 a	2.4 ac	1.0 d*	2.0 a*
	9	60.4ab		4.0 b	4.2 cd	4.7 a	4.4 bcd	2.6 b	2.1 c	3.0 a*	2.0 a*
	10	63.35ab		4.3 ab	4.5 bcd	4.6 a	4.9 bc	2.6 a	2.5 ab	2.0 c	2.0 a
Significance (*p* values)	Bio x pH	0.251		0.000		0.001		0.025		0.000	
	Bio	0.591		0.000		0.211		0.000		0.034	
	pH	0.007		0.000		0.000		0.000		0.000	
		Res	Sus	Res	Sus	Res	Sus	Res	Sus	Average	
NaCl concentration (mM)	0	64.2 a	65.8 a	4.8 a*	6.1 a*	4.9 ab	3.8 ab	2.7 c	1.8 a	1.3c	
	25	61.7 a*	52.5 b*	3.9 ab*	6.1 a*	5.9 ab*	2.3 b*	3.6 bc	1.5 a	1.4c	
	50	51.7 b	47.5 b	3.4 b	3.3 b	6.2 ab	5.4 a	3.5 bc	2.9 a	1.4c	
	75	50.8 b	45.8 b	3.0 b	3.6 b	5.5 ab*	3.1 ab*	3.2 bc	2.2 a	2.0b	
	100	30.83 c	31.7 c	1.6 c*	2.9 b*	6.1 ab*	2.8 ab*	4.2 abc*	1.7 a*	1.8bc	
	150	28.3 c	25.0 c	1.58 c	1.4 c	7.6 a*	4.2 ab*	6.2 a*	3.2 a*	2.3b	
	200	11.7 d*	5.8 d*	0.5 c	0.4 c	5.2 ab	3.5 ab	3.4 ab	3.0 a	3.5a	
	250	7.5 d	5.0 d	0.4 c	0.3 c	3.9 b	3.7 ab	3.0 bc	3.2 a	3.5a	
Significance (*p* values)	Bio x NaCl	0.146		0.000		0.056		0.495		0. 124	
	Bio	0.001		0.000		0.000		0.000		0.184	
	NaCl	0.000		0.000		0.010		0.022		0.000	

Table 7.2. Effect of different levels of pH, osmotic potential, NaCl concentration and burial depth on seed germination of resistant and susceptible biotypes of *Raphanus raphanistrum*. (Cont.)

Treatments		GP/SE		GI or EI		MGT/MET		T50/E50		TSG/TSE	
		Res.	Sus.	Res.	Sus.	Res.	Sus.	Res.	Sus.	Res	Sus
Osmotic potential (MPa)	0	65.0 a	67.5 a	3.7 a	3.3 a	5.5 a*	6.1 a*	3.31 b	4.0 a	2.0 c	2.2 bc
	-0.1	58.3 ab*	44.2 b*	3.0 b	3.3 a	7.4 a*	3.8 a*	4.1 b	3.0 a	2.2 c	1.3 b
	-0.2	50.8 b	45.8 b	3.8 a*	2.9 a*	3.5 a	4.2 a	2.0 b	3.0 a	2.0 c	2.0 bc
	-0.4	36.7 c*	25.8 c*	1.3 c	1.4 b	7.0 a*	3.8 a*	6.8 a*	3.3 a*	3.8 b	3.3 ac
	-0.6	10.8 d*	19.2 cd*	0.4 d	1.1 bc	6.0 a*	3.6 a*	6.2 a*	3.2 a*	4.2 b*	3.0 ac*
	-0.8	7.5 d	9.2 ed	0.2 d	0.5 c	7.1 a*	4.0 a*	6.6 a*	3.5 a*	6.7 a*	4.0 a*
	-1	8.3 d	0.0 e	0.2 d	NE	7.7 a*	NE*	7.0 a*	NE*	7.3 a*	NE*
Significance (*p* values)	Bio x PEG	0.002		0.000		0.000		0.000		0.000	
	Bio	0.025		0.833		0.000		0.000		0.000	
	PEG	0.000		0.000		0.023		0.007		0.000	
		Average		Res	Sus	Res	Sus	Res	Sus	Res	Sus
Burial depth (cm)	0	22.1bc		0.4 cb*	0.7 b*	10.7 a*	8.0 b*	8.8 ab	6.8 ab	5.8 c	5.5 c
	1	52.5a		1.1 a*	1.7 a*	10.3 a*	6.7 b*	8.8 b	5.7 b	6.5 bc*	5.0 c*
	2	26.7b		0.6 b	0.5 b	10.9 a	10.2 a	9.9 a	9.8 a	6.8 b*	7.5 b*
	4	15.0c		0.3 c	0.3 c	10.6 a	10.3 a	10.5 ab	8.5 ab	8.7 a*	9.0 a*
	6	NE		NE	NE	NE	NE	NE	NE	NE	NE
	8	NE		NE	NE	NE	NE	NE	NE	NE	NE
Significance (*p* values)	Bio x Dep	0.884		0.000		0.000		0.103		0.000	
	Bio	0.691		0.003		0.000		0.003		0.492	
	Dep	0.000		0.000		0.000		0.000		0.000	

Note: In the Table, Res. = resistant biotype; Sus. = Susceptible biotype; GP/SE = germination percentage/ seedling emergence percentage; GI/EI = germination/ emergence index; MGT/MET = mean germination/emergence time; T50/E50 = time taken for 50% germination/ emergence; TSG/TSE = time to start germination/emergence; NE = not emerged. Burial depth (Dep) is in cm below the surface. Different small letters within the columns indicate significant difference among the treatments ($p<0.05$), whilst the same letters indicate non-significant ($p>0.05$) differences. The asterisks indicate differences in the biotype for each level of the measure when interaction was significant ($p<0.05$).

7.4.2 Effect of pH

The effect of different pH levels on germination percentage was significant (*p*=0.007) but the effect of biotype (*p*=0.591) and the interaction effect of pH and biotype (*p*=0.251) were not significant (Table 7.2). Therefore, the GPs were averaged over the biotypes before analyses. The highest GP value, 67%, was observed in pH 8 which is slightly alkaline. But this was significantly different only from the pH 4 treatment which had the highest acidity level (*p*=0.034). Regardless of the pH level, all the treatments recorded a GP above 55% highlighting the ability of R. raphanistrum to grow over a wide range of soil types. The interaction effect of pH x biotype and the main effect of pH was significantly different in all the other parameters: germination index (GI), mean germination time (MGT), time for 50% germination (T50) and time to start germination (TSG) (*p*<0.05). In GI and MGT, the interaction was significant at pH levels 5, 6, 7. Additionally, GI showed a significant interaction in control but not MGT. The significant interaction in T50 was recorded at pH 5 and 7 whereas that was pH 4, 6, 8 and 9 for TSG. The effect of biotype also showed a significant influence (*p*<0.05) other than in MGT (*p*=0.211).

7.4.3 Effect of salt stress and osmotic potential

The effect of NaCl concentration and the biotype were significant (*p*<0.05) for GP, but their interaction effect was not significant (*p*=0.146)**.** Regardless of the treatment, the highest germination was observed in the non-saline control in both resistant (64.2%) and susceptible (65.8%) biotypes. Overall, the GP was reduced with increasing salt concentrations regardless of the biotype, with a higher GP in the resistant species. In susceptible seeds, the GP for the control was significantly different from the increasing NaCl concentrations (*p*<0.05). In resistant biotype, GP in control was similar to the GP in 25 mM treatment (*p*>0.05). Including GP, the effect of NaCl concentration was significantly different for all the other parameters evaluated (*p*<0.05). Similarly, the difference between biotypes was also significant in all the parameters except the time to start germination (TSG). Regardless of the biotype, the TSG increased with increasing NaCl concentration. T50 increased with increasing NaCl concentration in the susceptible biotype but the resistant biotype showed an increase only up to 150 mM (Table 7.2). The significant interaction effect in GI was observed at the NaCl concentrations of 25 mM, 100 mM and the control. Overall, in both biotypes, the germination index (GI) was reduced with increasing salt levels.

The interaction effect of biotype by polyethylene glycol (PEG) concentration was significant in GP (*p*=0.002). The differences in the two biotypes were observed in the low PEG concentrations up to 0.6 MPa other than in the 0.2 MPa treatment (*p*=0.213). The osmotic potential required to achieve a germination inhibition less than 50% in susceptible and resistant biotypes were -0.27 and -0.41 MPa respectively. This indicates the increased germinability in resistant biotype under water stressed conditions. Germination was completely inhibited at -1 MPa in the susceptible biotype whilst the resistant biotype showed a GP of 8.3%; this difference was, however, not significant (*p*=0.098). In the resistant biotype, GP of control was not significantly different (*p*=0.099) from the GP in -0.1 MPa but was significantly different from other osmotic potentials. In contrast, in the susceptible biotype, all the treatments were significantly different from the GP of control (*p*<0.05). TSG for both populations and the T50 in resistant biotype showed an increase with the increasing osmotic potential, but the T50 for susceptible biotype was not significantly different among any of the treatments. The GI showed a tendency of decreasing with increasing water stress (Table 2). Regardless of the biotype, MGT was stable across all the osmotic potentials with no significant difference among any PEG concentrations (*p*>0.05). Overall, the GP decreased with increasing osmotic potential in both populations. The significance in interaction effect for GI was observed in -0.2 and -0.6 MPa osmotic potentials, whereas for MGT, all osmotic potential levels other than the control were significant.

7.4.4 Effect of burial depth

The interaction effect between the biotype and the burial depth was not significant in terms of seedling emergence (SE) (*p*=0.884). Effect of burial depth on SE was not significantly affected by the biotype of the seeds (*p*=0.691). Therefore, the emergence percentages were averaged over the biotypes for analyses. All the parameters; SE, time to start seedling emergence (TSE), time taken for 50% seedling emergence (E50), mean emergence time (MET), emergence index (EI) were significantly determined by the burial depth (*p*<0.05). Similarly, the effect of biotype was also significant in all those parameters other than SE (*p*=0.691) and TSG (*p*=0.492). The significance of interaction effect for EI and MET was observed at the depths of 0 cm and 1 cm, whereas it was significant at 1 cm, 2 cm and 4 cm in TSE. The interaction effect was not significant in E50 (*p*=0.103). The highest SE (52.5%) was observed in the seeds buried at 1 cm depth with a significant difference from all the other depths (*p*<0.05). The seedlings from both biotypes could not emerge from the depths beyond 6 cm within the observed time period.

7.5 Discussion

7.5.1 Effect of light and temperature

The increased *R. raphanistrum* seed germination under dark conditions at alternating temperatures of 25ºC/15ºC in the current study, is in accordance with the study of Cheam (1986) and Mekenian and Willemsen (1975) which strongly suggests that the dark condition is an absolute factor for higher germination percentages of *R. raphanistrum.* Therefore, light limiting weed control strategies, such as shading, shallow inversion tillage, mulching, competition from larger species should be advisedly implemented as the dark conditions can increase seed germination of *R. raphanistrum,* especially the resistant biotype when compared to its germination under 12h light/dark condition in the current study. Under alternating photoperiods, at the lowest temperature range 17ºC/7ºC, the two biotypes showed similar germination, but a significant difference was prominent with the increasing temperatures with a higher germination percentage for the susceptible biotype. Although *R. raphanistrum* seed germination prefers cold autumn and winter conditions, this suggests the susceptible seeds are favoured even under hot summers compared to the resistant biotype. The similar time to start germination (TSG) in both biotypes for temperature or light conditions, implies the simultaneous germination of both biotypes regardless of the temperature and light environment. The overall germination reduction with the temperatures beyond 25ºC/15ºC suggests that germination success of both biotypes will reduce under hot summer conditions compared to the colder months. Therefore, the control strategies such as pre-emergent herbicide application, should be mainly focused around late autumn/early winter but should not be restricted to cold seasons, as *R. raphanistrum* seeds can germinate any time throughout the year when the moisture is adequate (GRDC, 2014).

7.5.2 Effect of pH

In this experiment, the highest GPs for both biotypes were observed in a pH 8 environment, showing its improved germinability in slightly alkaline soils. However, in both populations, the GP was maintained above 53% regardless of the pH level in both biotypes, highlighting the ability of *R. raphanistrum* to germinate in a wide range of soil types regardless of its herbicide resistance. The study of Chauhan *et al.* (2006c) has also shown a similar observation with *Rapistrum rugosum* (turnipweed) where germination was greater than 76% under a pH range varying from 4 to 10 with the highest germination in the control (6.2 pH) which has showed a significant difference only with the two extreme pH levels, pH 4 and 10. This was in contrast with *R. raphanistrum,* wherein our experiment germination was similar in all the pH levels except pH 4. The wide range of pH tolerance in Brassicaceae weeds was also evident in the study of Chauhan *et al.* (2006a) who examined *Brassica tournefortii* (wild turnip) seeds. Further, their study showed that germination under different pH levels is affected by the prevailing light conditions. *Myagrum perfoliatum* (musk weed), another Brassicaceae weed, also varied in accordance with these results, showing a GP of >46% over a pH range between 4 and 10 (Honarmand *et al.*, 2016). Considering the comprehensive adaptability of Brassicaceae weeds to germinate in a wide range of pH levels, this strongly suggests that soil pH is not a limiting factor to germination. In the current study, it was shown that, regardless of the herbicide resistance, both the populations germinated equally well over the pH range of 4 to 10.

7.5.3 Effect of salt stress and osmotic potential

In nature, continuous water evaporation from the soil surface and random salt depositions which may occur, lead to different salinity conditions in soil. Overcoming and surviving such unfavourable conditions will result in successful species distribution. In our experiment, the effect of salt stress on *R. raphanistrum* seed germination was significant between biotypes and for increasing salt concentrations with a higher tolerance in resistant biotype compared to the susceptible biotype.

The GP and the GI decreased, and the TSG increased with the increasing salt concentration in both biotypes, depicting the effect of unfavourable conditions for seed germination at higher salt concentrations. The Brassicaceae seeds of *Sinapsis alba* (white mustard) and *Brassica oleracea* (white cabbage) have also showed a similar reduced germination with increasing salt concentrations but showed complete inhibition for the concentrations beyond 200 mM and 400 mM concentrations for *S. alba* and *B. oleracea,* respectively (Bojović *et al.*, 2010). *Rapistrum rugosum* seeds from the same family have also showed a gradual reduction in GP with increasing NaCl concentrations, with a complete inhibition at 320 mM (Chauhan *et al.*, 2006c). This has also been evident in the *Myagrum perfoliatum* seeds, where a germination reduction was observed with an increasing NaCl concentration resulting in complete inhibition at 250 mM. Similarly, *Brassica tournefortii*, belonging to family Bracecaceae, also showed a GP of 18% at a NaCl concentration of 160 mM (Chauhan *et al.*, 2006a). In our experiment, under all the salinity concentrations evaluated, the seeds from both biotypes; resistant and susceptible, succeeded to germinate with a GP of 7.5% and 5% respectively at the highest NaCl concentration, 250 mM. The germinability, even under high salinity conditions may be a contributing factor in the success of *R. raphanistrum* as a problematic weed in southern Australia, where soil salinity is a prevailing problem (Rengasamy, 2002).

