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Biology of *Amaranthus retroflexus* and *Amaranthus viridis*

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Abstract

Amaranthus species are a key risk to farm production, biodiversity, and agriculture economics of more than 80 countries across the world. *A. retroflexus* and *A. viridis* are among the most noxious *Amaranthus* species. The current information available on the biology these species in Australian agroecosystem is not enough for suitable management. Therefore, several studies were conducted to explore biology of these two weeds in controlled and field conditions at University of Queensland, Australia.

A comparative study on two different Australia biotypes of *A. retroflexus* and *A. viridis* suggested that they are highly invasive in nature and both biotypes of these species are readily adaptive to changing climatic conditions and exhibit superior germination and reproductive ability. Overall, *A. retroflexus* and *A. viridis* germinated over a wide range of alternating day/night (15/5 to 35/25°C) temperatures, osmotic stress (0.0 to -0.6 MPa), salinity levels (0 to 200 mM of Sodium Chloride).

Amaranthus retroflexus and *A. viridis* tolerated moisture stress and produced seeds at 100%, 75%, 50% and 25% soil field capacity (FC) corresponding to no, light, moderate, and high moisture stress when grown in the pots in a ventilated screen house, respectively. Leaf production by *A. retroflexus* and *A. viridis* plants was inversely proportional to the water stress. The number of leaves produced by *A. retroflexus* and *A. viridis* plants at 25% of FC was 54% and 53% fewer than that at 100% of FC, respectively. Shoot biomass of both species declined linearly with increasing water stress. The highest shoot biomass of *A. retroflexus* (46 g plant^{-1}) and *A. viridis* (44 g plant^{-1}) was observed at 100% FC. No differences were observed between the shoot biomass of *A. retroflexus* at 100% and 75% FC. At 25% of FC, the shoot biomass of *A. retroflexus and A. viridis* plants were reduced by 73% and 76%, respectively, compared with the shoot biomass at 100% FC.

In conclusion, *A. retroflexus* and *A. viridis* have multifarious impacts on crop production. It is clear from the biology studies that invasiveness of this weed is associated with its ability to germinate over a wide range of environmental conditions and tolerate soil moisture stress through morphological and physiological adaptations. A prioritized multilateral integrated management plan must be devised to be implemented on a large scale to combat *A. retroflexus* and *A. viridis* in Australia to avoid the loss which these weeds have made in America.

Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly authored works that I have included in my thesis.

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NA

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No contributions by others.

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No works submitted towards another degree have been included in this thesis.

Research Involving Human or Animal Subjects

No research involving human and animal subject.

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Keywords

Amaranthus, invasive plant species, weed biology, germination ecology, integrated weed management, moisture stress, phenology

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DEDICATION

I dedicate this piece of my work to my father Late Mr. Miunsif Ali Khan who taught me the best values and priceless lessons of life

Submitted manuscripts included in this thesis

Asad MK, Mobli A. Jeff AW, Chauhan BS (2020) Germination ecology and seed persistent of redroot pigweed (*Amaranthus retroflexus*) and slender amaranth (*Amaranthus viridis*): emerging problem weeds of cotton farming systems in Australia. International Journal of Pest Management (Under review). *Based on Chapter 3*

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Mobli A, Manalil S, Khan AM, Jha P, Chauhan BS (2020) Effect of emergence time on growth and fecundity of *Rapistrum rugosum* and *Brassica tournefortii* in the northern region of Australia. Sci Rep 10. https://doi.org/10.1038/s41598-020-72582-7

Book chapters

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CHAPTER 1: General Introduction

Weeds are estimated to cost Australian agriculture more than $4 billion per year (Sinden *et al.* 2004). They have a significant economic, social and environmental impacts. They degrade natural vegetation, effect biodiversity and create long lasting negative effects on human health. Amaranthus species, native to the southwest United States of America, are one of the most noxious and aggressive weed species. Due to agricultural seed and equipment transport, Amaranthus species slowly become one of the most problematic weeds all over the world. These weeds have great economic importance as they create severe yield losses in several field crops, vegetables and orchards.

These species have adapted to a wide range of environmental conditions because of their unique set of biological features such as tolerance against moisture stress, prolific seed production and aggressive growth habits like rapid growth rate, genetic diversity, hybridization with closely related sub-species, ability to tolerate adverse conditions and high persistence levels make *Amaranthus* species dominant and difficult to control weeds. (Holm *et al.* 1997)

These species are equally problematic in conventional, reduced and no tillage systems. Different herbicides provide different degrees of control against these weeds; depending on the crop, application method, seasonal and agronomic practices used in conjugation with the application of herbicides belongs to same site of action. But due to continuous use of herbicides, these species have developed multiple resistance to herbicides used in the cotton *(Gossypium hirsutum),* maize (*Zia mays*) and soybean (*Glycine max*) crop. Glyphosate resistance in these weeds species increased following the widespread use of Roundup Ready crops Cotton, maize (*Zia mays*) and soybean (*Glycine max*) throughout the United States and Australia (Manalil *et al.* 2017). Some of the cultural practices such as mulching, hoeing and crop rotations help in controlling these species. The use of alternative herbicides in conjunction with cultural, mechanical, and biological control tactics may provide better control of these weed species.

Amaranthus retroflexus and *A. viridis* are among the most noxious *Amaranthus* species due to their aggressive growth habits. These species continue to be a significant problem for the cotton crop in Australia. Both weeds are very competitive and produce a lot of seeds (Holm *et al.* 1997). Growers use glyphosate to manage these weeds, but there are increased cases of development of resistance in weeds against glyphosate. This has increased interest in the development of integrated weed management (IWM) practices to manage weeds more effectively. The development of IWM practices, however, needs information on weed ecology and biology.

Since *A. retroflexus* and *A. viridis* are not uncommon weeds, significant research has been done in the United States on different aspects of its biology and management. Understanding the botanical features of the plant and management was the main focus around all studies conducted on biology of plants . However, no Australian studies are available on the biology of these two species, and this project has addressed this research gap. This study has used Australian populations of *A. retroflexus* and *A. viridis* to compare ecological and biological behaviour of both weeds. This project has focused on *A. retroflexus* and *A. viridis* dynamics at the eco-biological interface to gain the ability to understand its biology and to obtain insights for future management. Knowledge on the biology of these species will help growers and researchers to develop better management tools *A. retroflexus* and *A. viridis*. For Weed growth stages can be identified when weeds are most susceptible to control measures.

In short, this project adds significantly to our understanding of *A. retroflexus* and *A. viridis* biology, which is a pre-requisite for their effective management. The results presented in the chapters below answer the following broad questions with a focus on the mechanistic detail:

- How does different biotypes of *A. retroflexus* and *A. viridis* behave under different environmental condition during germination stage of the lifecycle?
- What is the impact of drought conditions on growth and propagation of these weeds' species?
- What is the impact of time of the year on germination and growth of these weeds' species?

Certainly, this work is not the end of the story but a huge leap forward in our understanding of these weeds of the modern era. The results of this thesis will help in predicting the behaviour of *A. retroflexus* and *A. viridis* under different geographic and climatic situations of Queensland (Gatton and Goondiwindi) and in their effective management in agro ecosystems.

CHAPTER 2: Literature Review

Description

Amaranthus retroflexus is commonly known as redroot pigweed, amarante à rough pigweed, racine rouge, and amarante pied rouge (Costea *et al.* 2004). Specimen collected from the United States of America and Canada showed that *A. retroflexus* has a 34-diploid chromosome (Grant 1959, Mulligan 1984, Murray 1940, Weaver and McWilliams 1980). However, few samples collected from Russia (Lomonosova *et al.* 2001) and California (Heiser and Whitaker 1948) have proved that the species can also have 2n=32 chromosomes. *Amaranthus retroflexus* is a monoecious member of Family *Amaranthaceae*.

Plant of *A. retroflexus* is erect in posture and have fine hair all over the stem and the branches. *A. retroflexus* branches freely and herbaceous in nature. The plant can grow up to two meter in height (Weaver and McWilliams 1980). The tap root is pink or red in color and the depth of tap root depends on the soil profile. Leaves of *A. retroflexus* alternate in symmetry and rhombic-ovate in shape. These leaves can go up to 10 cm in length with veins on underside. The margins of leaves are wavy, apex is sharp, and 1 - 5 cm blunt spikes are densely crowded on to 5 - 20 cm in length depending on the age of panicle. Bracteoles with rigid with awl shaped spiny tips surround the flowers. Flowers are numerous in number with one pistil and five stamens. Style branches can be erect or curved shaped. Fruit of *A. retroflexus* is membranous and flattened with a transverse line at the middle. Seed are somewhat flattened to oval shaped, brown or shiny black in colour an 1-1.2 mm in length (Weaver and McWilliams 1980).

Amaranthus viridis is another prominent member of family *Amaranthaceae*. It is known as green or slender amaranth. The specimen of *A. viridis* collected from China proved that it has 2n=32 chromosomes (Xu 1987). *A. viridis* is an annual herbaceous plant with distinct long petioles and under leaf venation. Stem is 6-100 cm tall, erect, and slightly hairy in nature. Leaves are glabrous to pubescent in shape with prominent veins at the lower surface (Holm *et al.* 1997). Flowers of *A. viridis are* green in colour, intermixed male and female, unisexual. Spikes are slender axillary in shape with a length of 2-12 and width of 2-5 mm. They may make dense axillary structure in the lower part of stem. Brackets are deltoid- to lanceolate-ovate in shape and membranous in nature. Stigma are 2-3 in number. Seeds of *A. viridis* are compressed, round, and dark brown to black in colour. The seed has paler thick boarder on it (Costea *et al.* 2004, Weaver and McWilliams 1980).

Origin, Distribution and Habitat

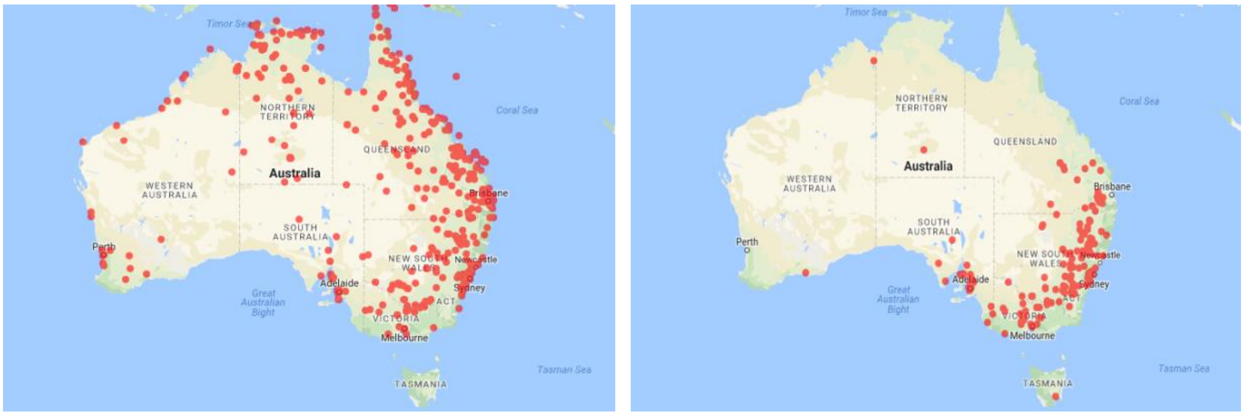
Fifty species in the genus *Amaranthus* are native to the Americas of which 10 are widely distributed in the great plain region of the USA (GPFA 1986). *A. retroflexus and A. viridis* are small seeded, broadleaf species of *Amaranthus* genus widely distributed throughout the USA, Canada and other parts of the world (Uva *et al.* 1997). Some scientists believe that *Amaranthus* originated from Americas and t spread to Europe from where it paved their way to Asia and Africa sometime after Europe colonization begins (Sauer 1967). Various parts of Africa and Asia welcomed and developed different Amaranthus species as ornamental varieties and food crops. From Canada to Argentina, *Amaranths* species can be found almost everywhere (Sauer 1950). *Amaranthus* originally belong to wild habitat (Sauer 1967, Weaver and McWilliams 1980) and after the early human settlement, *Amaranthus* started its journey from the disturbed area and waste ground and then invaded to cultivated fields.

Amaranthus retroflexus is a native of the central and eastern USA and adjacent regions of north-eastern Mexico and south-eastern Canada (Sauer 1967). It is widely distributed across the temperate regions of southern and northern hemispheres. The spread of these weeds then occurs to European regions particularly Greece, Spain, Germany, France and Portugal. Distribution of *A. retroflexus* is also extended in Australia. The major infestation in cotton crop has been observed in Queensland, New South Wales and Western Australia states of Australia (Holm *et al.* 1991, Lazarides *et al.* 1997).

Amaranthus retroflexus can grow well on wide range of soil types and textures especially fertile soils as it has very high nitrogen requirement. It tolerates varying soil pH from 4.2 to 9.1 (Feltner 1970) but does not commonly grow in highly acidic soils of the south-eastern USA, where *A. palmeri* is more extensively available. *A. retroflexus* needs longer growing season which reduces its availability at higher elevations and altitudes. As a common weed, it is commonly found in cultivated lands, gardens, roadsides, riverbanks, waste places, irrigation channels or any other disturbed habitats. However, due to sunlight is critical for its germination, it is seldom found in closed and shaded places (Weaver and McWilliams 1980). As per ecological preferences for temperature, water, light and nitrogen content of the soil, *A. retroflexus* is characterized as thermophyte, xerophyte, heliophyte and nitrophilous (Costea 1998). It can tolerate a wide range of soil pH levels and abundantly available in areas of high soil pH, nitrogen contents, organic matter and low phosphate and potassium levels (Dieleman *et al.* 1995).

Amaranthus viridis is extensively prevalent in plantation agriculture. It is an economic weed of 50 crops and widely distributed in 80 countries of the world (Holm et al. 1991). Originally native to the Southern United States, especially California and the adjacent regions of Mexico, it is becoming more previlant in the south-eastern United States, including North Carolina, Tennessee, and Mississippi, but it does not have high distribution in central and western regions of the United States or Canada (Holm *et al.* 1997). *A. viridis* can be found as far west as Arizona and as far north as Michigan, New Jersey, and New York (USDA, NRCS 2004). It is widely distributed in Australia with major problem seen in New South Wales, South Australia and Tasmania (Holm *et al.* 1991). *A. viridis* is troublesome weed of rice crop in several Asian countries (Moody 1989).

Amaranthus viridis grows in heavy organic to very sandy soils, including muck soils after the water has gone down for the season. *A. viridis* acclimatized to broad range pH level, soil types, and textures (Kigel 1994, Weaver and McWilliams 1980). *A viridis* has very high self-fertility which enables it to rapidly colonialize in distributed areas. A single plant of *A. viridis* can produce 7000 seeds and the seed bank of these seeds grows very fast in the absence of control (Holm *et al.* 1997, Schweizer and Zimdahl 1984). Unique seed ecology and efficient means of propagation play major role in the effective distribution of weed species (Bajwa *et al.* 2015, Chauhan and Johnson 2010).



Amaranthus viridis *Amaranthus retroflexus*

Plate 1: Distribution map of *A. retroflexus* and *A. viridis* (www.ala.org.au)

Seed production, dispersal and germination ecology

Amaranthus retroflexus is monoecious, self-compatible and self-pollinated weed specie (Costea *et al*. 2001). Most of the pollination occurs through the wind as the flower lack nectar glands and there are very fewer chances of insect pollination. Pollen grains are 18-25 µm in diameter and these pollen grains have 30-45 sunken apertures. These sunken apertures reduce the friction between air and pollen and help the pollen to cover more distance while pollinated through the wind (Franssen *et al.* 2001b). *A. retroflexus* has intermediate growth habit which allows flower formation, pollination, and seed set proceed simultaneously (Weaver 1984). *A. retroflexes* has high growth and competitive ability. Predation of the seeds is also very common because of dense terminal inflorescence and highly nutritious seeds (Nurse 2000). It takes minimum 30 days after fertilization to develop thin but structurally strong seeds.

Seed germination of *A. retroflexus* occurs within the top 2 cm of soil and stimulated by light and alternating high temperatures of 20/35°C (Weaver 1984, Weaver and McWilliams 1980). Seed viability of buried seeds decreases over the time (Purwanto and Poerba 1990). However, germination can be boosted by treating the seed with concentrated sulphuric acid. This treatment can alone imporove the germination rate by three-time and maximum germination can be obtained at 35°C (Ikenaga *et al.* 1975). Moreover, soil moisture of 85% and above decreases the germination percentage drastically and flooding normally kills the seeds (Yamamoto and Ohba 1977). Seeds can survive well when buried under 2.5 cm or below under the soil as compared to soil surface (Horng and Leu 1978). The propagation of *A. retroflexus* is carried out by seeds. The impermeable coat around the seeds is resistant to mechanical shock and chemicals (Kigel 1994)

Seed production largely depends on growing condition and a single vigorous plant of *A. retroflexus* may produce 100,000 seeds (Hanf 1983). Another study shows that it produces 25,300 seeds at a temperature of 22/28^{o}C and a photoperiod of 16 h and 43,150 at the temperature of 20/40^{0}C and photoperiod of 16 h in the absence of herbicides and crop competition (Weaver 1984). The presence of crop and herbicides significantly reduce the seed production by *A. retroflexus* (Weaver and Warwick 1982). Similarly, *A. retroflexus* produced 8085 to 90,175 seeds per plant at a temperature of 28/22^{o}C at a photoperiod of 14 h when atrazine herbicides were used to control it (Weaver and Warwick 1982). Mohler and Callaway (1995) reported that plants of *A. retroflexus* grown in a sweet corn (Zea mays) crop treated with herbicide in central New York State produced 200 to 500 seeds per plant. whereas species grown in the absence of any control produced 100,000 seeds per plant. Seed production per plant can be estimated from plant height and basal stem diameter.

Amaranthus species vary in seed size and weight. The size and weight may also vary between different biotypes of same species. McWilliams *et al.* (1968) reported that average weight of the *A. retroflexus* seed is positively corelated with the altitude in samples collected from North American. However, long term moisture condition creates a negative impact on the seed size and weight. Plants grown in continuous dry conditions produce larger and heavier seeds as compared to the plants grown in long term moisture condition (Chadoeuf-Hannel and Barralis 1982a, Schimpf 1977). Seeds of *A. retroflexus* are significantly lighter in weight than that of some other species of *Amaranthus* when grown in the same condition. (Weaver and McWilliams 1980). A long-term moisture stress condition creates a negative impact on seed size both in growth chamber and field (Chadoeuf-Hannel and Barralis 1982a, Schimpf 1977). Being smaller in size and lighter in weight, seed dispersal of *A. retroflexus* occurs through the wind, water, birds, spreading of manures, animals and farm machinery.

The seeds which remain on dried inflorescences in the winter are blown over the frozen snow through the wind rather than moving into small soil cracks (Blatchley 1930).