Osmotic potential, in the increasing PEG concentrations, showed a negative impact on *R. raphanistrum* seed germination. This indicated that available water for the seeds to imbibe and start germination is a limiting factor for *R. raphanistrum* seed germination. The resistant biotype showed a similar GP as the control at the lowest osmotic potential -0.1 MPa but subsequently the GP decrease was significant. While the decreasing trend was the same in the susceptible population, the GP for the control sample was significantly higher than for any other concentrations of PEG. The resistant population evidenced an 8% germination even at the highest osmotic potential of -1 MPA, but the susceptible population was completely inhibited above 0.8 MPa. Similarly, in the work of Chauhan *et al.* (2006a), *Brassica tournefortii* seeds showed an 8% seed germination at -1.0 MPa osmotic potential under dark conditions.

These observations suggest that the seeds of the resistant population are more adapted to water stress conditions than the susceptible biotype and hence will be able to germinate within a wide range of moisture stressed environments. This can influence its ability to germinate and grow in dryland farming areas (Gutterman & Gendler, 2005). It is also likely that this moisture tolerance can favour the growth of resistant *R. raphanistrum* seeds under the temporary soil moisture limiting conditions between the rainfall events that occur in southern Australia (Chauhan *et al.*, 2006a). It is noted that even a small GP such as 8% with the resistant biotype under moisture stressed conditions, will be beneficial for the resistant population's further establishment during late spring and early summer rain events in drier areas (Kleemann *et al.*, 2007).

7.5.4 Effect of burial depth

Overall, the two biotypes showed a similar behaviour in SE with respect to different burial depths. But the resistant biotype showed an increased mean germination time and E50, implying that the resistant seedlings emerge through an extended period of time compared to the susceptible biotype making its control problematic. In the current study, the seeds buried at 1 cm showed an increased SE compared to the seeds placed on soil surface. In contrast to this observation, the Brassicaceae weed *Myagrum perfoliatum* has also shown its highest germination when seeds were placed on the soil surface, with a reduced SE in higher depths and complete inhibition at 6 cm (Honarmand *et al.*, 2016). In line with these observations of decreased seedling emergence with increased burial depths, a controlling tactic for *R. raphanistrum* might be a no-till system or deep inversion tillage, which will reduce the emergence of both resistant and susceptible biotypes in cropping systems. However, the long-term viability of the buried seeds, which is evident even after a few years, does pose a problem because they can emerge at any time after soil disturbances. In this respect, Reeves *et al.* (1981), in their field study in Australia, showed that *R. raphanistrum* seeds buried at various depths ranging from 0 to 10 cm had a seed viability percentage of 43% even after four years when buried at 10 cm depth. It was also observed that the viability of seeds declines faster when placed closer to the soil surface, and depths between 5-10 cm have shown a reduced decline in viability, with the seed half-life of *R. raphanistrum* being found to be two years (Chancellor, 1986; Roberts & Boddrell, 1983). Therefore, the control of *R. raphanistrum* can also be achieved by occasional shallow tillage where the seeds are brought up to the topsoil to encourage seedling emergence and then to control them using herbicide or non-herbicide strategies.

As a summary both biotypes showed a significantly higher germination under a 24 h dark condition and a temperature regime of 25ºC/15ºC. Under moisture stress conditions, the resistant biotype showed an increased tolerance compared to the susceptible biotype, proving its ability to germinate even when the water is limited. Seeds from both biotypes showed a wide range of pH tolerance showing their adaptability to germinate in different soil types. The emergence was similar between both biotypes at different burial depths with the highest emergence for the seeds buried at 1 cm depth and no emergence beyond depths of 6 cm. As discussed above, these findings will help to understand the differential germination patterns of these two biotypes of *R. raphanistrum* for their early management under different abiotic conditions. Further studies should be conducted with two or more resistant and susceptible *R. raphanistrum* populations to confirm the findings of the current experiment.

CHAPTER 8 – Synthesis and conclusion

8.1 Introduction

The primary objective of the current project was to identify a potential herbicide MOA combination to control herbicide-resistant *Raphanus raphanistrum* in a herbicide-tolerant faba bean cultivar, *PBA Bendoc*. The secondary objectives were to evaluate the performance of faba bean cultivars, *PBA Bendoc* and *PBA Samira*, together with the sensitivity of *R. raphanistrum* biotypes upon the application of suggested herbicides either alone or in combination. These objectives were achieved through a stepwise approach described in the five experimental chapters, which also included a seed germination study of two biotypes of herbicide-resistant and susceptible *R. raphanistrum* biotypes to compare their germinability under different abiotic conditions (Figure 8.1). The key findings of the current study are discussed in this Chapter, together with recommendations for *R. raphanistrum* control. Future studies to advance this area are also discussed in this chapter.

8.2 Key research findings

8.2.1 Herbicide resistance in *Raphanus raphanistrum* biotypes

In Chapter Three, two *R. raphanistrum* biotypes, with known resistance and susceptibility to ALS-inhibiting herbicides, were assessed for their sensitivity towards two ALS-inhibiting herbicides; imazamox + imazapyr and imazethapyr, at four different growth stages; the 2-4 leaf stage, the 6 leaf stage, the 8 leaf stage and the flowering stage. Among the herbicide treatments, the most sensitive growth stage was identified as the 2-4 leaf stage regardless of the biotype or the herbicide treatment. At this growth stage, the herbicide-resistant biotype resulted in a percentage survival of 40% and 60% for the treatments of imazamox + imazapyr and imazethapyr, respectively. In contrast, the susceptible biotype showed a 100% mortality for both herbicides up to the 6 leaf stage treatment. The effectiveness of the herbicide was reduced with the advancing growth stage of the weed with a 100% survival in most of the herbicide treatments above the 8 leaf stage. It is noted that in all surviving herbicide-treatments, *R. raphanistrum* plants resulted in pod production, which flagged the possibility of viable seed set in the resistant plants. If seeds are produced, this can lead to these herbicide-resistant plants becoming dominant in subsequent generations in the future. Considering the results obtained in this experimental chapter, it was evident that the imazamox + imazapyr herbicide was more effective in controlling herbicide-resistant *R. raphanistrum,* compared to imazethapyr. With this notion, imazamox + imazapyr applied at the 2-4 leaf stage was identified as the best ALS-inhibiting herbicide treatment to incorporate in a potential herbicide MOA combination (Figure 8.1). However, despite being effective on weeds, before introducing such herbicide options in practice, evaluating the crop tolerance towards these herbicides is a critical aspect.

8.2.2 Crop sensitivity to the proposed herbicides and their combinations

In Chapter Four, the crop tolerance for ALS-inhibiting herbicide application was evaluated to investigate this system's potential to be incorporated as a safe in-crop application. In-crop herbicides provide an opportunity to achieve a better weed control throughout the growing season rather than relying on residual herbicide activity. The same two ALS-inhibiting herbicides used to assess *R. raphanistrum* sensitivity; imazamox + imazapyr and imazethapyr, were applied on the two faba bean cultivars; herbicide-tolerant *PBA Bendoc* and conventional *PBA Samira*. The herbicides were applied at four different crop growth stages; PSPE, 4 node, 8 node and flowering, to evaluate the crop performance.

Drought conditions that prevailed in 2018 at the trial sites in Horsham, Victoria had a significant impact on the results. Hence, the 2019 results were much more reliable, since the rainfall was abundant at the time of faba bean seedling establishment. In this experiment, *PBA Bendoc* proved its significantly increased tolerance towards the two ALS-inhibiting herbicides compared to the conventional *PBA Samira*, regardless of the herbicide or the growth stage at herbicide application. *PBA Bendoc* was equally tolerant of both herbicides, imazamox + imazapyr and imazethapyr, even when applied at the most advanced growth stage (the flowering stage). The increased tolerance in *PBA Bendoc* confirmed that these two herbicides are safe to apply in-crop at any of the four growth stages evaluated in this study; PSPE, 4 node, 8 node and flowering stage, as no yield loss was observed despite the slight increase in herbicide damage at later growth stage treatments (Figure 8.1.). The least crop injury was observed when herbicide was applied as PSPE. This proven broad tolerance to ALS-inhibiting herbicides provides a promising in-crop herbicide option to use for *PBA Bendoc* to achieve better weed control throughout the growing season. These results provide a solid background for the understanding of the ALS-inhibiting herbicide application window for these two faba bean cultivars in order to assure crop safety. This finding will also be advantageous in introducing another herbicide MOA to control weeds in this cropping system as the application time for ALS-inhibiting herbicides is quite flexible. Having this broad window for

ALS-inhibiting herbicide application, the crop sensitivity towards PS II-inhibiting metribuzin was then evaluated in Chapter Five allowing introduction of a second herbicide MOA in the potential herbicide MOA combination.

PS II-inhibiting metribuzin herbicide is not recommended for application in faba bean cropping systems as crop injury may occur. However, to evaluate the metribuzin sensitivity in the newly-released *PBA Bendoc* ALS-inhibiting herbicide-tolerant cultivar compared to conventional *PBA Samira*, and to investigate the introduction of metribuzin as a component in a herbicide MOA combination to control weeds in faba bean crops, five metribuzin rates; 0x, 1/8x (26.25 g/ha), 1/4x (52.5 g/ha), 1/2x (105 g/ha), 1x (210 g/ha) and 2x (420 g/ha) (210 g/ha = recommended rate as PSPE), were applied at the 4 node stage as the next step of our approach. The results of this experiment exhibited progressive herbicide damage which was associated with the increasing herbicide rates. Compared to two lower rates, 26.25 g/ha and 52.5 g/ha, significant herbicide damage was observed with the 105 g/ha rate, and complete plant mortality resulted at the two highest rates, 210 g/ha and 420 g/ha. Despite the nonsignificant effect on visual herbicide damage, the lowest herbicide rate, 26.25 g/ha caused a substantial reduction in the number of pods per plant in both faba bean cultivars. This suggests a yield reduction upon in-crop application of metribuzin even at the lowest rate (Figure 8.1). Therefore, this should be further investigated in real field conditions before making recommendations to use metribuzin as an in-crop herbicide for faba bean. Overall, both cultivars responded similarly across all metribuzin rates used. Consequently, considering the results of the current study, the PS II-inhibiting herbicide, metribuzin, is suggested to be included as a PSPE application following label recommendation in our proposed sequential herbicide MOA combination. This decision was supported by the increased tolerance and wide herbicide application window in *PBA Bendoc* for ALS-inhibiting herbicides.

The proposed sequential herbicide MOA combinations, metribuzin PSPE followed by imazamox + imazapyr at later growth stages, were then evaluated in Chapter Six with *PBA Bendoc* and herbicide-resistant *R. raphanistrum* to identify their efficacy in terms of weed control and crop safety. The herbicide combinations consisted of metribuzin PSPE, metribuzin PSPE followed by imazamox + imazapyr at each of the four different *PBA Bendoc*/ weed growth stages as carried out in the previous experiments. In *PBA Bendoc*, among all the evaluated parameters, only the visual herbicide damage showed a significant difference between the treatments. The least herbicide damage was observed in the herbicide treatments where both the herbicides were kept as PSPE, and in the PSPE metribuzin only treatment (0%), whilst the highest was in the metribuzin followed by imazamox + imazapyr flowering treatment (6%). All the other parameters, including survival, plant height, aboveground dry weight and number of pods, did not show any significant difference from the controls. Therefore, based on these observations, almost all the herbicide combinations evaluated could be recommended as safe to apply in the *PBA Bendoc* cropping system (Figure 8.1). Consequently, it allows an inclusive window for weed control decision making, which can be solely based on the weed growth stages without challenging crop safety. With regards to the weed, 100% *R. raphanistrum* control could be achieved with the metribuzin PSPE application in all treatments (Figure 8.1). Hence, no second herbicide MOA was required for its control in the current study. With respect to the findings of 100% *R. raphanistrum* control with PSPE metribuzin application, herbicide rotation can be recommended as a herbicide MOA combination strategy, to be incorporated in the *PBA Bendoc* cropping system with PSII-inhibiting metribuzin in one growing season, along with ALS-inhibiting herbicides or another potential herbicide (MOA preferably a low-risk herbicide).

Further studies should be carried out in identifying such potential herbicide MOAs, and this study can be used as a case study to conduct such investigations in the future. If the *R. raphanistrum* seedlings survive the metribuzin PSPE application, a sequential application can be recommended with a second herbicide application of imazamox + imazapyr at 2-4 leaf stage of *R. raphanistrum* plants, as this was identified as the most susceptible *R. raphanistrum* growth stage. This will make the herbicide MOA combination strategy a sequential application where both the applications take place in one growing season. The success of this strategy was not evident in the current study as metribuzin PSPE treatment itself resulted in a 100% control of *R. raphanistrum,* and hence no surviving seedlings were present to evaluate the efficacy of the second herbicide application. Therefore, further trials in real field conditions need to be conducted to recommend this sequential herbicide MOA combination to control *R. raphanistrum*.

8.2.3 Seed germination characteristics of herbicide resistant and susceptible *Raphanus raphanistrum*

In Chapter Seven, the differential germination success of *R. raphanistrum* with two biotypes, the ALS-inhibiting herbicide-resistant and susceptible, was investigated to identify possible management implications by addressing the germination requirements. The abiotic conditions affecting seed germination, such as photoperiod, temperature, moisture, pH and burial depth, were investigated for their effect on *R. raphanistrum* seed germination. The optimum germination conditions were identified as 25ºC/15ºC temperature range under 24 h dark, with the highest resulted germination percentages in both biotypes. Based on the results, the seed germination of both resistant and susceptible *R. raphanistrum* seeds was favoured by 24 hours of complete dark condition. The two biotypes differed significantly in their reaction to moisture tolerance, where the resistant biotype proved its high tolerance to moisture stress conditions imposed by osmotic potential and salinity compared to the susceptible biotype. This implies its adaptability to a wide range of soil types. Both biotypes responded similarly to different pH levels and burial depths with the highest germination percentage when buried at 1 cm depth. (Figure 8.1).

8.3 Recommendations and future studies

Given that chemical weed control is the primary practice in weed management strategies, care must be taken to reduce the inherent selection pressure on weeds. As a consequence, herbicide combination tactics are highly recommended for implementation in cropping systems. At the same time, adhering to strict stewardship guidelines when using a chemical product is mandatory to make their use sustainable in the future. With the results of the current project, PSPE metribuzin application is recommended to control *R. raphanistrum* in faba bean cropping areas, and if any seedlings survive this treatment, a follow-up application of imazamox + imazapyr is recommended at 2-4 leaf stage. The crop, *PBA Bendoc*, proved its tolerance for these herbicide treatments, and hence the crop safety is assured while controlling the weed. The increased herbicide damage observed with advancing growth stage, especially at the flowering stage was supported by the chemical registration of *PBA Bendoc*, for which it is recommended not to be applied beyond the 6-node stage (Seednet, 2020). Therefore, it appears that the safest window to apply ALS-inhibiting herbicides in *PBA Bendoc* to achieve a minimal herbicide damage is up to 6 node stage. In summary, two herbicide MOA combination strategies can be suggested as (i) herbicide MOA rotation with metribuzin in one year and either ALS-inhibiting herbicides or another potential herbicide MOA in the next year and (ii) sequential herbicide MOA application with metribuzin as PSPE and imazamox + imazapyr at the 2-4 leaf stage of survived *R. raphanistrum* plants (Figure 8.1). These strategies need to be further investigated in real field conditions to identify other potential herbicide MOAs to combine in these strategies. It is recommended that exploration of potential low-risk herbicide MOAs which are suitable to incorporate with the above herbicides be carried out, as it poses a possibility for further reduction in the rate of herbicide resistance evolution in weeds. It is also suggested that future studies could be conducted to evaluate the seed set and viability of the survived *R. Raphanistrum* plants upon the application of the above herbicide treatments. Analysis of the nutrition content and herbicide residue levels in faba bean grains is also advisable in order to assess the effect of these herbicides on crop grain quality.