Amaranthus viridis plant can produce 7000 seeds in its lifecycle (Mohler *et al.* 1999). Seed dispersal mainly occurs by water, wind, manure, birds, movement of farm machinery, as contaminated seed crop seeds (Mohler et al. 1999). *S*eeds of *A. viridis* loss viability over the time especially in the high temperature conditions. (Purwanto and Poerba 1990) and germination of *A. viridis* can be increased by treatment of seeds with concentrated sulphuric acid. This treatment increases the germination three time. However, 100% germination can be achieved at 35ºC. Time of sowing also impact the germination of seeds. Late sowing of seed can increase the germination percentage and reduce the time for germination (Ikenaga *et al.* 1975). Soil moisture also impacts the germination of *A. viridis* seeds. High moisture of more than 85% can increase the time of germination substantially and flooding may kill the seeds also (Yamamoto and Ohba 1977). Burial depth of seed below 2.5 cm can create a significant impact in germination of seeds. There was no change in the germination of seeds which were buried 2.5 cm below the surface and deeper for up to a year, indicating a survival technique used by this species (Horng and Leu 1978).

The irrigation canal also carries heavy quantities of seeds produced by these *Amaranthus* species (Wilson 1980). The *Amaranthus* seeds which were not damaged in the digestion process were found in the droppings of some mammals (Whitaker 1980). Similarly, many bird species (ornithochory) consume and spread *Amaranthus* seeds (Graaf *et al.* 1991, Terres 1980). Combine harvesters and threshers also spread seeds that remain on the plant at the time of harvest (Forcella 2000). Seeds of these two species can bear harsh environmental condition very well and exhibit the initial viability of more than 90%. (Weaver and McWilliams 1980). Seeds of *A. retroflexus* can remain viable for many years when buried in soil. Seeds burial depth, moisture, soil temperature, the rate of soil disturbance, and dormancy are five major factors that contribute to the long-term viability of the seeds (Weaver and McWilliams 1980). The increase in burial depth of seeds in these species increases the viability of this seed. The seeds dispersed on the soil surface have low viability because of decay and seeds predation (Omami *et al.* 1999, Weaver and McWilliams 1980). Large numbers of *Amaranthus* seeds were observed in the field used for agriculture purposes mainly because of amaranth's capacity of abundant production and long-term viability of these seeds (Cavers and Benoit 1989, Daniel 1992, Forcella 2000, Hoffman *et al.* 1998, Schweizer *et al.* 1988, Schweizer and Zimdahl 1984, Unger *et al.* 1999).

Seeds of both species have viability of more than 90% and the seed bank they form is very persistent (Weaver and McWilliams, 1980). Depth of burial, soil moisture and temperature, extent of soil disturbance, and dormancy are the factors which influence the viability of buried seeds. The viability of buried seeds of *A. retroflexus* vary from 6 to 10 years (Burnside *et al.* 1981). Similar experiment by other researchers showed that 2 % of the *A. retroflexus* seeds were viable even after 40 years of burial. (Telewski and Zeevaart 2002). However, one other study conducted in Mississippi proves that the viability of *A. retroflexus* declines to 1-2 % after 3 years of burial (Egley and Chandler 1983). Seed longevity has positive correlation with burial depth so increase with decrease in the depth of burial. Taylorson (1970) conducted an experiment on seed dormancy and found that dormant seeds of *A. retroflexus* exhibits more that 93% viability after 12 months of burial than non-dormant seeds which are only 25% viable. Similarly, Doroszewski (1997) reported that pre-treatment with far-red light to induce dormancy before burial increased the persistence of buried seeds of *A. retroflexus*.

Amaranthus retroflexus exhibits initial dormancy. The germination occurs at a minimum temperature of 30-35ºC for freshly developed seeds. Th wintered seeds in the field germinate at 20-25ºC (Frost 1971). However, seeds produced at the beginning of growing season by the same plant of these species show less dormancy and germinate at a lower temperature as compared with the seeds produced at the end of growing cycle (Costea 1998, Frost 1971, Kigel 1994). *A. retroflexus* seeds collected from the wintered dead plants above the soil demonstrated more dormancy as compared to the buried wintered seeds (Frost 1971). The difference in dormancy characteristics abolishes when the seeds are stored for a long time in dry conditions (Chadoeuf-Hannel and Barralis 1982a and 1982b). A seed bank study of *A. retroflexus* showed that when the seeds are placed at 0 to 60 cm depth of soil, they go through cyclic changes in dormancy due to changes in temperature throughout the season (Frost 1971).

Seed germination of *A. viridis* was influenced by both temperature and light. In the presence of light, seed germination of *A. viridis* was greater at 30/20 °C and 35/25°C than at 25/15°C. It took longer time for *A. viridis* seeds to reach 50% germination (T50) at 25/15°C compared with the other two temperatures (Chauhan and Johnson, 2009a).

Economic Importance

Interference

Interference is a phenomenon where one specie of plant is unable to germinate or grow at its actual pace, demonstrate the symptoms of damage or even does not survive when the other plant species exist in the same place. The detrimental effects of this weed interference with crop production involve reduction in crop yield and quality, release of allelopathic compounds, noxiousness to livestock, serving as an alternative host for insect and crop pathogens, and cause human allergic reaction by production of pollens (Bajwa *et al.* 2015, Bajwa 2014, Peerzada *et al.* 2016, Weaver and McWilliams 1980) Nitrates build up in the tissue is yet another problem affecting cattle. *A. retroflexus* and *A. viridis* cause lodging of infested crops which finally reduce the harvesting efficiency (Nave and Wax 1971).

A. retroflexs interference is the main cause of yield losses in Cotton (*Gossypium hirsutum*) (Smith *et al.* 1987), maize (*Zea mays*) (Massinga *et al.* 2001) and sorghum (*Sorghum bicolor*) (Moore *et al.* 1994). Surge in infestations caused by these species is related to excessive use of reduced and zero tillage systems, reduced dependence on soil-applied residual herbicides, varied response to postemergence herbicides, increase in herbicides resistance and shift of species from one area to another area (Jonathan *et al.* 1998, Michael and Loughin 2000, Michael and Peterson 1995). Sever infestation of *A. retroflexus* has been reported in many crops in most regions of USA and Canada (Holm *et al.* 1977). A significant reduction yield of soybean (*Glycine max)*, maize, cotton, beetroot

(*Beta vulgaris*), pea (*Pisum sativum*), and sorghum has been observed because of competition with *A. retroflexus* (Holm *et al.* 1977). High density of over 30 plants m^{-2} of *A. retroflexus* can cause a yield losses of up to 90% because of strong competition with crops for light (Joe *et al.* 1996, Légère and Schreiber 1989), water (Sage and Pearcy 1987) and nutrients (Santos *et al.* 1998, Teyker *et al.* 1991). Research shows that 0.5 plants m^{-2} of *A. retroflexus* is the economic threshold level for corn and soybeans crops (Cowan *et al.* 1998, Dieleman *et al.* 1995, Knezevic *et al.* 1994). At this level of infestation of *A. retroflexus* can cause a yield loss of 5% when emerge at the same time as the crop emerges and stay in the field for the whole crop cycle.

Stebbing *et al.* (2000) reported 12% reduction in beetroot yield in the season long interference of 1 plant per 2 to 3 m of row. The yield loss goes up to 31% at the densities of 1.5 plants m^{-2}. The decrease in row spacing from 76 cm to 46 cm of sugar beet can reduce the yield loss by 25%. Late emerging *A. retroflexus* does not compete very well in the full stand of sugar beet. However, these species can reduce the yield of poor stands by 50% (Dawson 1977).

In Michigan, season long interference of *A. retroflexus* with potato (*Solanum tuberosum*) reduced the marketable yields by 22 to 33% at a density of 1 plant m^{-1} of row (Mark and Karen 1990). In the first year of seeding, alfalfa (*Medicago sativa*). is susceptible to *A. retroflexus*. A yield reduction of 42% was seen in the state of Washington when the crop was sown in mid-August. But the delay in sowing of crop to October give excellent control of *A. retroflexus* as the frost kills most of weed seedlings and yield of alfalfa was not reduced in the following year (Fischer *et al.*1988).

Aguyoh and Masiunas (2003) conducted research in Illinois and found the reduction in yield of Snap bean (*Phaseolus vulgaris*) by 52% to 54% in the presence of season long interference of eight plants m^{-1} with *A. retroflexus*. In another study, density of 8 *A. retroflexus* plants per meter of row reduced the yield of snap been by 42% in early planted and 58% in late planted snap bean. While the yield reduction is recorded at 39% to 48%. The effect of redroot pigweed's emergence time and density on snap bean growth and yield was also studied. *A. retroflexus*, at four densities, was seeded with snap beans (early) or at the first trifoliate leaf stage (late). The percent yield loss depends on the year and time of weed emergence. The reduction in biomass and number of pods is directly related to the time of emergence of *A. retroflexus*. Early emerging *A. retroflexus* plants cause more reduction in yield as compared to the late emerging ones.

Amini *et al.* (2014) conducted field experiments to evaluate the competitive ability of kidney bean (*Phaseolus vulgaris*) against *A. retroflexus*. The research results showed that the *A. retroflexus* had greater plant height and growth rate than red kidney beans. But the red kidney bean had higher leaf area index than *A. retroflexus* plants. The results were consistent on all densities of 0, 4, 8, 16 and 32 plants m^{-2}. Higher densities of *A. retroflexus* decreased the seed yield, number of pods and over all yield of red kidney bean.