The seed germination success in herbicide-resistant and susceptible *R. raphanistrum* biotypes were different only in their response to moisture stress conditions where the resistant biotype proved to be more tolerant. Therefore, it appears that this resistant biotype of *R. raphanistrum* can be problematic even under rapidly drying top-soil conditions compared to the herbicide-susceptible species. Also, the results of moisture stress and a wide range of pH tolerance will be important when implementing weed control strategies as salinity and alkalinity are major constraints in most Australian soils (Dang *et al.*, 2015). As a parallel control strategy, a reduction in the *R. raphanistrum* soil seed bank could be achieved by allowing germination of the seeds buried within the topsoil, then applying management tactics such as herbicides or manual removal. Care must be taken with strategies including mulching, shading, and competition from large species since these will simulate dark conditions, which is favoured for the higher germination percentages of *R. raphanistrum* seeds. On the other hand, decreasing the germination rate can be achieved by ensuring seed burial below 4 cm and ensuring no further soil disturbances (Young & Cousens, 1999). Further suggested seed germination studies include the identification of the different morphological changes acquired with the herbicide resistance in *R. raphanistrum* compared to the susceptible seeds.

8.4 Conclusion

The primary objective of this study was related to the identification of a potential herbicide combination, and this was achieved as we demonstrated the effectiveness of two herbicide MOAs in controlling *R. Raphanistrum.* The secondary objectives were also executed as the crop safety was assured upon the application of proposed herbicide combination strategies. The increased ALS-inhibiting herbicide tolerance in the newly released *PBA Bendoc* faba bean cultivar permits a wide window for in-crop weed control to faba bean growers, the narrowness of which was previously a significant problem. This allows weed management to be carried out throughout the faba bean growing season, providing a solution in managing late emergence weeds.

The study also proved a 100% control of ALS-inhibiting herbicide-resistant *R. raphanistrum* with the PSPE metribuzin application. Both faba bean cultivars, *PBA Bendoc* and *PBA Samira* also proved their tolerance to PSPE metribuzin application, which highlighted the aptness of the finding in the incorporation of the second herbicide in the faba bean cropping system. Despite the ability to germinate and emerge throughout the year, *R. raphanistrum* emergence is mostly observed around late autumn/early winter period of the year, which is also the faba bean sowing time. According to Cheam and Code (1998), 73% of *R. raphanistrum* emerge during late autumn/early winter, while 3% and 21% emergence was observed in early autumn and late winter/early spring, respectively. Therefore, it is suggested that the results of this thesis are of significant importance in the practical control of *R. raphanistrum* in *PBA Bendoc* faba bean cropping activities. It has also indicated that the herbicide application timing for the ALS-inhibiting herbicide imazamox + imazapyr can be uniquely decided based on the perception of the weed growth stage, a possibility that has emerged because of the proven increased herbicide tolerance of the *PBA Bendoc* faba bean crop.

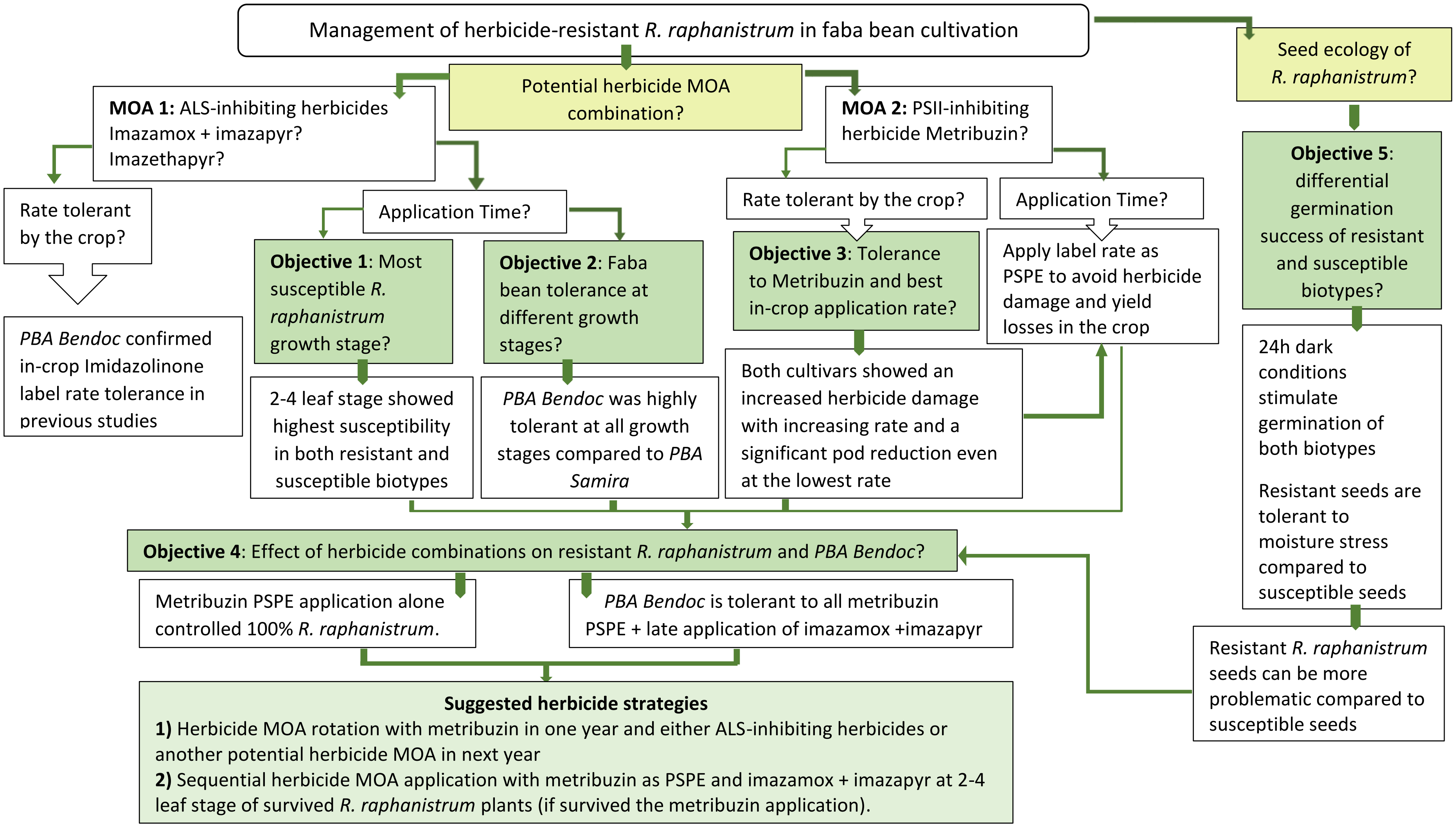


Figure 8.1. The conceptual framework of the current study

REFERENCES

ABARES. (2017). Agricutural commodities and trade data. Retrieved October 9, 2017, from Department of Agriculture and Water Resources http://www.agriculture.gov.au/abares/research-topics/agricultural-commodities/agricultural-commodities-trade-data#crops

ABARES. (2019). Agricutural commodities and trade data. Retrieved January 20, 2020, from Department of Agriculture and Water Resources https://www.agriculture.gov.au/abares/research-topics/agricultural-commodities/ACS2019_PulsesTables_v1.0.0

Agegnehu, G., & Fessehaie, R. (2006). Response of faba bean to phosphate fertilizer and weed control on nitisols of Ethiopian highlands. *Italian Journal of Agronomy, 1*(2), 281-290.

ALA. (2019). Atlas of living Australia; Raphanus raphanistrum. Retrieved March 26, 2020, from https://bie.ala.org.au/species/https://id.biodiversity.org.au/node/apni/2888495

Altuntaş, E., & Yıldız, M. (2007). Effect of moisture content on some physical and mechanical properties of faba bean (*Vicia faba* L.) grains. *Journal of Food Engineering, 78*(1), 174-183.

Amare, T., Sharma, J. J., & Zewdie, K. (2014). Effect of Weed Control Methods on Weeds and Wheat (*Triticum Aestivum L.*) Yield. *World Journal of Agricultural Research, 2*(3), 124-128.

Anderson, N. H. (1987). Spray retention: effects of surfactants and plant species. *Aspects of Applied Biology, 14*, 233-243.

AOSA, & SCST. (1993). Rules for testing seeds. *Seed Technology Journal, 16*, 1-113.

Australian Bureau of Statistics. (2019). Value of Agricultural Commodities Produced, Australia, 2015-16. Retrieved January 29, 2020, from Australian Bureau of Statistics https://www.abs.gov.au/ausstats/abs@.nsf/0/58529ACD49B5ECE0CA2577A000154456?Opendocument

Bagavathiannan, M., Singh, V., Govindasamy, P., Abugho, S. B., & Liu, R. (2017). Impact of concurrent weed or herbicide stress with other biotic and abiotic stressors on crop production. In M. Senthil-Kumar (Ed.), *Plant Tolerance to Individual and Concurrent Stresses* (pp. 33-45). New Delhi, India: Springer

Baltazar, A. M., & Smith Jr, R. J. (1994). Propanil-resistant barnyardgrass (*Echinochloa crus-galli*) control in rice (*Oryza sativa*). *Weed Technology, 8*(3), 576-581.

Barros, J. F., Basch, G., & de Carvalho, M. (2007). Effect of reduced doses of a post-emergence herbicide to control grass and broad-leaved weeds in no-till wheat under Mediterranean conditions. *Crop Protection, 26*(10), 1538-1545.

Beckie, H. J., Hall, L. M., Meers, S., Laslo, J. J., & Stevenson, F. C. (2004). Management practices influencing herbicide resistance in wild oat. *Weed Technology, 18*(3), 853-859.

Beckie, H. J. (2006). Herbicide-resistant weeds: management tactics and practices. *Weed Technology, 20*(3), 793-814.

Beckie, H. J., Leeson, J. Y., Thomas, A. G., Hall, L. M., & Brenzil, C. A. (2008). Risk assessment of weed resistance in the Canadian prairies. *Weed Technology, 22*(4), 741-746.

Beckie, H. J., & Reboud, X. (2009). Selecting for weed resistance: herbicide rotation and mixture. *Weed Technology, 23*(3), 363-370.

Beckie, H. J., & Tardif, F. J. (2012). Herbicide cross resistance in weeds. *Crop Protection, 35*, 15-28.

Beckie, H. J., & Harker, K. N. (2017). Our top 10 herbicide-resistant weed management practices. *Pest Management Science, 73*(6), 1045-1052.

Benbrook, C. (2001). Do GM crops mean less pesticide use? *Pesticide Outlook, 12*(5), 204-207.

Bertholet, J., & Clark, K. W. (1985). Effect of trifluralin and metribuzin on faba bean growth, development, and symbiotic nitrogen fixation. *Canadian Journal of Plant Science, 65*(1), 9-21.

Blackshaw, R. E., Lemerle, D., Mailer, R., & Young, K. R. (2002). Influence of wild radish on yield and quality of canola. *Weed Science, 50*(3), 344-349.

Bojović, B., Đelić, G., Topuzović, M., & Stanković, M. (2010). Effects of NaCl on seed germination in some species from families Brassicaceae and Solanaceae. *Kragujevac Journal of Science, 32*, 83-87.

Bonny, S. (2016). Genetically modified herbicide-tolerant crops, weeds, and herbicides: overview and impact. *Environmental Management, 57*(1), 31-48.

Borger, C. P., & Hashem, A. (2007). Evaluating the double knockdown technique: sequence, application interval, and annual ryegrass growth stage. *Australian Journal of Agricultural Research, 58*(3), 265-271.

Borowiec, S., Grinn, U., & Kutyna, I. (1972). The influence of soil conditions and kinds of crops on the constancy of occurrence of weeds. *Ekologia Polska, 20*(20), 199-217.

Boutsalis, P., & Powles, S. (1995). Resistance of dicot weeds to acetolactate synthase (ALS)-inhibiting herbicides in Australia. *Weed Research, 35*(3), 149-155.

Bradley, C. A., Hartman, G. L., Wax, L. M., & Pedersen, W. L. (2002). Influence of herbicides on Rhizoctonia root and hypocotyl rot of soybean. *Crop Protection, 21*(8), 679-687.

Bradshaw, L. D., Padgette, S. R., Kimball, S. L., & Wells, B. H. (1997). Perspectives on glyphosate resistance. *Weed Technology, 11*(1), 189-198.

Brand, J. (2012). *Interpreting variable data from herbicide tolerance trials on pulse cultivars for on-farm applicability*. Paper presented at the 16th Agronomy Conference 2012, Yunusa. Armidale, NSW.

Broster, J. C., Koetz, E. A., Shephard, A. J., & Wu, H. (2014 September). *Extent of herbicide resistance in three broadleaf weed species in southern New South Wales*. Paper presented at the 19th Australasian Weeds Conference Hobart, Tasmania. https://researchoutput.csu.edu.au/en/publications/extent-of-herbicide-resistance-in-three-broadleaf-weed-species-in-2

Brunskill, R. (1956). *Physical factors affecting the retention of spray droplets on leaf surfaces.* Paper presented at the Third British Weed Control Conference, Blackpool, England.

Buhler, D. D., & Burnside, O. C. (1983). Effect of spray components on glyphosate toxicity to annual grasses. *Weed Science, 31*(1), 124-130.

Bukun, B., Nissen, S. J., Shaner, D. L., & Vassios, J. D. (2012). Imazamox absorption, translocation, and metabolism in red lentil and dry bean. *Weed Science, 60*(3), 350-354.

Bullock, D. S., & Nitsi, E. I. (2001). Roundup ready soybean technology and farm production costs: measuring the incentive to adopt genetically modified seeds. *American Behavioral Scientist, 44*(8), 1283-1301.

Burke, J. J., Gamble, P. E., Hatfield, J. L., & Quisenberry, J. E. (1985). Plant morphological and biochemical responses to field water deficits: I. Responses of glutathione reductase activity and paraquat sensitivity. *Plant Physiology, 79*(2), 415-419.

Burnet, M. W., Hildebrand, O. B., Holtum, J. A., & Powles, S. B. (1991). Amitrole, triazine, substituted urea, and metribuzin resistance in a biotype of rigid ryegrass (*Lolium rigidum*). *Weed Science, 39*(3), 317-323.

Busconi, M., Rossi, D., Lorenzoni, C., Baldi, G., & Fogher, C. (2012). Spread of herbicide-resistant weedy rice (red rice, *Oryza sativa* L.) after 5 years of Clearfield rice cultivation in Italy. *Plant Biology, 14*(5), 751-759.