Buchanan *et al.* (1980) conducted a research study on cotton, by planting different densities of *A. retroflexus* ranging from 0 to 32 plants per 5 m of row. He found a linear decrease in yield seed cotton with increasing densities of *A. retroflexus* plants. Loss of 21 to 38 kg/ha was seen in yield of hand harvested seed cotton in the presence of one plant per 15 m of row. The comparison of hand harvest and mechanically harvested cotton was also conducted in the same weed densities. The results showed no significant difference in yield of seed cotton. However, higher percentage of trash increased at higher densities of weeds. Gale and Earl (1971) conducted experiment in Albama to investigate the impact *A. retroflexus* on yield of cotton. *A. retroflexus*, at a density of 48 plants per 7.31 m of row reduced cotton yields less than 50% in Norfolk soil. While a yield reduction of 90% was observed in the presence of 48 plants per 7.31 m of row in Lucedale soils. Ma *et al.* (2015) conducted research experiment on *A. retroflexus*. Densities of 0, 0.125, 0.25, 0.5, 1, 2, 4, and 8 plants m^{-1} of row was used to see its competition with cotton. *A. retroflexus* shaded the cotton and remain taller and thicker than cotton through the cropping season. Stem diameter and height of cotton reduced with increasing densities of weed. The major impact observed in the presence of 1-8 *A. retroflexus* plants per meter of row, where the cotton maturity was delayed. Moreover, the seed number per boll and boll weight reduction was also observed. A yield loss 50% was estimated in the presence of 0.20– 0.33 weed plant per meter of cotton row. Densities of one *A. retroflexus* m^{-1} of row and above reduces the cotton fiber length, uniformity and micronaire. *A. retroflexus* produced about 626, seed at a density of 1 plant m^{-1} of row which could cause sever infestation in the next crop.

Bukun (2011) evaluated the impact of *A. retroflexus* on the yield of peanut (*Arachis hypogaea*) The *A. hypogaea* yield decreases linearly with increasing densities of *A. retroflexus*. Total yield reduction observed was 4.1% and 63.9% at the densities of 0.2 and 4.7 plants m^{-1} of row. The study proved that the *A. retroflexus* showed strong competition with peanut and should be controlled as early as possible.

Fu (2000) studied the interference of *A. retroflexus* in pepper (*Capsicum annuum*). Naturally occurring densities of 0, 1, 2, 4, 8, 16, and 32 weed plants m^{-1} were established in the field of pepper. Impact on yield was observed at two different emergence times of *A. retroflexus* (3days and 2 weeks) after transplantation. Maximum yield loss of 99% was observed in case of early emerging *A. retroflexus* at a density of 32 plants per meter of row. Kaveh *et al.* (2009) studied the impact of different densities of *A. retroflexus* on growth and yield of corn. *A. retroflexus* was planted at 0, 35, 50, 65, and 80 plants m^{-1} in different densities of 4, 5, 6 and 7 plants m^{-1} of corn. A reduction in growth, total dry matter and leaf area index was observed due to competition with *A. retroflexus*. Six corn plants per meter of row were suggested as an optimum density to compete with the *A. retroflexus* and to produce better yield. Vazin (2012) conducted research experiment to access the economic damage threshold of *A. retroflexus* on maize. The comparison of maize at densities of 7.5, 8.5 and 9.5 plants m^{-2} were made with *A. retroflexus* densities of 0, 2, 4, 6 and 8 plants m^{-2} were conducted in a field experiment. The result of study depicted that increase in *A. retroflexus* density reduce the grain and biological yield of maize. However, increasing the maize plants density as compared to *A. retroflexus* increased the grain yield and biological yield of maize. *A. retroflexus* densities of 0.09 to 0.13 plants m^{-2} was noted as the economic threshold level in maize.

Mark and Karen (1990) studied the impact of *A. retroflexus* on yield of Atlantic and Russet Burbank potatoes (*Solanum tuberosum*). The tuber yield losses range between 19% to 33 % in the presence of 1 *A. retroflexus* plants per meter of row when planted 6-7 weeks after planting. However, neither specific gravity nor tuber quality was altered at presence of any density of *A. retroflexus*.

Mirshekari *et al.* (2010) conducted field experiment to observe the impact of *A. retroflexus* on the yield of hybrid green been (*Phaseolus vulgaris*). The focus of the study was to consider the yield loss and to determine the critical period of redroot pigweed control in the hybrid green bean. The crop was infested with weeds at 2, 4, 6, 8, 10, and 14 weeks after been emergence and the yield losses were observed. The results of study showed that the green bean biomass decreased when the *A. retroflexus* plants emerged early in the crop cycle. But no significant reduction was observed if the weeds emerged 10 weeks after green bean emergence. When redroot pigweed interference lasted for 4 weeks after green bean emergence, the green bean yield was reduced significantly. Highest level of green bean yield was obtained when the *A. retroflexus* emergence occurred after 14 weeks of green bean germination. The critical period of *A. retroflexus* control was observed between 19 to 52 days. The result of the study recommends that the weed management practice should be started 3 weeks after the germination of green bean and should continue until at least 8 weeks to get the maximum yield.

A. viridis is quite to very common and can be a serious weed in any crop. Since it usually occurs with various other weeds, losses cannot be directly attributed to *A. viridis* alone. There is not much information available on *A. viridis* interference with different crops in Australia.

Allelopathic Effect

Allelopathy is a major form of interference. It is mediated by the release of phytotoxin in the surrounding environment of the plant. Allelopathic compounds released by weeds are responsible for hampering growth and development of plants (Qasem and Foy 2001, Romero-Romero *et al.* 2005). When a plant with allelopathic potential is a weed species, such as the case of *Amaranthus*, it may cause problems in agricultural production, depending on the implemented management in the production area. *A. retroflexus* and *A. viridis* are allelopathic in nature and several parts of these plants produce allelopathic compounds, which impact the native crops (Bhowmik and Doll 1983). These allelochemicals can influence the germination of seeds of various crops, affect their photosynthetic rate, and reduce their growth and, consequently, their productivity (Suma *et al.* 2002). These allelochemicals affect specific plant processes such as cell division and elongation, the action of inherent growth regulators, mineral uptake, photosynthesis, respiration, stomatal opening, protein synthesis, membrane permeability and specific action (Rice 1984). Some scientists reported that allelochemicals released by *Amaranthus* plants influence the germination and the growth of seedlings of various species (Einhellig and Rasmussen 1978, Glass and Bohm 1971). *A. retroflexus* dry shoot residues release 31 volatile allelopathic compounds which hamper the germination (Connick *et al.* 1989). It also has a severe allelopathic effect on snapbean when both were planted in a Mexican polyculture system (Anaya *et al.* 1987). Extracts form the *A. retroflexus also* can reduce the hypocotyl elongation and nodulation process in soybean which in turn reduces the development, growth, and yield of the crop (Bhowmik and Doll 1983, Mallik and Watson 1998) *Amaranthus* extracts of 10% (v/v) added to Murashige and Skoog (MS) basal medium can reduce the fresh weight of Melrose plant by 71%. Similarly, 1% of *A. retroflexus* impacts soybean by reducing of callus fresh weight of the hypocotyls to 28 and 16%, respectively (Chaniago *et al.* 2003). The allelopathic effect of aqueous extracts of *A. retroflexus* leaf, stem, and root parts caused a considerable reduction in seed germination and growth of wheat cultivars HAR-1685 and durum wheat (Gella *et al.* 2013).

Allelopathic potential of these species requires more studies related to the effects of their allelochemicals over cultivated plants and other weed plants. Isolation and identification of allelochemical compounds from these plants could provide means to minimize their negative effects on the crops and potentially could provide structural models for the development of bioherbicides or semisynthetic herbicides.

The biomass of *A. viridis* incorporated in the soil reduced the rice productivity (Gaffar *et al.* 1998). Burned residues of *A. viridis* diminished the growth and the productivity of millet (*Pennisetum americanum*) (Sighal and Sen 1981). Marques (1992) reported that the emergence velocity index of cotton was stimulated when small quantities of residue of *A. viridis* were incorporated in amended AQd soils and in LR soils without amendments. The decomposition of larger quantities of residue showed inhibitory effect on germination and growth of cotton, which were more pronounced in sandy soils. The allelopathic substances affected the absorption of nitrogen and water, promoted reduction of transpiration and affected the accumulation of dry matter by the plants.

Alternative host for insects and pests

Amaranthus retroflexus and A. viridis serve as alternate hosts of several pests and insects that attack the crops. In most cases, the damage to the native crops is rarely severe enough to be used as biological control agents. These Amaranthus species also serve as a host for several fungi that create pathogenic impact on major crops such as tomato (*Lycopersicon esculentum*) and sugarbeet. *A. retroflexus* is an alternative host for members of the parasitic nematode genus Meloidogyne and for tobacco mosaic tomabovirus (Holm *et al.* 1977, Tedford and Fortnum 1988). It also serves as a host for *Colletotrichum capsici*, which causes anthracnose on tomato fruit and cotton seedlings (McLean and Roy 1991).

Implications for management

Management of weed species needs an understanding of their history, their impacts, and the progress of the weed has made in the region where management is to be attempted. Since *A. retroflexus* and *A. viridis* have been successful in a wide range of geographic and climatic conditions, devising suitable management options for these weeds is not simple. Along with the chemical control of *Amaranthus* species, creation of environmental conditions which are not favorable for its growth, may offer another way of control. An increase in use of preventive measures, such as reducing the use or cleaning of vehicles in infested areas, reducing the use of farm machinery, reducing the transportation of agricultural commodities, and the use of better crop husbandry, can reduce weed seed dispersal and prevent *A. retroflexus* and *A. viridis* introduction to new areas. The use of natural enemies for classical biological control is another important option. The competitive displacement or allelopathic suppression of *A. retroflexus* and *A. viridis* through potential suppressive plant species is another management prospect. Chemical herbicides could be used to clear up new biotypes at invasion front. None of these management options may provide effective control alone, and, therefore, an integrated approach should be opted. The integration of biological agents with suppressive competitive plant species was found promising for *A. retroflexus* and *A. viridis* management.

Conclusion

It is clear from the information above that *A. retroflexus* and *A. viridis* are problematic plant species due to their multiple negative impacts on the agriculture. Strong morphological features and a unique reproductive biology enables these weeds to adapt to a wide range of environmental conditions. The superior morpho-physiological traits make these weeds a good competitor in their introduced range. An efficient photosynthesis mechanism and tolerance to numerous abiotic stresses and edaphic constraints are parts of the physiological component of these weed's invasion. In addition to these morphological and physiological aspects, improvement in growth and reproductive capacity under a changing climate is also thought to be an important factor contributing towards their invasion success. Management of *A. retroflexus* and *A. viridis* require an understanding of their introduction history, invasive biology, impacts, and the progress in the regions where management is to be attempted. Since *A. retroflexus* and *A. viridis* has been successful in a wide range of geographic and climatic conditions, devising suitable management options for this weed is not simple. Studying their biology in relation to different environmental conditions and interference dynamics is essential to determine the weak points of this species that can be exploited for their effective management.

Research Gaps and Direction of Present Thesis

There are several research gaps which hinder the understanding of (1) the extent and actual impact of *A. retroflexus* and *A. viridis* on agro eco-systems and (2) their biology in relation to major environmental factors. Therefore, a comprehensive project has been designed to reveal the ambiguities surrounding *A. retroflexus* and *A. viridis*. Several laboratory and screen house, and field studies were carried out to investigate the following major aspects of *A. retroflexus* and *A. viridis* weed biology to achieve the underlying broad objectives.

The germination ecology of two discrete Australian biotypes (Goondiwindi and Gatton) of *A. retroflexus* and *A. viridis* was studied under different levels of environmental factors including light, temperature, moisture and salinity. The objective was to determine the relative germination ability of two biotypes as a key contributing factor towards the differential establishment of these biotypes in diverse environmental conditions (Chapter 3).

The biological responses (morphological, physiological and reproductive output) of both biotypes under different levels of soil moisture were also studied. The objective was to determine the biological responses and adaptive strategies of these biotypes under varying soil moisture levels to gain an understanding of the potential links between biological plasticity and invasion success of *A. retroflexus* and *A. viridis* (Chapter 4).

The emergence, vegetative growth, seed production and phenological behaviour of different weed biotypes could vary according to environmental conditions. This study was conducted to evaluate the effect of weed emergence time on growth, flowering and seed production of two biotypes (Goondiwindi and Gatton) of *A. retroflexus and A. viridis* (Chapter 5)

CHAPTER 3: Germination and seed persistence of *Amaranthus retroflexus* and *Amaranthus viridis:* two emerging problem weeds in Australian cotton

Publications included in this chapter

Asad MK**,** Mobli A, Jeff AW, Chauhan BS (2020) Germination ecology and seed persistent of *Amaranthus retroflexus* and *Amaranthus viridis*: emerging problem weeds of cotton farming systems in Australia. International Journal of Pest Management (In Review)

Available online: *No*

Introduction

Amaranthus retroflexus and *Amaranthus viridis* are becoming problem weeds of cotton (*Gossypium hirsutum*) cropping systems in Australia (Manalil *et al.* 2017, Osten *et al.* 2007, Walker *et al.* 2005). *A. retroflexus* is native to the central and eastern USA and nearby regions of Canada, but it has naturalized to the temperate regions of Asia, Africa, Europe and Australia (Holm *et al.* 1991). *A. retroflexus* classified as competitive broad leaf weed in row crops such as cotton, soybean (*Glycine max*), maize (*Zea mays*), sugar beet (*Beta vulgaris*), sorghum (*Sorghum bicolor*) and several vegetable crops (Hao *et al.* 2017, Holm *et al.*1997). The presence of 8 plants m^{-2} row could reduce snap bean (*Phaseolus vulgaris*) yield by 39-58% (Aguyoh and Masiunas 2003). *A. retroflexus* is a prolific seed producer; a single vigorous plant of *A. retroflexus* can produce up to 300,000 seeds (Costea *et al.* 2004, McLachlan *et al.* 1995).

Amaranthus viridis is an extensively prevalent weed in plantation agriculture. It is a weed in 50 crops and widely distributed in 80 countries of the world (Holm *et al.* 1991). It is native to the USA and a very common weed of tropics, subtropics, and warm temperate regions all over the world (Holm *et al.* 1991). It is also widely distributed in Australia, and is considered a major problem in New South Wales, South Australia and Tasmania (Holm and Plucknet 1991). The presence of 12 plants m^{-2} of this weed reduced the leaf area, leaf biomass, and stem biomass of red pepper (*Capsicum baccatum*) by 25%, 72%, and 74%, respectively (Barbasso *et al.* 2018). A single plant of *A. viridis* (self-fertile) can produce 7,000 seeds and the seedbank of *A. viridis* builds-up very fast in the absence of control (Holm *et al.* 1977)

The *Amaranthus* specie’s prolific growth and wide distribution are favoured by their germination potential and high seed production ability in varied environmental conditions (Hao *et al.* 2017). Besides having a C$_4$ photosynthetic pathway and have a highly competitive potential (Carvalho and Christoffoleti 2008), allelopathic compounds from these species could further enhance their invasiveness (Rezaie and Yarnia 2009, Menon and Thakker 2019). Although there are no reports on herbicide-resistant biotypes of these weeds in Australia, resistance to acetolactate synthase and photosystem II inhibitor herbicides has brought new challenges for crop production worldwide (Heap 2020).

High seed production of *Amaranthus* species may excessively enrich the soil seedbank, which ensures their regeneration despite biotic and abiotic constraints and contributes to further infestations over time and locations (Chauhan and Johnson 2009, Korres *et al.* 2018). Weed seedbanks get depleted through predation, decay, and fatal germination (Long *et al.* 2015). Although studies on seedbank persistence of the *Amaranthus* species showed that less than 5% of the seeds are viable for more than 4 years, it seems that their ubiquitous presence contributes to their existence in many cropping systems worldwide (Jha *et al.* 2014, Korres *et al.* 2018; Steckel *et al.* 2007). Weed seeds in a persistent seedbank could remain viable for a long time in unpredictable conditions and emerge in several flushes during favourable conditions (Long *et al.* 2015). Therefore, studying seedbank persistence and their germination could be very important for the development of efficient weed management strategies.

Seed germination biology plays a significant role in the emergence and establishment of invasive weeds in an agroecosystem by impacting the availability of viable seeds in seedbanks (Chauhan and Johnson 2010, Hao *et al.* 2017). Environmental factors such as temperature, light, moisture availability, and salinity influence the ability of weed seeds to germinate, survive and establish in certain agroecosystems (Chauhan and Johnson 2010; Guo and Al-Khatib 2003). Breaking dormancy and germination rate of many weed species is highly associated with temperature fluctuation and mean temperature (Benech-Arnold *et al.* 2000). It has been reported that a light requirement also may inhibit the germination in soil depth and under crop canopies in some species (Benech-Arnold *et al.* 2000; Chauhan and Johnson 2010). Water deficiency and soil salinity are major problems throughout Australia (Rengasamy 2002), therefore, the evaluation of the germination responses of weed species to these key environmental conditions is crucial. Weed seedling emergence depends largely on seed burial depth (Baskin and Baskin 1998, Chauhan and Johnson 2010). Thereby, weed density and growth can be suppressed by increasing the burial depth of seeds through deep tillage (Chauhan and Johnson 2010).

The viability and germination behaviour of weed seeds in a wide range of environmental conditions determines the level of infestation and the demographics within a cropping system. Although the *A. retroflexus* and *A. viridis* are members of the same family, their germination behaviour may be different under different environmental conditions. (Baskin and Baskin 1998, Chauhan and Johnson 2010). A comparison of the germination behaviour of *A. retroflexus* and *A. viridis* in various environmental conditions highlights their superiority for potential invasion and distribution in new areas (Hao *et al.* 2017). Although there is some information on seedbank persistence and germination behaviour of *A. retroflexus* and *A. viridis* collected from Asia and the United States (Chauhan and Johnson 2009, Ghorbani *et al.* 1999, Guo and Al-Khatib 2003, Jha *et al.* 2014, Korres *et al.* 2018, Thomas *et al.* 2006), the information on Australian biotypes is not available, and comprehensive studies are needed before these weeds become problematic. The objective of this comparative study was to investigate seed bank persistence and germination behaviour of Australian biotypes of *A. retroflexus* and *A. viridis* under different temperatures, osmotic potentials, salt stress, and burial depths conditions.

Material and Methods

Seed Collection

Mature seeds of *A. retroflexus* and *A. viridis* were collected from Queensland’s regions of Goondiwindi (28.41°S, 150.23°E) and Gatton (27.45°S, 152.21°E) in March 2017. Goondiwindi is 256 km (aerial distance) from Gatton and its elevation is 217 m above sea level, whereas the elevation of Gatton is 94 m above the sea level. Long-term (1918–2019) mean precipitation and maximum and minimum temperature data for both locations are shown in Figure 3.1 Gatton receives 765 mm of average annual rainfall whereas Goondiwindi receives an average annual rainfall of 620 mm. Fully mature seeds were collected from cotton cropping fields by tapping the inflorescence gently in a tray.