Busi, R., & Beckie, H. (2019). *Herbicide resistance in focus paddocks of WA champion farmers.* Paper presented at the GRDC Research updates Perth, WA, Australia.

Busi, R., Powles, S. B., Beckie, H. J., & Renton, M. (2020). Rotations and mixtures of soil-applied herbicides delay resistance. *Pest Management Science, 76*(2), 487-496.

Byker, H. P., Van Wely, A. C., Jhala, A. J., Soltani, N., Robinson, D. E., Lawton, M. B., & Sikkema, P. H. (2018). Preplant followed by postemergence herbicide programs and biologically effective rate of metribuzin for control of glyphosate-resistant common ragweed (*Ambrosia artemisiifolia*) in soybean. *Canadian Journal of Plant Science, 98*(4), 809-814.

Bzour, M., Zuki, F. M., Mispan, M. S., Jodeh, S., & Abdel-Latif, M. (2019). Determination of the leaching potential and residues activity of Imidazolinone herbicide in clearfield rice soil using High-Performance Liquid Chromatography. *Bulletin of Environmental Contamination and Toxicology, 103*(2),

CABI. (2019). Invasive species compendium. Retrieved June 22, 2018, from Centre for Agriculture and Bioscience International https://www.cabi.org/isc/datasheet/2312

Caracuta, V., Barzilai, O., Khalaily, H., Milevski, I., Paz, Y., Vardi, J., . . . Boaretto, E. (2015). The onset of faba bean farming in the Southern Levant. *Scientific Reports, 5*, 14370.

Carpenter, J. E., & Gianessi, L. P. (1999). Herbicide tolerant soybeans: why growers are adopting Roundup Ready varieties. *AgBioForum, 2*(2), 65-72.

Caseley, J. C., & Coupland, D. (1985). Environmental and plant factors affecting glyphosate uptake, movement and activity. In E. Grossbard & D. Atkinson (Eds.), *The herbicide glyphosate* (pp. 92-123). London, UK: Butterworth & Co.

Cechin, J., Vargas, L., Agostinetto, D., Zimmer, V., Pertile, M., & Dal Magro, T. (2017). Fitness costs of susceptible and resistant radish biotypes to ALS-inhibitor herbicides. *Comunicata Scientiae, 8*(2), 281-286.

Chachalis, D., & Reddy, K. N. (2000). Factors affecting *Campsis radicans* seed germination and seedling emergence. *Weed Science, 48*(2), 212-216.

Champion, G., May, M., Bennett, S., Brooks, D., Clark, S., Daniels, R., . . . Heard, M. (2003). Crop management and agronomic context of the Farm Scale Evaluations of genetically modified herbicide–tolerant crops. *Philosophical Transactions of the Royal Society of London B: Biological Sciences, 358*(1439), 1801-1818.

Chancellor, R. J. (1986). Decline of arable weed seeds during 20 years in soil under grass and the periodicity of seedling emergence after cultivation. *Journal of Applied Ecology, 23*, 631-637.

Charudattan, R. (2005 December). *Use of plant pathogens as bioherbicides to manage weeds in horticultural crops.* Paper presented at the 118th Annual Meeting of the Florida State Horticultural Society, Florida, USA.

Chauhan, B. S., Gill, G., & Preston, C. (2006a). African mustard (*Brassica tournefortii*) germination in southern Australia. *Weed Science, 54*(5), 891-897.

Chauhan, B. S., Gill, G., & Preston, C. (2006b). Factors affecting seed germination of annual sowthistle (*Sonchus oleraceus*) in southern Australia. *Weed Science, 54*(5), 854-860.

Chauhan, B. S., Gill, G., & Preston, C. (2006c). Factors affecting turnipweed (*Rapistrum rugosum*) seed germination in southern Australia. *Weed Science, 54*(6), 1032-1036.

Chauhan, B. S., Gill, G., & Preston, C. (2006d). Influence of environmental factors on seed germination and seedling emergence of rigid ryegrass (*Lolium rigidum*). *Weed Science, 54*(6), 1004-1012.

Chauhan, B. S., & Abugho, S. B. (2012). Effect of growth stage on the efficacy of postemergence herbicides on four weed species of direct-seeded rice. *The Scientific World Journal, 2012*, 7.

Cheam, A., & Lee, S. (2004 September). *Diflufenican resistance in wild radish (Raphanus raphanistrum L.): its discovery and consequences for the lupin industry.* Paper presented at the 14th Australian Weeds Conference, Wagga Wagga, New South Wales, Australia.

Cheam, A. H. (1986). Seed production and seed dormancy in wild radish (*Raphanus raphanistrum* L.) and some possibilities for improving control. *Weed Research, 26*(6), 405-414.

Cheam, A. H., & Code, G. R. (1995). The biology of Australian weeds. 24. *Raphanus raphanistrum* L. *Plant Protection Quarterly, 10*(1), 2-13.

Cheam, A. H., & Code, G. R. (1998). *Raphanus raphanistrum*. In F. C. Panetta, R. H. Groves, & R. C. H. Shepherd (Eds.), *The Biology of Australian Weeds* (Vol. 2, pp. 207-224). Melbourne, Australia: RG and FJ Richardson.

Cheam, A. H., Storrie, A. M., Koetz, E. A., Holding, D. J., Bowcher, A. J., & Barker, J. A. (2008). *Managing wild radish and other brassicaceous weeds in Australian cropping systems*. CRC for Australian Weed Management, Adelaide, Australia.

Chejara, V. K., Kristiansen, P., Whalley, R. D., Sindel, B. M., & Nadolny, C. (2008). Factors affecting germination of coolatai grass (*Hyparrhenia hirta*). *Weed Science, 56*(4), 543-548.

Cheng, X., & Ni, H. (2013). Weed control efficacy and winter wheat safety of a novel herbicide HW02. *Crop Protection, 43*, 246–250.

Chikwenhere, G. P., & Keswani, C. (1997). Economics of biological control of Kariba weed (*Salvinia molesta* Mitchell) at Tengwe in north-western Zimbabwe-a case study. *International Journal of Pest Management, 43*(2), 109-112.

Clapham, A. R., Tutin, T. G., & Moore, D. M. (1990). *Flora of the British isles*. Cambridge, UK. Cambridge University Press.

Code, G. R., & Donaldson, T. W. (1996). Effect of cultivation, sowing methods and herbicides on wild radish populations in wheat crops. *Australian Journal of Experimental Agriculture, 36*(4), 437-442.

Coetzer, E., Al-Khatib, K., & Peterson, D. E. (2002). Glufosinate efficacy on Amaranthus species in glufosinate-resistant soybean (Glycine max). *Weed Technology, 16*(2), 326-331.

Colbach, N., Darmency, H., Fernier, A., Granger, S., Le Corre, V., & Messéan, A. (2017). Simulating changes in cropping practices in conventional and glyphosate-resistant maize. II. Weed impacts on crop production and biodiversity. *Environmental Science and Pollution Research, 24*(14), 13121-13135.

Condon, G. (2020 March). *New chemistry - waht's new, whats coming and how to keep them for longer.* Paper presented at the Grain research update - Driving profit through research, Barellan New South wales.

Congreve, M., & Cameron, J. (2018). *Understanding post-emergent herbicide weed control in Australian farming systems - A National Reference Manual for Agronomic Advisers.* Retrieved from https://grdc.com.au/__data/assets/pdf_file/0028/367534/Understanding-post-emergent-herbicide-weed-control-in-Australian-farming-systems.pdf

Coolbear, P., Francis, A., & Grierson, D. (1984). The effect of low temperature pre-sowing treatment on the germination performance and membrane integrity of artificially aged tomato seeds. *Journal of Experimental Botany, 35*(11), 1609-1617.

Costa, L. O., & Rizzardi, M. A. (2014). Resistance of *Raphanus raphanistrum* to the herbicide metsulfuron-methyl. *Planta daninha, 32*(1), 181-187.

Croplife Australia. (2017). Herbicide mode of action groups. Retrieved October 18, 2017 from https://www.croplife.org.au/resistance-strategy/2017-herbicide-mode-of-action-groups/

Croplife Australia. (2018). List of herbicide resistant weeds in Australia. Retrieved October 01, 2019 from https://www.croplife.org.au/wp-content/uploads/2017/06/2018-Herbicide-Resistant-Weeds.pdf

Cui, Y. C., Hoffman, T., Zhou, N., Novak, S. N., Colon, J., Parkhurst, D. M., . . . Held, B. (2020). USA Patent No. 20200093129. Corteva Agriscience & MS Technologies Inc.

Dang, Y. P., Moody, P. W., Bell, M. J., Seymour, N. P., Dalal, R. C., Freebairn, D. M., & Walker, S. R. (2015). Strategic tillage in no-till farming systems in Australia's northern grains-growing regions: II. Implications for agronomy, soil and environment. *Soil and Tillage Research, 152*, 115-123.

Dao, T. H., & Lavy, T. L. (1978). Extraction of soil solution using a simple centrifugation method for pesticide adsorption-desorption studies. *Soil Science Society of America Journal, 42*(2), 375-377.

Das, T. K., Baghel, J. K., & Raj, R. (2019). Herbicide Tolerant Crops: Challenges and prospects for weed management. In B. S. Parmar, S. B. Singh, & S. Walia (Eds.), *Pesticides and Pests* (pp. 218). UK: Cambridge scholars publishing.

Dauer, J. T., Luschei, E. C., & Mortensen, D. A. (2009). Effects of landscape composition on spread of an herbicide-resistant weed. *Landscape Ecology, 24*(6), 735-747.

Davidson, B., Cook, T., & Chauhan, B. S. (2019). Alternative options to Glyphosate for control of large *Echinochloa colona* and *Chloris virgata* plants in cropping fallows. *Plants, 8*(8), 245.

Davis, A. S., & Frisvold, G. B. (2017). Are herbicides a once in a century method of weed control? *Pest Management Science, 73*(11), 2209-2220.

de Carvalho, S. J. P., Nicolai, M., Ferreira, R. R., Figueira, A. V. D. O., & Christoffoleti, P. J. (2009). Herbicide selectivity by differential metabolism: considerations for reducing crop damages. *Scientia Agricola, 66*(1), 136-142.

de Oliveira Jr, R. S., & Inoue, M. H. (2011). Seletividade de herbicidas para culturas e plantas daninhas. In R. S. de Oliveira Jr, J. Constantin, & M. H. Inoue (Eds.), *Biologia e manejo de plantas daninhas* (pp. 243-261): Pix Bureau Grafica e Editora.

Debeaujon, I., Léon-Kloosterziel, K. M., & Koornneef, M. (2000). Influence of the testa on seed dormancy, germination, and longevity in *Arabidopsis*. *Plant Physiology, 122*(2), 403-414.

Defelice, M. S., Brown, W. B., Aldrich, R. J., Sims, B. D., Judy, D. T., & Guethle, D. R. (1989). Weed control in soybeans (*Glycine max*) with reduced rates of postemergence herbicides. *Weed Science, 37*(3), 365-374.

Délye, C., Jasieniuk, M., & Le Corre, V. (2013). Deciphering the evolution of herbicide resistance in weeds. *Trends in Genetics, 29*(11), 649-658.

Dennis, M., Hembree, K. J., Bushoven, J. T., & Shrestha, A. (2016). Growth stage, temperature, and time of year affects the control of glyphosate-resistant and glyphosate-paraquat resistant *Conyza bonariensis* with saflufenacil. *Crop Protection, 81*, 129-137.

Dentzman, K. (2018). “I would say that might be all it is, is hope”: The framing of herbicide resistance and how farmers explain their faith in herbicides. *Journal of Rural Studies, 57*, 118-127.

Devine, M. D. (2005). Why are there not more herbicide-tolerant crops? *Pest Management Science, 61*(3), 312-317.

Devlin, D. L., Long, J. H., & Maddux, L. D. (1991). Using reduced rates of postemergence herbicides in soybeans (*Glycine max*). *Weed Technology, 5*(4), 834-840.

Dickson, R. L., Andrews, M., Field, R. J., & Dickson, E. L. (1990). Effect of water stress, nitrogen, and gibberellic acid on fluazifop and glyphosate activity on oats (*Avena sativa*). *Weed Science, 38*(1), 54-61.

Diggle, A. J., Neve, P. B., & Smith, F. P. (2003). Herbicides used in combination can reduce the probability of herbicide resistance in finite weed populations. *Weed Research, 43*(5), 371-382.

Dill, G. M., CaJacob, C. A., & Padgette, S. R. (2008). Glyphosate-resistant crops: adoption, use and future considerations. *Pest Management Science: formerly Pesticide Science, 64*(4), 326-331.

Djebali, N., Scott, J. K., Jourdan, M., & Souissi, T. (2009). Fungi pathogenic on wild radish (*Raphanus raphanistrum* L.) in northern Tunisia as potential biocontrol agents. *Phytopathologia mediterranea, 48*(2), 205-213.

Donaldson, T. W. (1986). Wild radish (*Raphanus raphanistrum* L.): a review of research on its biology and control in Victoria, 1976–1982. *Plant Protection Quarterly, 1*(1), 160-162.

Duggleby, R. G., McCourt, J. A., & Guddat, L. W. (2008). Structure and mechanism of inhibition of plant acetohydroxyacid synthase. *Plant Physiology and Biochemistry, 46*(3), 309-324.

Duhoux, A., Carrère, S., Gouzy, J., Bonin, L., & Délye, C. (2015). RNA-Seq analysis of rye-grass transcriptomic response to an herbicide inhibiting acetolactate-synthase identifies transcripts linked to non-target-site-based resistance. *Plant Molecular Biology, 87*(4-5), 473-487.

Duke, S. O., Cerdeira, A. L., & Matallo, M. B. (2006). Herbicide effects on plant disease. *Outlooks on Pest Management, 18*, 36-40.

Duke, S. O. (2012). Why have no new herbicide modes of action appeared in recent years? *Pest Management Science, 68*(4), 505-512.

Dupont, Y. L., Strandberg, B., & Damgaard, C. (2018). Effects of herbicide and nitrogen fertilizer on non-target plant reproduction and indirect effects on pollination in *Tanacetum vulgare* (Asteraceae). *Agriculture, Ecosystems & Environment, 262*, 76-82.

Dyer, W. E. (2018). Stress-induced evolution of herbicide resistance and related pleiotropic effects. *Pest Management Science, 74*(8), 1759-1768.

Eberlein, C. V., Guttieri, M. J., Berger, P. H., Fellman, J. K., Mallory-Smith, C. A., Thill, D. C., . . . Belknap, W. R. (1999). Physiological consequences of mutation for ALS-inhibitor resistance. *Weed Science, 47*(4), 383-392.

Ebrahimi, E., & Eslami, S. (2012). Effect of environmental factors on seed germination and seedling emergence of invasive *Ceratocarpus arenarius*. *Weed Research, 52*(1), 50-59.

Egan, J. F. (2014). Herbicide-resistant crop biotechnology: potential and pitfalls. In A. Ricroch, S. Chopra, & S. Fleischer (Eds.), *Plant Biotechnology* (pp. 143-154): Springer, Cham.

Ehler, L. E. (2006). Integrated pest management (IPM): definition, historical development and implementation, and the other IPM. *Pest Management Science, 62*(9), 787-789.

Ellis, J. M., & Griffin, J. L. (2002). Benefits of soil-applied herbicides in glyphosate-resistant soybean (*Glycine max*). *Weed Technology, 16*(3), 541-547.

Ellis, R. H., & Roberts, E. H. (1981). The quantification of ageing and survival in orthodox seeds. *Seed Science and Technology, 9*, 373-409.

Eslami, S. V., Gill, G., Bellotti, W., & McDonald, G. (2004). *Comparative growth and competition of wild radish (Raphanus raphanistrum L.) and wheat.* Paper presented at the 4th International Crop Science Congress, Brisbane, Australia.

Eslami, S. V., Gill, G. S., Bellotti, B., & McDonald, G. (2006). Wild radish (*Raphanus raphanistrum*) interference in wheat. *Weed Science, 54*(4), 749-756.

Evans, J. A., Tranel, P. J., Hager, A. G., Schutte, B., Wu, C., Chatham, L. A., & Davis, A. S. (2016). Managing the evolution of herbicide resistance. *Pest management science, 72*(1), 74-80.

Faccini, D., & Puricelli, E. (2007). Efficacy of herbicide dose and plant growth stage on weeds present in fallow ground. *Agriscientia, 24*(1).

FAOSTAT. (2019). Crops. Retrieved October 13, 2020, from Food and Agriculture Organization of the United Nations http://www.fao.org/faostat/en/#data/QC

Ferrari, F. N., & Parera, C. A. (2015). Germination of six native perennial grasses that can be used as potential soil cover crops in drip-irrigated vineyards in semiarid environs of Argentina. *Journal of Arid Environments, 113*, 1-5.

Fish, J. C., Webster, E. P., Blouin, D. C., & Bond, J. A. (2015). Imazethapyr co-application interactions in imidazolinone-resistant rice. *Weed Technology, 29*(4), 689-696.

Fish, J. C., Webster, E. P., Blouin, D. C., & Bond, J. A. (2016). Imazamox plus propanil mixtures for grass weed management in imidazolinone-resistant rice. *Weed Technology, 30*(1), 29-35.

Foley, J. A., DeFries, R., Asner, G. P., Barford, C., Bonan, G., Carpenter, S. R., . . . Gibbs, H. K. (2005). Global consequences of land use. *Science, 309*(5734), 570-574.

Frenda, A. S., Ruisi, P., Saia, S., Frangipane, B., Di Miceli, G., Amato, G., & Giambalvo, D. (2013). The critical period of weed control in faba bean and chickpea in Mediterranean areas. *Weed Science, 61*(3), 452-459.

Friends of Queens Park Bushland. (2011). *Raphanus raphanistrum*. Retrieved September 14, 2017 from https://www.friendsofqueensparkbushland.org.au/raphanus-raphanistrum/

Ghersa, C., & Martınez-Ghersa, M. (2000). Ecological correlates of weed seed size and persistence in the soil under different tilling systems: implications for weed management. *Field Crops Research, 67*(2), 141-148.

Gianessi, L. P. (2005). Economic and herbicide use impacts of glyphosate-resistant crops. *Pest Management Science: formerly Pesticide Science, 61*(3), 241-245.

Gill, G. S., Cousens, R. D., & Allan, M. R. (1996). Germination, growth, and development of herbicide resistant and susceptible populations of rigid ryegrass (*Lolium rigidum*). *Weed Science, 44*(2), 252-256.

Gonzini, L. C., Hart, S. E., & Wax, L. M. (1999). Herbicide combinations for weed management in Glyphosate-resistant soybean (*Glycine max*). *Weed Technology, 13*(2), 354-360.

Gossen, B. D., Peng, G., Wolf, T. M., & McDonald, M. R. (2008). Improving spray retention to enhance the efficacy of foliar-applied disease-and pest-management products in field and row crops. *Canadian Journal of Plant Pathology, 30*(4), 505-516.

GRDC. (2003). Faba bean variety development. *Online farm trials.* Retrieved June 12, 2019 from https://www.farmtrials.com.au/trial/13700

GRDC. (2013). Emerging issues with diseases weeds and pests supplement. Retrieved November 25, 2019 from https://grdc.com.au/resources-and-publications/groundcover/ground-cover-supplements/link.aspx

GRDC. (2014). Wild radish fact sheet. Retrieved November 14, 2019 from https://grdc.com.au/__data/assets/pdf_file/0016/117205/grdc_fs_wildradish_low-res-pdf.pdf.pdf

GRDC. (2017). GRDC Grow notes: Faba bean. Retrieved May 11th, 2019 from https://grdc.com.au/__data/assets/pdf_file/0027/292833/GRDC-GrowNotes-Faba-Southern.pdf

Green, J. M., & Owen, M. D. (2010). Herbicide-resistant crops: utilities and limitations for herbicide-resistant weed management. *Journal of Agricultural and Food Chemistry, 59*(11), 5819-5829.

Green, J. M. (2018). The rise and future of glyphosate and glyphosate-resistant crops. *Pest Management Science, 74*(5).

Gressel, J. (1995). *Creeping resistances: the outcome of using marginally-effective or reduced rates of herbicides.* Paper presented at the Brighton Crop Protection Conference-Weeds, Brighton, UK.

Grey, T. L., Wehtje, G. R., Walker, R. H., & Paudel, K. P. (1995). Comparison of imazethapyr and paraquat-based weed control systems in peanut (*Arachis hypogaea*). *Weed Technology, 9*(4), 813-818.

Guimarães, A. C. D., Mendes, K. F., dos Reis, F. C., Campion, T. F., Christoffoleti, P. J., & Tornisielo, V. L. (2018). Role of soil physicochemical properties in quantifying the fate of diuron, hexazinone, and metribuzin. *Environmental Science and Pollution Research, 25*(13), 12419-12433.

Gundel, P. E., Martínez-Ghersa, M. A., & Ghersa, C. M. (2008). Dormancy, germination and ageing of *Lolium multiflorum* seeds following contrasting herbicide selection regimes. *European Journal of Agronomy, 28*(4), 606-613.

Gunier, R. B., Bradman, A., Harley, K. G., & Eskenazi, B. (2017). Will buffer zones around schools in agricultural areas be adequate to protect children from the potential adverse effects of pesticide exposure? *PLoS Biology, 15*(12).

Gutterman, Y., & Gendler, T. (2005). Annual rhythm of germination of seeds of *Mesembryanthemum nodiflorum* 32 years after collection. *Seed Science Research, 15*(3), 249-253.

Hahns-Vaughn, D. L. (2017). *Applied Multivariate Statistical Concepts*. New York. Routledge.

Halford, C., Hamill, A. S., Zhang, J., & Doucet, C. (2001). Critical period of weed control in no-till soybean (*Glycine max*) and corn (*Zea mays*). *Weed Technology, 15*(4), 737-744.

Hall, J. C., Van Eerd, L. L., Miller, S. D., Owen, M. D., Prather, T. S., Shaner, D. L., . . . Weller, S. C. (2000). Future research directions for weed science. *Weed Technology, 14*(3), 647-658.

Hall, M. R., Swanton, C. J., & Anderson, G. W. (1992). The critical period of weed control in grain corn (*Zea mays*). *Weed Science, 40*(3), 441-447.

Hamill, A. S., & Zhang, J. (1997). Rate and time of bentazon/atrazine application for broadleaf weed control in corn (*Zea mays*). *Weed Technology, 11*(3), 549-555.

Hammerton, J. L. (1967). Environmental factors and susceptibility to herbicides. *Weeds, 15*(4), 330-336.

Hammond, C. M., Luschei, E. C., Boerboom, C. M., & Nowak, P. J. (2017). Adoption of integrated pest management tactics by wisconsin farmers. *Weed Technology, 20*(3), 756-767.

Harker, K. N., Blackshaw, R. E., Kirkland, K. J., Derksen, D. A., & Wall, D. (2000). Herbicide-tolerant canola: weed control and yield comparisons in western Canada. *Canadian Journal of Plant Science, 80*(3), 647-654.

Harker, K. N., & O'donovan, J. T. (2013). Recent weed control, weed management, and integrated weed management. *Weed Technology, 27*(1), 1-11.

Harper, J. L. (1956). *The evolution of weeds in relation to resistance to herbicides.* Paper presented at the Third British Weed Control Conference, Blackpool, England.

Harries, M., Flower, K. C., Scanlan, C. A., Rose, M. T., & Renton, M. (2020). Interactions between crop sequences, weed populations and herbicide use in Western Australian broadacre farms: findings of a six-year survey. *Crop and Pasture Science, 71*(5), 491-505.

Hashem, A., Bowran, D., Piper, T., & Dhammu, H. (2001a). Resistance of Wild Radish (*Raphanus raphanistrum*) to Acetolactate Synthase-Inhibiting Herbicides in the Western Australia Wheat Belt1. *Weed Technology, 15*(1), 68-74.

Hashem, A., Dhammu, H. S., Powles, S. B., Bowran, D. G., Piper, T. J., & Cheam, A. H. (2001b). Triazine resistance in a biotype of wild radish (*Raphanus raphanistrum*) in Australia. *Weed Technology, 15*(4), 636-641.

Hashem, A., & Dhammu, H. S. (2002). Cross-resistance to imidazolinone herbicides in chlorsulfuron-resistant *Raphanus raphanistrum*. *Pest Management Science, 58*(9), 917-919.

Hashem, A., & Wilkins, N. (2002). *Competitiveness and persistence of wild radish (Raphanus raphanistrum L.) in a wheat-lupin rotation.* Paper presented at the 13th Australian weeds conference, WA, Australia.

Hashem, A., Pathan, S., & French, B. (2006). *Wild radish-lupin competition: difference in the competitive ability of lupin cultivars.* Paper presented at the 15th Australian Weeds Conference, WA, Australia.

Heap, I. (2014). Herbicide resistant weeds. In D. Pimentel & R. Peshin (Eds.), *Integrated Pest Management* (pp. 281-301). Dordrecht, Netherlands: Springer.

Heap, I. (2017). The International Survey of Herbicide Resistant Weeds. Retrieved October 10, 2017, from weedscience.org.au http://www.weedscience.org/Summary/CountrySummary.aspx

Heap, I., & Duke, S. O. (2018). Overview of glyphosate-resistant weeds worldwide. *Pest Management Science, 74*(5), 1040-1049.

Heap, I. (2019). The International Survey of Herbicide Resistant Weeds. Retrieved November 13, 2019, from weedscience.org.au http://www.weedscience.org/Graphs/ChronologicalIncrease.aspx

Heap, I. (2020). The International Survey of Herbicide Resistant Weeds. Retrieved February 20, 2020, from weedscience.org.au www.weedscience.com

Hill, J. P., & Lord, E. M. (1987). Dynamics of pollen tube growth in the wild radish *Raphanus raphanistrum* (Brassicaceae). II. Morphology, cytochemistry and ultrastructure of transmitting tissues, and path of pollen tube growth. *American Journal of Botany, 74*(7), 988-997.

Hobbs, P. R. (2007). Conservation agriculture: what is it and why is it important for future sustainable food production? *The Journal of Agricultural Science, 145*(2), 127.

Holm, F. A., Kirkland, K. J., & Stevenson, F. C. (2000). Defining optimum herbicide rates and timing for wild oat (*Avena fatua*) control in spring wheat (*Triticum aestivum*). *Weed Technology, 14*(1), 167-175.

Holm, L. G. (1997). *World weeds: natural histories and distribution*. New York, USA. John Wiley & Sons.

Honarmand, S. J., Nosratti, I., Nazari, K., & Heidari, H. (2016). Factors affecting the seed germination and seedling emergence of muskweed (*Myagrum perfoliatum*). *Weed Biology and Management, 16*(4), 186-193.

Hunt, N., & Gilkes, B. (1992). Farm Monitoring Handbook: A practical down-to-earth manual for farmers and other land users. The University of Western Australia, Nedlands.

Hurley, T. M., & Frisvold, G. (2016). Economic barriers to herbicide-resistance management. *Weed Science, 64*(SP1), 585-594.

Husain, M. M., Reid, J. B., Othman, H., & Gallagher, J. N. (1990). Growth and water use of faba beans (*Vicia faba*) in a sub-humid climate I. Root and shoot adaptations to drought stress. *Field Crops Research, 23*(1), 1-17.

Iqbal, N., Manalil, S., Chauhan, B. S., & Adkins, S. W. (2019). Investigation of alternate herbicides for effective weed management in glyphosate-tolerant cotton. *Archives of Agronomy and Soil Science, 65*(13), 1885-1899.

Javaid, A. (2007). Efficacy of some common herbicides against Parthenium weed. *Pakistan Journal of Weed Science Research, 13*(1-2), 93-98.

Jefferies, M. L., Willenborg, C. J., & Tar'an, B. (2016). Response of chickpea cultivars to imidazolinone herbicide applied at different growth stages. *Weed Technology, 30*(3), 664-676.

Jensen, P. K. (1993). Tolerance to foliage-applied herbicides in combining peas: effect of growth stage, cultivar type and herbicide. *Crop Protection, 12*(3), 214-218.

Johnson, D. B., & Norsworthy, J. K. (2014). Johnsongrass (*Sorghum halepense*) management as influenced by herbicide selection and application timing. *Weed Technology, 28*(1), 142-150.

Johnson, D. E., Wopereis, M. C. S., Mbodj, D., Diallo, S., Powers, S., & Haefele, S. M. (2004). Timing of weed management and yield losses due to weeds in irrigated rice in the Sahel. *Field Crops Research, 85*(1), 31-42.

Kaloumenos, N. S., Capote, N., Aguado, A., & Eleftherohorinos, I. G. (2013). Red rice (Oryza sativa) cross-resistance to imidazolinone herbicides used in resistant rice cultivars grown in northern Greece. *Pesticide Biochemistry and Physiology, 105*(3), 177-183.

Kavaliauskaitė, D., & Bobinas, Č. (2006). Determination of weed competition critical period in red beet. *Agronomy Research, 4*(Sp), 217-220.

Kavurmaci, Z., Karadavut, U., Kokten, K., & Bakoglu, A. (2010). Determining critical period of weed-crop competition in faba bean (*Vicia faba*). *International Journal of Agriculture Biology, 12*(2), 318-320.

Kebaso, L., Frimpong, D., Iqbal, N., Bajwa, A. A., Namubiru, H., Ali, H. H., . . . Chauhan, B. S. (2020). Biology, ecology and management of *Raphanus raphanistrum* L.: a noxious agricultural and environmental weed. *Environmental Science and Pollution Research, 27*(15), 17692-17705.

Kieloch, R., & Domaradzki, K. (2011). The role of the growth stage of weeds in their response to reduced herbicide doses. *Acta Agrobotanica, 64*(4), 259-266.

Kiran, Y. D., Subramanyam, D., & Sumathi, V. (2010). Growth and yield of transplanted rice (*Oryza sativa*) as influenced by sequential application of herbicides. *Indian Journal of Weed Science, 42*(3), 226-228.

Kirkland, K. J., Holm, F., & Stevenson, F. C. (2000). Appropriate crop seeding rate when herbicide rate is reduced. *Weed Technology, 14*(4), 692-698.

Kishore, G. M., Padgette, S. R., & Fraley, R. T. (1992). History of herbicide-tolerant crops, methods of development and current state of the art–emphasis on glyphosate tolerance. *Weed Technology, 6*(3), 626-634.