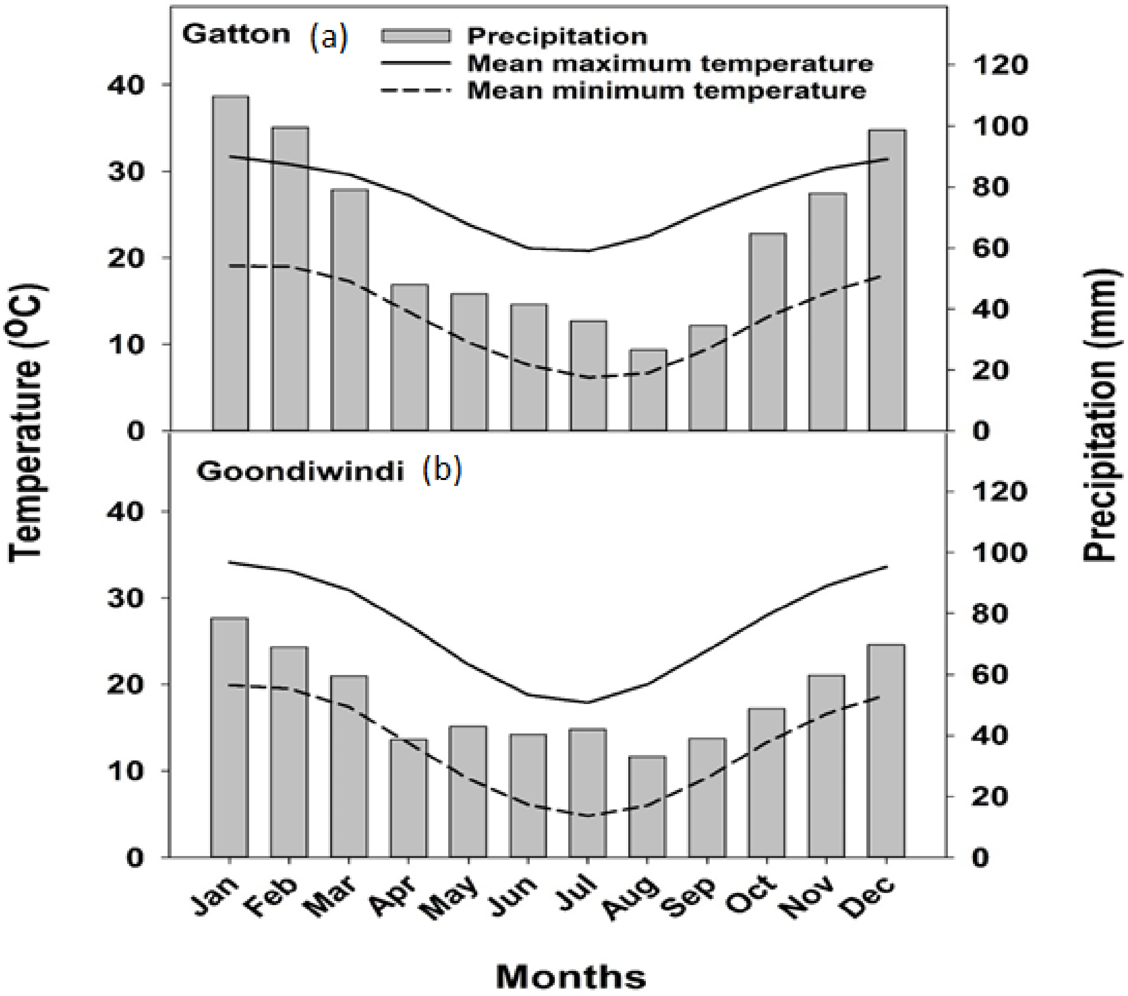


Figure 3.1 Long-term (100 years; 1918–2019) monthly mean precipitation and maximum and minimum temperatures for Gatton and Goondiwindi, Queensland, Australia.

Each population was collected from 40-50 plants dispersed in a field of 3-4 hactare. The seeds were dried, cleaned and stored in paper bags. In September 2017, these seeds were planted at Gatton (both biotypes of Gatton and Goondiwindi), and the fresh seeds were harvested in January 2018 to remove the effect of maternal environmental conditions (Mobli *et al.* 2019). The seeds of both species were stored at room temperature (25^{o}C) in a separate paper bag until the start of the experiment. The germination status of seeds during the experiment was evaluated at a day/night temperature of 35/25^{o}C for *A. retroflexus* and 30/20^{o}C for *A. viridis* under a 12-hour light/dark regime. At harvest, seeds of both weeds showed a level of seed dormancy; however, germination improved over time (Figure 3.2).

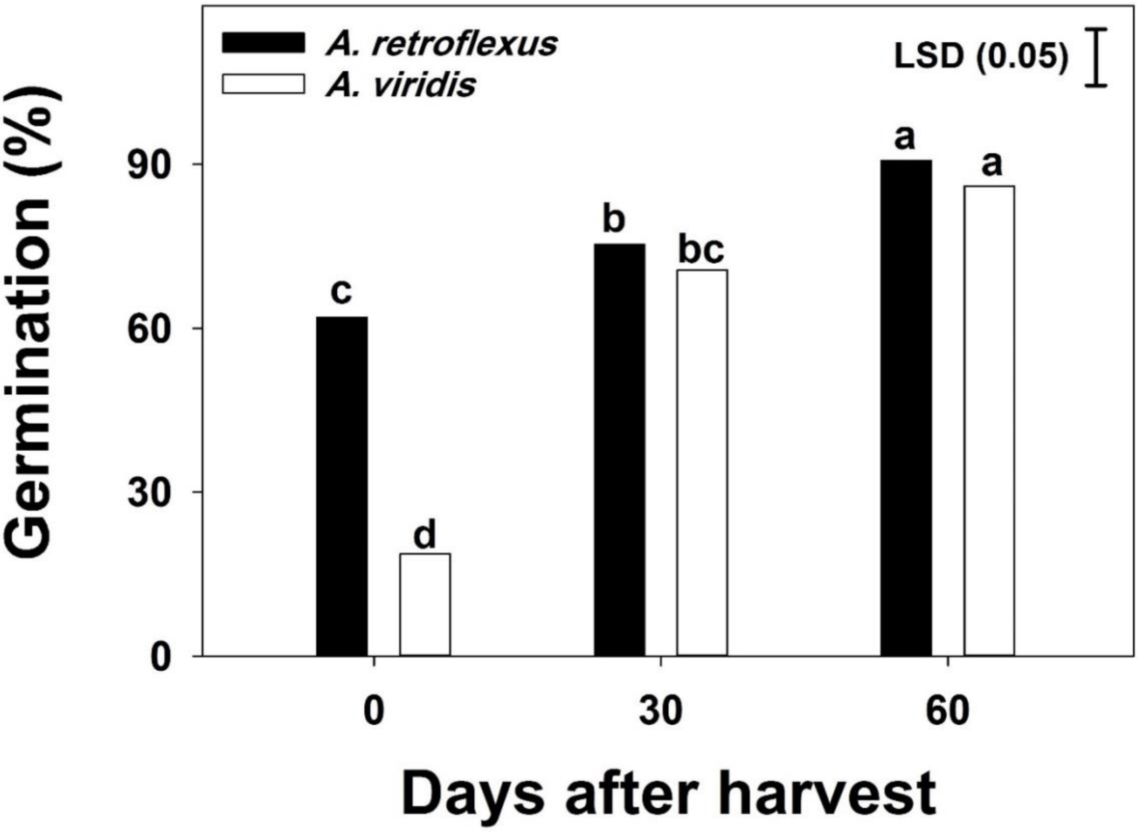


Figure 3.2 Seed germination of *A. retroflexus* and *A. viridis* after harvest. Each bar of the graph shows the mean value of data [pooled across biotypes (Gatton and Goondiwindi) and experimental repeats, n=12]. Vertical bars depict the least significant difference (LSD) values at the 5% level of probability and the letters above bars show group differences between means.

Germination Protocol

All the studies were conducted in the weed science facilities of the Queensland Alliance for Agriculture and Food Innovation (QAAFI), University of Queensland, Gatton, Australia. Twenty-five seeds of both biotypes of *A. retroflexus* and *A. viridis* were evenly arranged in 9 cm Petri dishes on a double layer of filter paper. The Petri dishes were moistened with 5 ml of sterile deionized water or the required test solution. The Petri dishes were sealed in zip-lock plastic bags to avoid loss of moisture. Except for the temperature and light experiments, a day/night temperature (12 hour photoperiod) of 35/25^{o}C sfor *A. retroflexus* and 30/20^{o}C for *A. viridis* was maintained during the experiment. The number of seeds, replications, and the quantity of water or appropriate solute were the same for every experiment. An aggregate germination count for 28-days was converted into a germination percentage for each replication.

Study 1: Effect of environmental conditions on germination and emergence of A. retroflexus and A. viridis

The comparative study was conducted in a factorial arrangement (species × population × temperature × light) to access effects of five different alternating temperature regimes of 15/5, 20/10, 25/15, 30/20, and 35/25°C under different light treatments (12-h photoperiod and continuous darkness) on germination of *A. retroflexus*, and *A. viridis* seeds. The seeds were incubated in five growth chambers separately. The growth chambers were calibrated to provide the 12 h/12 h light/dark period. In continuous dark conditions, the Petri dishes were covered with two layers of aluminium foil to create a dark environment. Eighteen Petri dishes were used per treatment with 25 seed each.

Osmotic and Salt Stress

A germination test was performed to evaluate the effect of osmotic potentials of 0.0, -0.1, -0.2, -0.4, -0.8, and -1.6 MPa on seed germination *A. retroflexus and A. viridis* biotypes. To obtain these osmotic potential concentrations, an appropriate amount of polyethylene glycol 8000 was dissolved into distilled water, following the method described by Michel and Radcliffe (1995).