Kleemann, S. G., Chauhan, B. S., & Gill, G. S. (2007). Factors affecting seed germination of perennial wall rocket (*Diplotaxis tenuifolia*) in Southern Australia. *Weed Science, 55*(5), 481-485.

Kleinman, Z., Ben-Ami, G., & Rubin, B. (2016). From sensitivity to resistance–factors affecting the response of *Conyza* spp. to glyphosate. *Pest Management Science, 72*(9), 1681-1688.

Klingaman, T. E., King, C. A., & Oliver, L. R. (1992). Effect of application rate, weed species, and weed stage of growth on imazethapyr activity. *Weed Science, 40*(2), 227-232.

Knezevic, S. Z., & Cassman, K. G. (2003). Use of herbicide-tolerant crops as a component of an integrated weed management program. *Crop Management, 2*(1), 1-7.

Knezevic, S. Z., Datta, A., Scott, J., Klein, R. N., & Golus, J. (2009). Problem weed control in glyphosate-resistant soybean with glyphosate tank mixes and soil-applied herbicides. *Weed Technology, 23*(4), 507-512.

Knowler, D., & Bradshaw, B. (2007). Farmers' adoption of conservation agriculture: A review and synthesis of recent research. *Food Policy, 32*(1), 25-48.

Koger, C. H., Reddy, K. N., & Poston, D. H. (2004). Factors affecting seed germination, seedling emergence, and survival of texasweed (*Caperonia palustris*). *Weed Science, 52*(6), 989-995.

Kremer, R. J. (2017). Soil and environmental health after twenty years of intensive use of glyphosate. *Advances in Plants and Agriculture Research, 6*(5), 00224.

Kruidhof, H. M., Bastiaans, L., & Kropff, M. J. (2008). Ecological weed management by cover cropping: effects on weed growth in autumn and weed establishment in spring. *Weed Research, 48*(6), 492-502.

Kudsk, P., & Kristensen, J. L. (February 1992). *Effect of environmental factors on herbicide performance*. Paper presented at the 1st international weed control congress, Victoria, Australia. https://agris.fao.org/agris-search/search.do?recordID=AU9201347

Kudsk, P. (2008). Optimising herbicide dose: a straightforward approach to reduce the risk of side effects of herbicides. *The Environmentalist, 28*(1), 49-55.

Kuk, Y. I., Burgos, N. R., & Shivrain, V. K. (2008). Natural tolerance to imazethapyr in red rice (*Oryza sativa*). *Weed Science, 56*(1), 1-11.

Kurre, D. K., Bharati, V., Singh, A., Kumar, M., & Prasad, S. (2017). Impact of herbicides on yield, economics and phytotoxicity in kharif maize. *Pharma Innovation, 6*(11), 190-192.

Lagator, M., Vogwill, T., Mead, A., Colegrave, N., & Neve, P. (2013). Herbicide mixtures at high doses slow the evolution of resistance in experimentally evolving populations of *Chlamydomonas reinhardtii*. *New Phytologist, 198*(3), 938-945.

Lamichhane, J. R., Dachbrodt-Saaydeh, S., Kudsk, P., & Messéan, A. (2016). Toward a reduced reliance on conventional pesticides in European agriculture. *Plant Disease, 100*(1), 10-24.

Lamichhane, J. R., Devos, Y., Beckie, H. J., Owen, M. D., Tillie, P., Messéan, A., & Kudsk, P. (2017). Integrated weed management systems with herbicide-tolerant crops in the European Union: lessons learnt from home and abroad. *Critical reviews in biotechnology, 37*(4), 459-475.

Lanclos, D. Y., Webster, E. P., & Zhang, W. (2002). Glufosinate tank-mix combinations in glufosinate-resistant rice (*Oryza sativa*). *Weed Technology, 16*(3), 659-663.

Légère, A., Beckie, H. J., Stevenson, F. C., & Thomas, A. G. (2000). Survey of management practices affecting the occurrence of wild oat (*Avena fatua*) resistance to acetyl-CoA carboxylase inhibitors. *Weed Technology, 14*(2), 366-376.

Lemerle, D., & Hinkley, R. B. (1991). Tolerances of canola, field pea, lupin and faba bean cultivars to herbicides. *Australian Journal of Experimental Agriculture, 31*(3), 379-386. doi:https://doi.org/10.1071/EA9910379

Livingston, M., Fernandez-Cornejo, J., Unger, J., Osteen, C., Schimmelpfennig, D., Park, T., & Lambert, D. M. (2015). *The economics of glyphosate resistance management in corn and soybean production*. Retrieved from https://papers.ssrn.com/sol3/papers.cfm?abstract_id=2690579

Llewellyn, R. S., Lindner, R. K., Pannell, D. J., & Powles, S. B. (2004). Grain grower perceptions and use of integrated weed management. *Australian Journal of Experimental Agriculture, 44*(10), 993-1001.

Llewellyn, R. S., David, R., Michael, C., Steve, W., & Allan, M. (2016). *Impact of Weeds on Australian Grain Production: the cost of weeds to Australian grain growers and the adoption of weed management and tillage practices*. Retrieved from https://grdc.com.au/__data/assets/pdf_file/0027/75843/grdc_weeds_review_r8.pdf.pdf

Loss, S. P., Siddique, K. H. M., & Tennant, D. (1997). Adaptation of faba bean (*Vicia faba* L.) to dryland Mediterranean-type environments III. Water use and water-use efficiency. *Field Crops Research, 54*(2-3), 153-162.

Lu, H., Yu, Q., Han, H., Owen, M. J., & Powles, S. B. (2019). A novel psbA mutation (Phe274–Val) confers resistance to PSII herbicides in wild radish (*Raphanus raphanistrum*). *Pest Management Science, 75*(1), 144-151.

Maalouf, F., Naoum, A. A., El Shamaa, K., & Patil, S. B. (2016). *Wide range of genetic variabilrty for herbicide tolerance in Faba bean*. Paper presented at the International Conference on Pulses, Marrakesh, Morocco.

Madafiglio, G. P. (2002). *Population management of Raphanus raphanistrum L.(wild radish) by regulating seed production.* (Ph. D. thesis), University of Western Sydney, NSW, Australia. Retrieved from https://researchdirect.westernsydney.edu.au/islandora/object/uws:339/

Madafiglio, G. P., Medd, R. W., Cornish, P. S., & Ven, R. v. d. (2006). Seed production of *Raphanus raphanistrum* following herbicide application during reproduction and effects on wheat yield. *Weed Research, 46*(1), 50-60.

Mahmood, A. H., Florentine, S. K., Chauhan, B. S., McLaren, D. A., Palmer, G. C., & Wright, W. (2016). Influence of various environmental factors on seed germination and seedling emergence of a noxious environmental weed: green galenia (*Galenia pubescens*). *Weed Science, 64*(3), 486-494.

Malefyt, T., Quakenbush, L., Shaner, D. L., & O'Connor, S. L. (1991). Influence of environmental factors on the biological activity of the imidazolinone herbicides. In S. D. L & S. L. O'Connor (Eds.), *The Imidazolinone Herbicides* (pp. 103-127). Boca Raton, Florida: CRC Press

Malik, M. S., Norsworthy, J. K., Riley, M. B., & Bridges Jr, W. (2010). Temperature and light requirements for wild radish (*Raphanus raphanistrum*) germination over a 12-month period following maturation. *Weed Science, 58*(2), 136-140.

Manickavelu, A., Nadarajan, N., Ganesh, S. K., Gnanamalar, R. P., & Babu, R. C. (2006). Drought tolerance in rice: morphological and molecular genetic consideration. *Plant Growth Regulation, 50*(2-3), 121-138.

Manley, B. S., Wilson, H. P., & Hines, T. E. (1998). Characterization of imidazolinone-resistant smooth pigweed (*Amaranthus hybridus*). *Weed Technology, 12*(4), 575-584.

Mao, D., Paull, J., Preston, C., Dyson, C., Yang, S., & McMurray, L. (2015). *Improving herbicide tolerance in pulses to support the diversification of Australian crop rotations.* Paper presented at the Building Productive, Diverse and Sustainable Landscapes, 17th Australian Agronomy Conference, Hobart, Australia.

Mao, D., Michelmore, S., Paull, J., Preston, C., Sutton, T., Oldach, K., . . . McMurray, L. (2019). Phenotypic and molecular characterisation of novel Vicia faba germplasm with tolerance to acetohydroxyacid synthase-inhibiting herbicides (AHAS) developed through mutagenesis techniques. *Pest Management Science, 75*(10), 2698-2705.

Marcano, L., Hernandez, J., Zapata-Vivenes, E., & Leon, A. (2017). Effects of contaminated natural soil by Glyphosan SL on biochemical responses of the earthworm *Eisenia* sp. *Journal of Toxicology and Environmental Health Sciences, 9*(10), 92-97.

Martin, A. R., Roeth, F. W., & Lee, C. (2000). *Herbicide resistant weeds*. Retrieved from Lincon, USA: https://digitalcommons.unl.edu/extensionhist/91/

Martin, D. A., Miller, S. D., & Alley, H. P. (1990). Spring wheat response to herbicides applied at three growth stages. *Agronomy Journal, 82*(1), 95-97.

Marwat, K. B., Muhammad, S., Zahid, H., & Bakhtiar, G. (2008). Study of various herbicides for weed control in wheat under irrigated conditions. *Pakistan Journal of Weed Science Research, 14*, 1-8.

Maseko, I., Ncube, B., Mabhaudhi, T., Tesfay, S., Chimonyo, V., Araya, H., . . . Du Plooy, C. (2019). Moisture stress on physiology and yield of some indigenous leafy vegetables under field conditions. *South African Journal of Botany, 126*, 85-91.

Matthews, P., & Marcellos, H. (2003). *Faba bean*. Retrieved from https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0004/157729/faba-bean-pt1.pdf

Maxwell, A., & Scott, J. K. (2008). Pathogens on wild radish, *Raphanus raphanistrum* (Brassicaceae), in south-western Australia—implications for biological control. *Australasian Plant Pathology, 37*(5), 523-533.

Maxwell, B. D., Roush, M. L., & Radosevich, S. R. (1990). Predicting the evolution and dynamics of herbicide resistance in weed populations. *Weed Technology, 4*(1), 2-13.

McGillion, T., & Storrie, A. (2006). *Integrated weed management in Australian cropping systems–a training resource for farm advisors*. Adelaide, SouthAustralia. CRC for Australian Weed Management.

McGonigle, B., Lau, S. C., Jennings, L. D., & O'Keefe, D. P. (1998). Homoglutathione selectivity by soybean GlutathioneS-Transferases. *Pesticide Biochemistry and Physiology, 62*(1), 15-25.

McMurray, L., Mao, D., Preston, C., Sutton, T., Michelmore, S., Yang, S. Y., . . . Paull, J. (2016 September). *Herbicide tolerance to enhance pulse contribution to the farming system.* Paper presented at the Australian Pulse Conference, Australia.

McWhorter, C. G., Jordan, T. N., & Wills, G. D. (1980). Translocation of 14 C-glyphosate in soybeans (*Glycine max*) and johnsongrass (*Sorghum halepense*). *Weed Science, 28*(1), 113-118.

Mekenian, M. R., & Willemsen, R. W. (1975). Germination characteristics of *Raphanus raphanistrum*. I. Laboratory studies. *Bulletin of the Torrey Botanical Club*, 243-252.

Metzger, B. A., Soltani, N., Raeder, A. J., Hooker, D. C., Robinson, D. E., & Sikkema, P. H. (2019). Influence of application timing and herbicide rate on the efficacy of tolpyralate plus atrazine. *Weed Technology, 33*(3), 448-458.

Michel, B. E. (1983). Evaluation of the water potentials of solutions of Polyethylene Glycole 8000 both in the absence and presence of other solutes. *Plant Physiology*, *72*, 66-70.

Minkey, D. (2006). Wild radish (*Raphanus raphanistrum* L) development and seed production in response to time of emergence, crop-topping and sowing rate of wheat. *Plant Protection Quarterly, 21*(1), 25.

Minton, B. W., Kurtz, M. E., & Shaw, D. R. (1989). Barnyardgrass (*Echinochloa crus-galli*) Control with Grass and Broadleaf Weed Herbicide Combinations. *Weed Science, 37*(2), 223-227.

Moll, S. (1997). *Commercial experience and benefits from glyphosate-tolerant crops*. Paper presented at the Brighton Crop Potection Conference Weeds, Brighton, UK.

Monfreda, C., Ramankutty, N., & Foley, J. A. (2008). Farming the planet: 2. Geographic distribution of crop areas, yields, physiological types, and net primary production in the year 2000. *Global Biogeochemical Cycles, 22*(1).

Morishita, D. W. (2017). Weed management in grain legume cultivation. In S. Sivasankar, D. Bergvinson, P. Gaur, S. K. Agrawal, S. Beebe, & M. Tamo (Eds.), *Achieving sustainable cultivation of grain legumes* (Vol. 1, pp. 321-335). Cambridge, UK: Burleigh Dodds Science Publishing Limited.

Mortensen, D. A., Egan, J. F., Maxwell, B. D., Ryan, M. R., & Smith, R. G. (2012). Navigating a critical juncture for sustainable weed management. *BioScience, 62*(1), 75-84.

Mortimer, A. (1984). Population ecology and weed science. In R. Dirzo & J. Sarukhan (Eds.), *Perspectives on Plant Population Ecology* (pp. 363–388). Sunderland, MA: Sinauer Associate.

Moss, S. (2010). *Non-chemical methods of weed control: benefits and limitations.* Paper presented at the 17th Australasian Weed Conference, Christchurch, New Zealand.

Moss, S. (2017). Herbicide Resistance in Weeds. In *Weed Research: Expanding Horizons* (pp. 181-214). Hoboken, New Jersey: Jon Wiley & Sons Ltd.

Moss, S. (2019). Integrated weed management (IWM): why are farmers reluctant to adopt non-chemical alternatives to herbicides? *Pest Management Science, 75*(5), 1205-1211.

Muzik, T. (1976). Influence of environmental factors on toxicity to plants. In L. J. Audus (Ed.), *Herbicides: Physiology, Biochemistry, Ecology* (pp. 204-247). New York: Academic Press.

Myers, N. (2001). *Perverse subsidies: how tax dollars can undercut the environment and the economy*. Washington, DC. Island Press.

Nabipour, M., Ahmadpour, S. R., Mesgarbashi, M., Rajabi-Memari, H., & Farzane, M. (2017). Mechanisms of resistance to metribuzin in new resistant biotype of jungle rice (*Echinochloa colona*) in sugarcane fields of hot semi-arid climates. *Australian Journal of Crop Science, 11*(7), 868.

Nath, C. P., Das, T. K., Rana, K. S., Bhattacharyya, R., Paul, S., Singh, S. B., . . . Hazra, K. K. (2018). Tillage and nitrogen management effects with sequential and ready-mix herbicides on weed diversity and wheat productivity. *International journal of pest management, 64*(4), 303-314.

Neve, P., Diggle, A. J., Smith, F. P., & Powles, S. B. (2003). Simulating evolution of glyphosate resistance in *Lolium rigidum* I: population biology of a rare resistance trait. *Weed Research, 43*(6), 404-417.

Neve, P. (2008). Simulation modelling to understand the evolution and management of glyphosate resistance in weeds. *Pest Management Science: formerly Pesticide Science, 64*(4), 392-401.

Newman, P. (2003). *The looming threat of wild radish.* Retrieved from http://archive.lls.nsw.gov.au/__data/assets/pdf_file/0010/495289/archive-wild_radish.pdf

Newman, P. (2005). *Knockdowns for large wild radish, single and double.* Paper presented at the Agribusiness Crop Updates 2005, Department of Agriculture, Western Australia. Perth.

Newman, P., & Adam, G. (2006). *Croptopping of wild radish in lupins and barley, how long is a piece of string*. Perth, Australia. Department of Agriculture and Food Western Australia.

Newman, P. (2013). *We can kill wild radish in lupins if we have enough spray capacity.* Paper presented at the Agribusiness Crop Updates 2013, Western Australia. Perth.

Niknam, S., Moerkerk, M., & Cousens, R. (September 2002). *Weed seed contamination in cereal and pulse crops.* Paper presented at the 13th Australian Weeds Conference, Perth, WA, Australia.

Norsworthy, J. K., & Oliveira, M. J. (2006). Sicklepod (*Senna obtusifolia*) germination and emergence as affected by environmental factors and seeding depth. *Weed Science, 54*(5), 903-909.

Norsworthy, J. K., Ward, S. M., Shaw, D. R., Llewellyn, R. S., Nichols, R. L., Webster, T. M., . . . Barrett, M. (2012). Reducing the Risks of Herbicide Resistance: Best Management Practices and Recommendations. *Weed Science, 60*(sp1), 31-62.

Nufarm. (2020). Nufarm Intercept herbicide. Retrieved July 20, 2020 from https://nufarm.com/au/product/intercept/

Olson, B. L. S., Al-Khatib, K., Stahlman, P., & Isakson, P. J. (2000). Efficacy and metabolism of MON 37500 in *Triticum aestivum* and weedy grass species as affected by temperature and soil moisture. *Weed Science, 48*(5), 541-548.

Owen, M. D. K., & Zelaya, I. A. (2005). Herbicide-resistant crops and weed resistance to herbicides. *Pest Management Science, 61*(3), 301-311.

Owen, M. D. K., Beckie, H. J., Leeson, J. Y., Norsworthy, J. K., & Steckel, L. E. (2015). Integrated pest management and weed management in the United States and Canada. *Pest Management Science, 71*(3), 357-376.

Owen, M. D. K. (2016). Diverse Approaches to Herbicide-Resistant Weed Management. *Weed Science, 64*(sp1), 570-584.

Owen, M. J., Walsh, M. J., Llewellyn, R. S., & Powles, S. B. (2007). Widespread occurrence of multiple herbicide resistance in Western Australian annual ryegrass (*Lolium rigidum*) populations. *Australian Journal of Agricultural Research, 58*(7), 711-718.

Owen, M. J., Martinez, N. J., & Powles, S. B. (2015). Multiple herbicide-resistant wild radish (*Raphanus raphanistrum*) populations dominate Western Australian cropping fields. *Crop and Pasture Science, 66*(10).

Oyarzabal, E. S. (1991). *Effect of weed water stress on postemergence herbicide activity.* (Ph. D. Thesis), Iowa State University, Iowa State University Digital Repository.

Pandolfo, C. E., Presotto, A., Poverene, M., & Cantamutto, M. (2013). Limited occurrence of resistant radish (*Raphanus sativus*) to AHAS-inhibiting herbicides in Argentina. *Planta daninha, 31*(3), 657-666.

Pandolfo, C. E., Presotto, A., Moreno, F., Dossou, I., Migasso, J. P., Sakima, E., & Cantamutto, M. (2016). Broad resistance to acetohydroxyacid-synthase-inhibiting herbicides in feral radish (*Raphanus sativus* L.) populations from Argentina. *Journal of Pest Management Science, 72*(2), 354-361.

Parsons, W. T., & Cuthbertson, E. (2001). *Noxious weeds of Australia*. Victoria, Australia. CSIRO.

Pathan, S., French, R. J., & Hashem, A. (September 2006). *Competitive effects of wild radish (Raphanus raphanistrum L.) on lupin cultivars (Lupinus angustifolius L.).* Paper presented at the 13th Australian Agronomy Conference Perth, WA, Australia

Patterson, D. T. (1995). Effects of environmental stress on weed/crop interactions. *Weed Science, 43*(3), 483-490.

PBA. (2018). PBA Bendoc Faba Bean. Retrieved May 12, 2019 from https://static1.squarespace.com/static/5892d8953a0411ada0658a98/t/5ba2e96888251ba163909600/1537403251838/Bendoc+Faba+Bean+18+-+PBA+factsheet.pdf

Peerzada, A. M., O'Donnell, C., & Adkins, S. (2019). Optimizing herbicide use in herbicide-tolerant crops: challenges, opportunities, and recommendations. In M. Hasanuzzaman (Ed.), *Agronomic Crops* (pp. 283-316). Singapore: Springer.

Peng, S., Huang, J., Sheehy, J. E., Laza, R. C., Visperas, R. M., Zhong, X., . . . Cassman, K. G. (2004). Rice yields decline with higher night temperature from global warming. *Proceedings of the National Academy of Sciences, 101*(27), 9971-9975.

Perotti, V. E., Larran, A. S., Palmieri, V. E., Martinatto, A. K., & Permingeat, H. R. (2020). Herbicide resistant weeds: A call to integrate conventional agricultural practices, molecular biology knowledge and new technologies. *Plant Science, 290*.

Phipps, R. H., & Park, J. (2002). Environmental benefits of genetically modified crops: global and European perspectives on their ability to reduce pesticide use. *Journal of Animal and Feed sciences, 11*(1), 1-18.

Piggin, C. M., Reeves, T. G., Brooke, H. D., & Code, G. R. (1978). *Germination of wild radish (Raphanus raphanistrum L.).* Paper presented at the 1st Conference of the Australian Weed Science Societies, Victoria, Australia.

Plaza, G., Osuna, M., Prado, R., & Heredia, A. (2006). Absorption and translocation of imazethapyr as a mechanism responsible for resistance of *Euphorbia heterophylla* L. biotypes to acetolactate synthase (ALS) inhibitors. *Agronomía Colombiana, 24*, 302-305.

Powles, S. B., Preston, C., Bryan, I. B., & Jutsum, A. R. (1996). Herbicide resistance: impact and management. In D. L. Sparks (Ed.), *Advances in Agronomy* (Vol. 58, pp. 57-93): Academic Press.

Powles, S. B. (1997). *Success from adversity: herbicide resistance can drive changes to sustainable weed management systems.* Paper presented at the Brighton Crop Protection Conference- Weeds, Franham, UK.

Powles, S. B., Monjardino, M., Llewellyn, R. S., Pannell, D., & Légére, A. (2000). *Proactive versus reactive herbicide resistance management: understanding the economic sense of herbicide conservation versus exploitation.* Paper presented at the 3rd International Weed Science Congress, Foz Do Iguassu, Brazil.

Powles, S. B., & Yu, Q. (2010). Evolution in action: plants resistant to herbicides. *Annual Review of Plant Biology, 61*(1), 317-347.

Powles, S. B., & Gaines, T. A. (2016). Exploring the potential for a regulatory change to encourage diversity in herbicide use. *Weed Science, 64*(sp1), 649-654.

Prasad, P. V., Boote, K. J., & Allen Jr, L. H. (2006). Adverse high temperature effects on pollen viability, seed-set, seed yield and harvest index of grain-sorghum [*Sorghum bicolor* (L.) Moench] are more severe at elevated carbon dioxide due to higher tissue temperatures. *Agricultural and Forest Meteorology, 139*(3-4), 237-251.

Pulse Australia. (2016). Faba bean production: Southern and Western region. Retrieved October 12, 2017 from http://pulseaus.com.au/growing-pulses/bmp/faba-and-broad-bean/southern-guide

Rajcan, I., & Swanton, C. J. (2001). Understanding maize–weed competition: resource competition, light quality and the whole plant. *Field Crops Research, 71*(2), 139-150.

Ramankutty, N., Evan, A. T., Monfreda, C., & Foley, J. A. (2008). Farming the planet: 1. Geographic distribution of global agricultural lands in the year 2000. *Global Biogeochemical Cycles, 22*(1).

Ranjan, P. N., Ram, C. J., Anurag, T., Nilesh, J., Kumar, P. B., Suresh, Y., . . . Rahul, K. (2020). Breeding for herbicide tolerance in crops: a review. *Research Journal of Biotechnology, 15*(4), 154-162.

Rasche, E., & Gadsby, M. (November 1997). *Glufosinate ammonium tolerant crops-international commercial developments and experiences.* Paper presented at the Brighton Crop Protection Conference Weeds, Brighton, UK.

Ray, T. B. (1984). Site of Action of Chlorsulfuron: Inhibition of Valine and Isoleucine biosynthesis in plants. *Plant Physiology, 75*(3), 827-831.

Reeg, J., Schad, T., Preuss, T. G., Solga, A., Körner, K., Mihan, C., & Jeltsch, F. (2017). Modelling direct and indirect effects of herbicides on non-target grassland communities. *Ecological Modelling, 348*, 44-55.

Reeves, T. G., Code, G. R., & Piggin, C. M. (1981). Seed production and longevity, seasonal emergence and phenology of wild radish (*Raphanus raphanistrum* L.). *Australian Journal of Experimental Agriculture, 21*(112), 524-530.

Reid, J. B. (1990). Growth and water use of faba beans (*Vicia faba*) in a subhumid climate II. Simulation analysis of crop responses to drought. *Field Crops Research, 23*(1), 19-38.

Rengasamy, P. (2002). Transient salinity and subsoil constraints to dryland farming in Australian sodic soils: an overview. *Australian Journal of Experimental Agriculture, 42*(3), 351-361.

Rey-Caballero, J., Menéndez, J., Osuna, M. D., Salas, M., & Torra, J. (2017). Target-site and non-target-site resistance mechanisms to ALS inhibiting herbicides in *Papaver rhoeas*. *Pesticide Biochemistry and Physiology, 138*, 57-65.

Riar, D. S., Norsworthy, J. K., Steckel, L. E., Stephenson, D. O., Eubank, T. W., & Scott, R. C. (2013). Assessment of weed management practices and problem weeds in the midsouth United States—soybean: a consultant's perspective. *Weed Technology, 27*(3), 612-622.

Roberts, H., & Boddrell, J. E. (1983). Seed survival and periodicity of seedling emergence in eight species of Cruciferae. *Annals of Applied Biology, 103*(2), 301-309.

Roberts, P., Mao, D., Aggarwal, N., Day, S., Michelmore, S., Sutton, T., . . . Gutsche, A. (2020). Spotlight on pulses. Retrieved Aril 4th, 2020, from GRDC https://grdc.com.au/resources-and-publications/grdc-update-papers/tab-content/grdc-update-papers/2020/02/spotlight-on-pulses

Robinson, M. A., Letarte, J., Cowbrough, M. J., Sikkema, P. H., & Tardif, F. J. (2015). Winter wheat (*Triticum aestivum* L.) response to herbicides as affected by application timing and temperature. *Canadian Journal of Plant Science, 95*(2), 325-333.

Rotteveel, T. J., de Goeij, J. W., & van Gemerden, A. F. (1997). Towards the construction of a resistance risk evaluation scheme. *Pesticide science, 51*(3), 407-411.

Rubiales, D., Pérez-de-Luque, A., Fernández-Aparico, M., Sillero, J. C., Román, B., Kharrat, M., . . . Riches, C. (2006). Screening techniques and sources of resistance against parasitic weeds in grain legumes. *Euphytica, 147*, 187-199.

Saari, L. L., Cotterman, J. C., & Thill, D. C. (1994). Resistance to acetolactate synthase inhibiting herbicides. In S. B. Powles & J. A. M. Holtum (Eds.), *Herbicide Resistance in Plants: Biology and Biochemistry.* (pp. 83-140). Ann Arbor, Michigan: Lewis.

Saatkamp, A., Affre, L., Dutoit, T., & Poschlod, P. (2011). Germination traits explain soil seed persistence across species: the case of Mediterranean annual plants in cereal fields. *Annals of Botany, 107*(3), 415-426.

Sammons, R. D., Heering, D. C., Dinicola, N., Glick, H., & Elmore, G. A. (2007). Sustainability and stewardship of glyphosate and glyphosate-resistant crops. *Weed Technology, 21*(2), 347-354.

Sampson, D. (1964). A one-locus self-incompatibility system in *Raphanus raphanistrum*. *Canadian Journal of Genetics and Cytology, 6*(4), 435-445.

Samunder, S., & Singh, M. (2004). Effect of growth stage on Trifloxysulfuron and Glyphosate efficacy in twelve weed species of citrus groves. *Weed Technology, 18*(4), 1031-1036.

Sandström, J., Broyer, A., Zoia, D., Schilt, C., Greggio, C., Fournier, M., . . . Monnet-Tschudi, F. (2017). Potential mechanisms of development-dependent adverse effects of the herbicide paraquat in 3D rat brain cell cultures. *Neurotoxicology, 60*, 116-124.

Sankula, S., Marmon, G., & Blumenthal, E. (2005). *Biotechnology-derived crops planted in 2004: impacts on US agriculture*. Retrieved from National Center for Food and Agricultural Policy, Washington, DC, http://ucbiotech.org/biotech_info/PDFs/Sankula_2005_BiotechnologyDerivedCropsPlantedin2004.pdf

Scarabel, L., Pernin, F., & Délye, C. (2015). Occurrence, genetic control and evolution of non-target-site based resistance to herbicides inhibiting acetolactate synthase (ALS) in the dicot weed *Papaver rhoeas*. *Plant Science, 238*, 158-169.

Schroeder, J., Barrett, M., Shaw, D. R., Asmus, A. B., Coble, H., Ervin, D., . . . Creech, C. F. (2018). Managing wicked herbicide-resistance: Lessons from the field. *Weed Technology, 32*(4), 475-488.

Scott, J. K., Julien, M., McFadyen, R., & Cullen, J. (2012). *Raphanus raphanistrum* L.-wild radish. *Biological control of weeds in Australia*, 486-493.

Scott, J. K., Vitou, J., & Jourdan, M. (2002). *Review of the potential for biological control of wild radish (Raphanus raphanistrum) based on surveys in the Mediterranean region.* Paper presented at the 13th Australian Weeds Conference. Plant Protection Society of WA, Perth.

Seednet. (2020). PBA Bendoc. Retrieved April 20, 2020 from https://www.seednet.com.au/product/pba-bendoc

Sellers, B. A., Ferrell, J. A., MacDonald, G. E., & Kline, W. N. (2009). Dogfennel (*Eupatorium capillifolium*) size at application affects herbicide efficacy. *Weed Technology, 23*(2), 247-250.

Shaner, D. L., & Mallipudi, N. (1991). Mechanisms of selectivity of the imidazolinones. In D. L. Shaner & S. L. O'Conner (Eds.), *The Imidazolinone Herbicides*. Boca Raton, Florida: CRC Press.