The effect of salt stress was determined using six concentrations (0, 50, 100, 150, 200, and 250 mM) of sodium chloride (NaCl). An appropriate amount of NaCl was dissolved into distilled water to prepare the different NaCl concentrations (Poljakoff-Mayber *et al.* 1994).

Burial Depth

The effect of seed burial depth on *A. retroflexus* and *A. viridis* was determined by placing the seeds at soil depths of 0 (soil surface), 1, 2, 4, 6 and 8 cm. The experiment was conducted in a rainout shelter facility of the University of Queensland, Gatton. A grey vertosol soil (bulk density 1.32 g cm^{-3}, pH 7.2, and organic matter 2.3%) was used. The soil was sieved using a 4 mm sieve and oven-dried at 100 C for 96 h to kill seeds already present in the soil. Plastic pots (10 cm diameter × 10 cm height, 1 L) were filled with soil, and 50 seeds per pot were placed at the appropriate depth. Pots were sub-irrigated during the experiment.

Study 2: Seedbank persistence of A. retroflexus and A. viridis

A comparative field study was conducted at the Research Farm of the University of Queensland, Gatton, Australia, to evaluate the germinability and seedbank depletion of *A. retroflexus* and *A. viridis*. The experiment was a factorial arrangement of species, biotype, burial depth and burial durations arranged in a randomized complete block design. Right after seed collection, 50 seeds of both biotypes of *A. retroflexus* and *A. viridis* were placed in separate permeable nylon bags (5 cm by 4 cm). Permeable bags were used to create conditions close to natural soil conditions (water and air diffusion and microorganisms). The bags were placed at 0 (soil surface), 2, and 10 cm of soil depths. Wooden sticks were used to tie the surface bags to stop the wind from moving them away. Nine bags were exhumed every three months (three replications), and the seeds were retrieved from the bags and cleaned in the laboratory before the germination tests. The seeds that lost their structural rigidity were labelled as deteriorated, decayed, or germinated. The viability of remaining seeds was evaluated using the germination test described in the germination protocol section. The viability of seed embryos of non-germinated seeds was determined by the crush test (Borza *et al.* 2007, Taylor *et al.* 2004). This study was continued until 24-months after seed placement.

Data analyses

All germination tests were laid out in a completely randomized design with three replications and were repeated once after compilation of the first run. The normality (Shapiro-Wilk) and homogeneity (Breusch-Pagan) assumptions of the data were checked, and the original data was used. The significance of any treatment and their interaction with each other was evaluated using ANOVA (GENSTAT 16th edition; VSN International, Hemel Hempstead, United Kingdom) at a probability level of 0.05. In the first study, no significant differences were observed between each repeat and treatment; therefore, data was pooled across the experimental runs (n=6). In the both studies, the data was pooled across the biotypes as no significant differences were observed between biotypes (therefore, n=12). After combination of insignificant variables, again the data was subjected to analysis of variance and means was compared. The mean data of temperature, light, burial depth and dormancy status were compared using the least significant difference (LSD) test at a probability level of 0.05. A three-parameter sigmoidal model was fitted to salt stress and water potential data (equation 1) using SigmaPlot 14 (Systat Software, San Jose, CA, United States).

$$F = \frac{X_{max}}{1 + e^{\frac{-(X - X_{50})}{b}}} \qquad \text{(equation 1)}$$

F is germination (%) or seed depletion (%) at a given osmotic potential, salt concentration or months after seed placement in the field, X_{max} is the maximum germination (%) or seed depletion (%); X_{50} is the osmotic potential, salt concentration or months after seed placement, which inhibited the maximum germination by 50% or depleted 50% of seeds and b is the slope. A three-parameter logistic model was fitted to the seedbank germination data as follows:

$$F = \frac{X_{max}}{1 + (\frac{T}{T_{50}})^{b}} \qquad \text{(equation 2)}$$

F is germination percentage at a given months after seed placement (T), $Xmax$ is the maximum germination; T_{50} is the months after seed placement at which the maximum germination (%) reduced by 50% and b is the slope. Parameter estimates of the salt, osmotic stress, seed viability, and seed depletion experiments were compared with the standard error of means.

Results and Discussion

No differences between biotypes were observed in either species. The effect of seed storage and weed species and their interactions was significant ($p<0.05$) on germination. Immediately after seed harvest, poor germination was observed in *A. viridis* seeds (19%), while 62% of *A. retroflexus* seeds germinated (Figure 2). After 2-months storage, no significant differences were observed between weed species and germination of *A. retroflexus* and *A. viridis* reached 93% and 86%, respectively. After 2-months, no significant variation in seed dormancy was observed for both species (data not shown).

The results showed that both *Amaranthus* species exhibited primary dormancy, which eased with an after-ripening period. Dormancy is responsible for the lack of germination in viable seeds in favorable conditions, which facilitates the seed dispersal over time and location (Baskin and Baskin 1998). Cristaudo *et al.* (2007) reported that after-ripening is a common phenomenon in *Amaranthus* species. Initial dormancy of these species may result in periodic germination over the growing season, which could lead to failures in predictions of the germination times and intensities (Jha *et al.* 2010; Karimmojeni *et al.* 2014).

Study 1: Effect of environmental conditions on germination and emergence of *A. retroflexus* and *A. viridis*

Effect of alternating temperature and light on germination of *A. retroflexus* and *A. viridis*

Germination was affected ($p < 0.05$) by the effect of weed species, alternating temperature, light treatment and their interactions (Table 3.1). Both species showed very poor germination at 15/5°C. In a day/night temperature range of 25/15°C to 35/25°C, both weeds had more than 40% germination under both light treatments. In a 12-h photoperiod, the highest germination of *A. retroflexus* (93%) and *A. viridis* (86%) was observed at 35/25 and 30/25°C day/night temperature, respectively. Although lower germination was observed in the dark treatment, it was not an inhibiting factor for the germination of both species.

Table 3.1 The effect of alternating day/night temperatures and light treatments on germination of *A. retroflexus* and *A. viridis* (study 1).

Species		Alternating day/night temperatures				
	Light treatments	15/5	20/10	25/15	30/20	35/25
		Germination				
A. retroflexus	Light & dark	0.7	28.5	65.5	81.8	93.3
	Dark	0.3	19.8	47	64.5	80.5
A. viridis	Light & dark	0.7	8.3	51.7	85.7	73
	Dark	0.0	6	33.5	64.2	53.2
		LSD (0.05)= 6.36				

Data was pooled across biotypes (Gatton and Goondiwindi) and experimental runs (n=12).

Temperature is an important factor that impacts seed germination by regulating activities of different enzyme systems and synthesis of hormones responsible for germination of seeds (Baskin and Baskin 1998). In the current study, the effect of various alternating day/night temperature treatments on both weeds showed that *A. retroflexus* and *A. viridis* could germinate more than 40% between the temperature range of 25/15 to 35/25^{o}C. Poor germination of both species in day/night temperature ranges of 15/5 and 20/10^{o}C could be a mechanism to ensure the germination of these weeds in favorable conditions. Guo and Al-Khatib (2003) reported that germination of *A. retroflexus* was highest at day/night temperature 35/30^{o}C, and germination at 15/10 ^{o}C. Similarly, the highest germination of both Goondiwindi and Gatton biotypes of *A. viridis* was observed in temperature ranges of 30/20 to 35/25 ^{o}C (Chauhan and Johnson 2009). In cotton-growing regions of Australia (New South Wales and Queensland), except for the short winter season, the temperature ranges of 15 to 35^{o}C is favorable for the germination of these species (Figure 3.1). It could be concluded that *A. retroflexus* and *A. viridis* could germinate in warm cotton farming regions of Australia and create problems for most summer season crops. The persistent seedbank, initial dormancy, and ability to germinate in a wide range of temperatures may result in periodic germination of this weed during the growing season (Jha *et al.* 2014; Steckel *et al.* 2007). Therefore, seeds of these species could theoretically germinate in several flushes and may escape from conventional weed management strategies.

Although darkness constrained the germination significantly, the light was not an absolute requirement for the germination of *A. retroflexus* and *A. viridis*. Cristaudo *et al.* (2007) reported that although the dark did not inhibit the germination of *A. viridis*, maximum germination occurred in the presence of light. In *Amaranthus* species, a cyclic change in the requirement of temperature and light has been reported during the time, but the darkness could not inhibit the germination completely (Jha *et al.* 2010). These findings suggest *A. retroflexus* and *A. viridis* are capable of germinating even in dark conditions, allowing germination of these species to occur under crop canopy or shallow soil depths.

Effect of osmotic stress on germination of *A. retroflexus* and *A. viridis*

Osmotic potential significantly ($p<0.05$) affected the seed germination of both species (Figure 3.3). Germination (%) declined with decreasing osmotic potential, and the greatest germination of both species was observed at 0 MPa. A three-parameter sigmoid model estimated that the germination of *A. retroflexus* and *A. viridis* was inhibited by 50% (X_{50} parameter) at osmotic potentials -0.26 and -0.18 MPa, respectively. At the osmotic potential of -0.8 MPa, *A. viridis* germination was inhibited completely, while 11% germination was observed for *A. retroflexus*.