Shaner, D. L., Howard, S., & Chalmers, I. (1999). *Effectiveness of mode of action labelling for resistance management: a survey of Australian farmers.* Paper presented at the Brighton Crop Protection Conference- Weeds, Franham, UK.

Shaner, D. L. (2000). The impact of glyphosate-tolerant crops on the use of other herbicides and on resistance management. *Pest Management Science: formerly Pesticide Science, 56*(4), 320-326.

Shaw, D. R., Mack, R. E., & Smith, C. A. (1991). Redvine (*Brunnichia ovata*) germination and emergence. *Weed Science, 39*(1), 33-36.

Sheets, T. (1970). Persistence of triazine herbicides in soils. *Residue Reviews, 32*, 287-310.

Singh, G., & Wright, D. (2002). In vitro studies on the effects of herbicides on the growth of rhizobia. *Letters in Applied Microbiology, 35*(1), 12-16.

Singh, S., & Singh, M. (2004). Effect of growth stage on trifloxysulfuron and glyphosate efficacy in twelve weed species of citrus groves1. *Weed Technology, 18*(4), 1031-1036.

Skelton, J. J., Ma, R., & Riechers, D. E. (2016). Waterhemp (*Amaranthus tuberculatus*) control under drought stress with 2,4-dichlorophenoxyacetic acid and glyphosate. *Weed Biology and Management, 16*(1), 34-41.

Skylas, D. J., Paull, J. G., Hughes, D. G. D., Gogel, B., Long, H., Williams, B., . . . Quail, K. J. (2019). Nutritional and anti-nutritional seed-quality traits of faba bean (*Vicia faba*) grown in South Australia. *Crop and Pasture Science, 70*(5), 463-472.

Smit, A. L., & Groenwold, J. (2005). Root characteristics of selected field crops: data from the Wageningen Rhizolab (1990–2002). *Plant and Soil, 272*, 365-384.

Smit, J. J., & Cairns, A. L. P. (2001). Resistance of *Raphanus raphanistrum* to chlorsulfuron in the Republic of South Africa. *Weed Research, 41*(1), 41-47.

Smitchger, J. A., Burke, I. C., & Yenish, J. P. (2012). The critical period of weed control in lentil (*Lens culinaris*) in the Pacific Northwest. *Weed Science, 60*(1), 81-85.

Soltani, N., Nurse, R. E., & Sikkema, P. H. (2016). Biologically effective dose of glyphosate as influenced by weed size in corn. *Canadian Journal of Plant Science, 96*(3), 455-460.

Stanton, M. L. (1984). Seed variation in wild radish: effect of seed size on components of seedling and adult fitness. *Ecology, 65*(4), 1105-1112.

Steptoe, P. J., Vencill, W. K., & Grey, T. L. (2006). Influence of moisture stress on herbicidal control of an invasive weed, Benghal dayflower (*Commelina benghalensis*). *Journal of Plant Diseases and Protection, 20*, 907-914.

Stewart, C. L., Nurse, R. E., Van Eerd, L. L., Vyn, R. J., & Sikkema, P. H. (2011). Weed control, environmental impact, and economics of weed management strategies in glyphosate-resistant soybean. *Weed Technology, 25*(4), 535-541.

Stoddard, F. (1991). Pollen vectors and pollination of faba beans in southern Australia. *Australian Journal of Agricultural Research, 42*(7), 1173-1178.

Stoltenberg, D. E. (2002). *Weed management and agronomic risks associated with glyphosate-resistant corn and soybean cropping systems.* Paper presented at the Wisconsin Fertilizer, Aglime and Pest Management Conference 2002, Wisconsin, US.

Switzer, C. (1957). *The existence of 2, 4-D resistant strains of wild carrot.* Paper presented at the Northeast Weed Control Conference, Columbia.

Tan, M. K., & Medd, R. W. (2002). Characterisation of the acetolactate synthase (ALS) gene of *Raphanus raphanistrum* L. and the molecular assay of mutations associated with herbicide resistance. *Plant Science, 163*(2), 195-205.

Tang, W., Xu, X., Shen, G., & Chen, J. (2015). Effect of environmental factors on germination and emergence of aryloxyphenoxy propanoate herbicide-resistant and-susceptible Asia minor bluegrass (*Polypogon fugax*). *Weed Science, 63*(3), 669-675.

Tawaha, A. M., & Turk, M. A. (2001). Crop-weed competition studies in faba bean (*Vicia faba* L.) under rainfed conditions. *Acta Agronomica Hungarica, 49*(3), 299-303.

Taylor, C., Craig, S., Dolman, F., & Preston, C. (2015). *Herbicide resistant Wild Radish: Take back control*. Retrieved from https://sciences.adelaide.edu.au/agriculture-food-wine/system/files/docs/2016-wild-radish.pdf

Teixeira, E. I., Fischer, G., Van Velthuizen, H., Walter, C., & Ewert, F. (2013). Global hot-spots of heat stress on agricultural crops due to climate change. *Agricultural and Forest Meteorology, 170*, 206-215.

Torres-García, J. R., Uscanga-Mortera, E., Trejo, C., Conde-Martínez, V., Kohashi-Shibata, J., Núñes-Farfán, J., & Martínez-Moreno, D. (2015). Effect of herbicide resistance on seed physiology of Phalaris minor (*littleseed canarygrass*). *Botanical Sciences, 93*(3), 661-667.

Tranel, P. J., & Wright, T. R. (2002). Resistance of weeds to ALS-inhibiting herbicides: what have we learned? *Weed Science, 50*(6), 700-712.

Tu, Y. Q., Lin, Z. M., & Zhang, J. Y. (1986). The effect of leaf shape on the deposition of spray droplets in rice. *Crop Protection, 5*(1), 3-7.

Tursun, N., Datta, A., Sakinmaz, M. S., Kantarci, Z., Knezevic, S. Z., & Chauhan, B. S. (2016). The critical period for weed control in three corn (*Zea mays* L.) types. *Crop Protection, 90*, 59-65.

Tuti, M. D., Das, T. K., Sairam, R. K., Annapurna, K., & Singh, S. B. (2015). Effect of sequential application of metribuzin on selectivity and weed control in soybean. *International Journal of Pest Management, 61*(1), 17-25.

United States Department of Agriculture. (2017). Plants database. Retrieved December 4, 2017, from USDA https://plants.usda.gov/java/ClassificationServlet?source=profile&symbol=VIFA&display=31

Van Assche, J., Van Nerum, D., & Darius, P. (2002). The comparative germination ecology of nine *Rumex* species. *Plant Ecology, 159*(2), 131-142.

Van Waes, J. M., & Debergh, P. C. (1986). Adaptation of the tetrazolium method for testing the seed viability, and scanning electron microscopy study of some Western European orchids. *Physiologia Plantarum, 66*(3), 435-442.

VanGessel, M. J., Scott, B. A., Johnson, Q. R., & White-Hansen, S. E. (2009). Influence of glyphosate-resistant horseweed (*Conyza canadensis*) growth stage on response to glyphosate applications. *Weed Technology, 23*(1), 49-53.

Varanasi, A., Prasad, P. V., & Jugulam, M. (2016). Impact of climate change factors on weeds and herbicide efficacy. In D. L. Sparks (Ed.), *Advances in Agronomy* (Vol. 135, pp. 107-146): Academic Press.

Vencill, W. K. (2002). *Herbicide Handbook*. Lawrence, USA. Weed Science Society of America.

Vila-Aiub, M. M., Neve, P., & Powles, S. B. (2005a). Resistance cost of a cytochrome P450 herbicide metabolism mechanism but not an ACCase target site mutation in a multiple resistant *Lolium rigidum* population. *New Phytologist, 167*(3), 787-796.

Vila-Aiub, M. M., Neve, P., Steadman, K. J., & Powles, S. B. (2005b). 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*(2), 288-298.

Vila-Aiub, M. M., Gundel, P. E., & Preston, C. (2015). Experimental methods for estimation of plant fitness costs associated with herbicide-resistance genes. *Weed Science, 63*(SP1), 203-216.

Wakweya, K., & Dargie, R. (2017). Effect of Different Weed Management Practices on Growth, Yield and Yield Components of Faba Bean (*Vicia faba* L.) in Bale Highland Conditions, Southeastern Ethiopia. *American-Eurasian Journal of Agriculture and Environmental Science, 17*(5), 383-391.

Walker, S., Boucher, L., Cook, T., Davidson, B., McLean, A., & Widderick, M. (2012). Weed age affects chemical control of *Conyza bonariensis* in fallows. *Crop Protection, 38*, 15-20.

Wall, D. A. (1997). Effect of crop growth stage on tolerance to low doses of thifensulfuron: tribenuron. *Weed Science, 45*(4), 538-545.

Walsh, M., & Newman, P. (2007). Burning narrow windrows for weed seed destruction. *Field Crops Research, 104*, 24-30.

Walsh, M., Newman, P., & Powles, S. (2013). Targeting weed seeds in-crop: a new weed control paradigm for global agriculture. *Weed Technology, 27*(3), 431-436.

Walsh, M. J., Duane, R. D., & Powles, S. B. (2001). High frequency of chlorsulfuron-resistant wild radish (*Raphanus raphanistrum*) populations across the Western Australian wheatbelt. *Weed Technology, 15*(2), 199-203.

Walsh, M. J. (2004). Multiple-herbicide resistance across four modes of action in wild radish (*Raphanus raphanistrum*). *Weed Science, 52*(1), 8-13.

Walsh, M. J., Newman, P., & Chitty, D. (2005). Destroy wild radish and annual ryegrass seeds by burning narrow windrows. *Agribusiness Crop Updates. Adelaide, South Australia: Grains Research and Development Corporation (GRDC)*, 159-163.

Walsh, M. J., Friesen, S., & Powles, S. (2006). *Frequency, distribution and mechanisms of herbicide resistance in Western Australian wild radish (Raphanus raphanistrum L.) populations: a review*. Paper presented at the 15th Australian Weeds Conference, WA, Australia.

Walsh, M. J., Owen, M. J., & Powles, S. B. (2007). Frequency and distribution of herbicide resistance in *Raphanus raphanistrum* populations randomly collected across the Western Australian wheatbelt. *Weed Research, 47*(6), 542-550.

Walsh, M. J., & Powles, S. B. (2007). Management strategies for herbicide-resistant weed populations in Australian dryland crop production systems. *Weed Technology, 21*(2), 332-338.

Walsh, M. J., Stratford, K., Stone, K., & Powles, S. B. (2012). Synergistic effects of atrazine and mesotrione on susceptible and resistant wild radish (*Raphanus raphanistrum*) populations and the potential for overcoming resistance to triazine herbicides. *Weed Technology, 26*(2), 341-347.

Warwick, S. I., Beckie, H. J., & Small, E. (1999). Transgenic crops: new weed problems for Canada? *Phytoprotection, 80*(2), 71-84.

Weller, S. L., Florentine, S. K., Mutti, N. K., Jha, P., & Chauhan, B. S. (2019). Response of *Chloris truncata* to moisture stress, elevated carbon dioxide and herbicide application. *Scientific Reports, 9*(1), 1-10.

Wells, H. (2017, 08.02.2017). Faba bean flounders amid tepid Egyptian demand. Retrieved December 18, 2018 from https://www.graincentral.com/cropping/pulses/faba-bean-flounders-amid-tepid-egyptian-demand/

Werth, J., Preston, C., Taylor, I. N., Charles, G. W., Roberts, G. N., & Baker, J. (2008). Managing the risk of glyphosate resistance in Australian glyphosate- resistant cotton production systems. *Pest management science, 64*(4), 417-421.

Werth, J., Walker, S., Boucher, L., & Robinson, G. (2010). Applying the double knock technique to control *Conyza bonariensis*. *Weed Biology and Management, 10*(1), 1-8.

Wheeler, T. R., Craufurd, P. Q., Ellis, R. H., Porter, J. R., & Prasad, P. V. (2000). Temperature variability and the yield of annual crops. *Agriculture, Ecosystems & Environment, 82*(1-3), 159-167.

Whitcomb, C. E. (1999). An introduction to ALS-inhibiting herbicides. *Toxicology and Industrial Health, 15*(1-2), 232-240.

Whitehead, C. W., & Switzer, C. M. (1963). The differential response of strains of wild carrot to 2, 4-D and related herbicides. *Canadian Journal of Plant Science, 43*(3), 255-262.

Wijewardana, C., Reddy, K. R., & Bellaloui, N. (2019). Soybean seed physiology, quality, and chemical composition under soil moisture stress. *Food Chemistry, 278*, 92-100.

Wilcut, J. W., Walls Jr, F. R., & Horton, D. N. (1991). Imazethapyr for broadleaf weed control in peanuts (*Arachis hypogaea*). *Peanut Science, 18*(1), 26-30.

Wilson, R. S., Hooker, N., Tucker, M., LeJeune, J., & Doohan, D. (2009). Targeting the farmer decision making process: a pathway to increased adoption of integrated weed management. *Crop Protection, 28*(9), 756-764.

Wu, X., Zhang, T., Pan, L., Wang, L., Xu, H., & Dong, L. (2016). Germination requirements differ between fenoxaprop-P-ethyl resistant and susceptible Japanese foxtail (*Alopecurus japonicus*) biotypes. *Weed Science, 64*(4), 653-663.

Yamaji, Y., Honda, H., Hanai, R., & Inoue, J. (2016). Soil and environmental factors affecting the efficacy of pyroxasulfone for weed control. *Journal of Pesticide Science, 41*(1), 1-5.

Ye, H., Roorkiwal, M., Valliyodan, B., Zhou, L., Chen, P., Varshney, R. K., & Nguyen, H. T. (2018). Genetic diversity of root system architecture in response to drought stress in grain legumes. *Journal of Experimental Botany, 69*(13), 3267-3277.

Young, K., & Cousens, R. (1999). *Factors affecting the germination and emergence of wild radish (Raphanus raphanistrum) and their effect on management options.* Paper presented at the 12th Australian weeds conference, Horbart, Tasmania, Australia.

Young, K. R. (2001). *Germination and emergence of wild radish (Raphanus raphanistrum L.).* (Ph. D. thesis), University of Melbourne Australia.

Yu, Q., Zhang, X. Q., Hashem, A., Walsh, M. J., & Powles, S. B. (2003). ALS gene proline (197) mutations confer ALS herbicide resistance in eight separated wild radish (*Raphanus raphanistrum*) populations. *Weed Science, 51*(6), 831-838.

Yu, Q., Han, H., Li, M., Purba, E., Walsh, M. J., & Powles, S. B. (2012). Resistance evaluation for herbicide resistance–endowing acetolactate synthase (ALS) gene mutations using *Raphanus raphanistrum* populations homozygous for specific ALS mutations. *Weed Research, 52*(2), 178-186.

Yu, Q., & Powles, S. B. (2014). Resistance to AHAS inhibitor herbicides: current understanding. *Pest Management Science, 70*(9), 1340-1350.

Yuan, J. S., Tranel, P. J., & Stewart Jr, C. N. (2007). Non-target-site herbicide resistance: a family business. *Trends in Plant Science, 12*(1), 6-13.

Zeleke, K., & Nendel, C. (2019). Growth and yield response of faba bean to soil moisture regimes and sowing dates: Field experiment and modelling study. *Agricultural Water Management, 213*, 1063-1077.