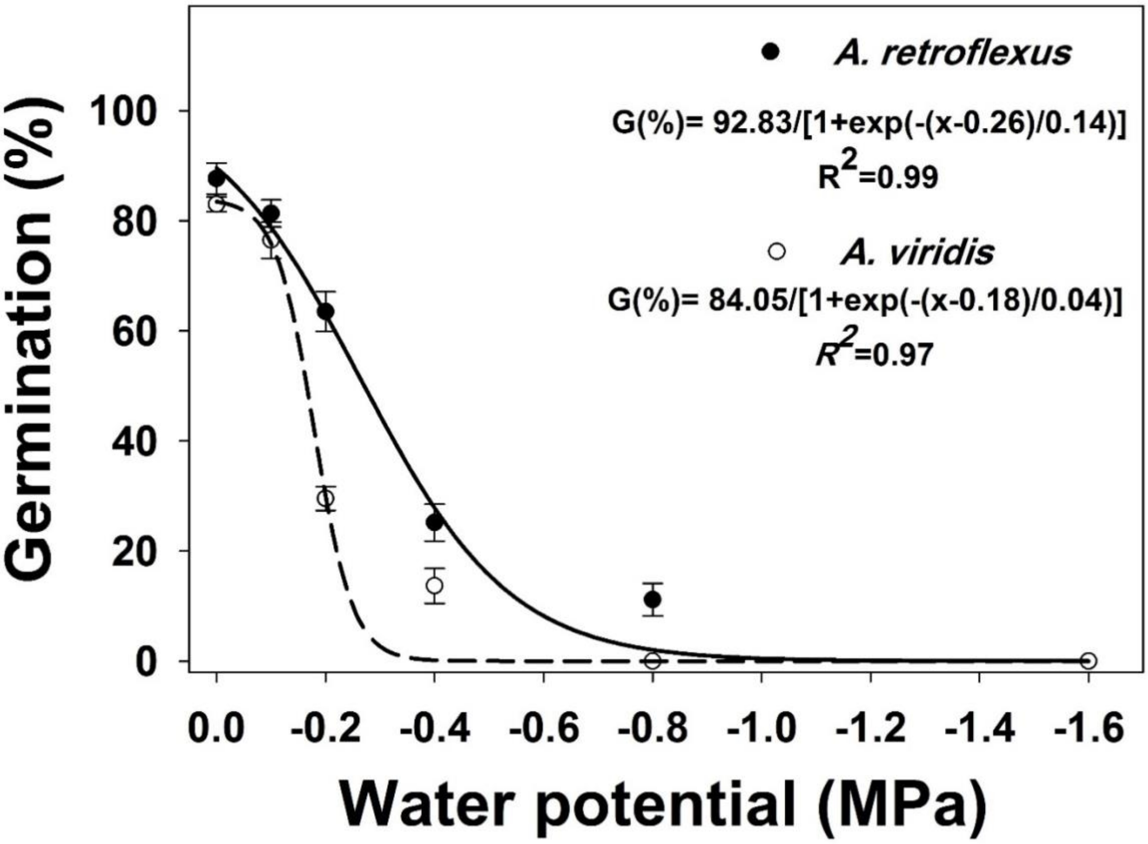


Figure 3.3 Effect of osmotic potential on germination of *A. retroflexus* and *A. viridis*. Each point of the graph shows the mean value of data [pooled across biotypes (Gatton and Goondiwindi) and experimental repeats, n=12]. A three-sigmoidal model was fitted to data. Vertical bars are the standard error of means (study 1).

The results depict that *A. retroflexus* may germinate under a wide range of moisture stress conditions, as 11% germination was observed at an osmotic potential of -0.8 MPa. In contrast, *A. viridis* could not germinate at this osmotic potential. A previous study in the Philippines also reported the lack of *A. viridis* germination at a water potential -0.8 MPa (Chauhan and Johnson 2009). Hao *et al.* (2017) showed that germination of the Chinese biotypes of *A. retroflexus* and *A. viridis* completely inhibited at -0.8 and -0.4 MPa, respectively. Although the germination of these species may be inhibited under high osmotic stress, the abundant summer rainfall in cotton-growing regions of Australia (New South Wales and Queensland). The lack of germination under high osmotic potential could be a defence mechanism for these species to survive under drought conditions (Chauhan and Johnson 2009).

Effect of salt stress on germination of *A. retroflexus* and *A. viridis*

Germination of both species was significantly ($p<0.05$) influenced by salt stress (Figure 3.4). Germination was highest at NaCl concentration 0 mM and germination (%) declined with increasing NaCl concentration. NaCl concentrations of 102 and 77 mM inhibited 50% germination of *A. retroflexus* and *A. viridis*, respectively. No germination was observed in both species at the NaCl concentration of 200 mM and above as shown in Figure 5

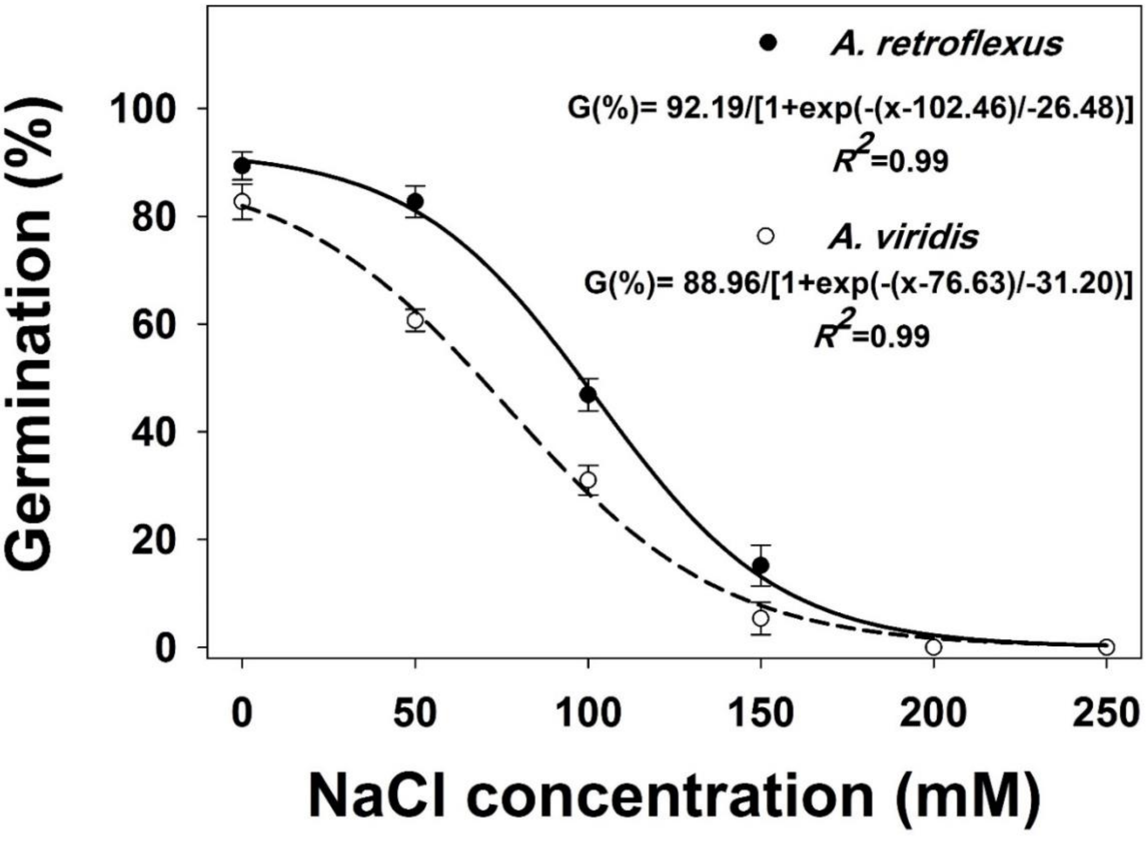


Figure 3.4 Effect of salt stress on germination of *A. retroflexus* and *A. viridis*. Each point of the graph shows the mean value of data [pooled across biotypes (Gatton and Goondiwindi) and experimental

repeats, n=12]. A three-sigmoidal model was fitted to data. Vertical bars show the standard error of means (study 1).

Our study showed that germination of both species was inhibited completely at 200 mM NaCl concentration. Similarly, no germination was observed in the Chinese biotypes of *A. retroflexus* and *A. viridis* at 200 mM NaCl (Hao *et al.* 2017). Drought, high evaporation and low rainfall conditions may result in the accumulation of salts in the soil profile. In Australia, 30% of the land is saline, and 67% of the agricultural area is potentially associated with saline water irrigation (Rengasamy 2006) and these species could sustain their germination under moderate saline conditions.

Effect of seed burial depth on emergence of *A. retroflexus* and *A. viridis*

Seedling emergence was influenced ($p < 0.05$) by the seed burial depth, weed species and their interaction (Figure 3.5). The highest emergence of *A. retroflexus* (67%) was observed at 1 cm burial depth, while the highest emergence of *A. viridis* (72%) was observed on the soil surface (Figure 5). A sharp decline in emergence was observed with increaseing burial depths, and the emergence of both species was less than 10% at 4 cm. The emergence of *A. retroflexus* and *A. viridis* was completely inhibited at 6 to 8 cm, respectively.

In the current study, the adverse effect of burial depth on the emergence of these species was observed. Information from studies conducted on other *Amaranthus* species indicates a similar trend of germination and emergence owing to the restricted carbohydrate supplies available in small-seeded broadleaf weeds (Chauhan and Johnson 2009; Ghorbani *et al.* 1999; Hao *et al.* 2017). Ghorbani *et al.* (1999) studied the emergence trends of *A. retroflexus* and found that the optimum burial depth was between 0.5 and 3 cm, with no emergence at 5 cm. As *A. retroflexus* and *A. viridis* seedlings could not emerge from burial depths greater than 4 cm, deep tillage could be used as an option to halt their emergence.

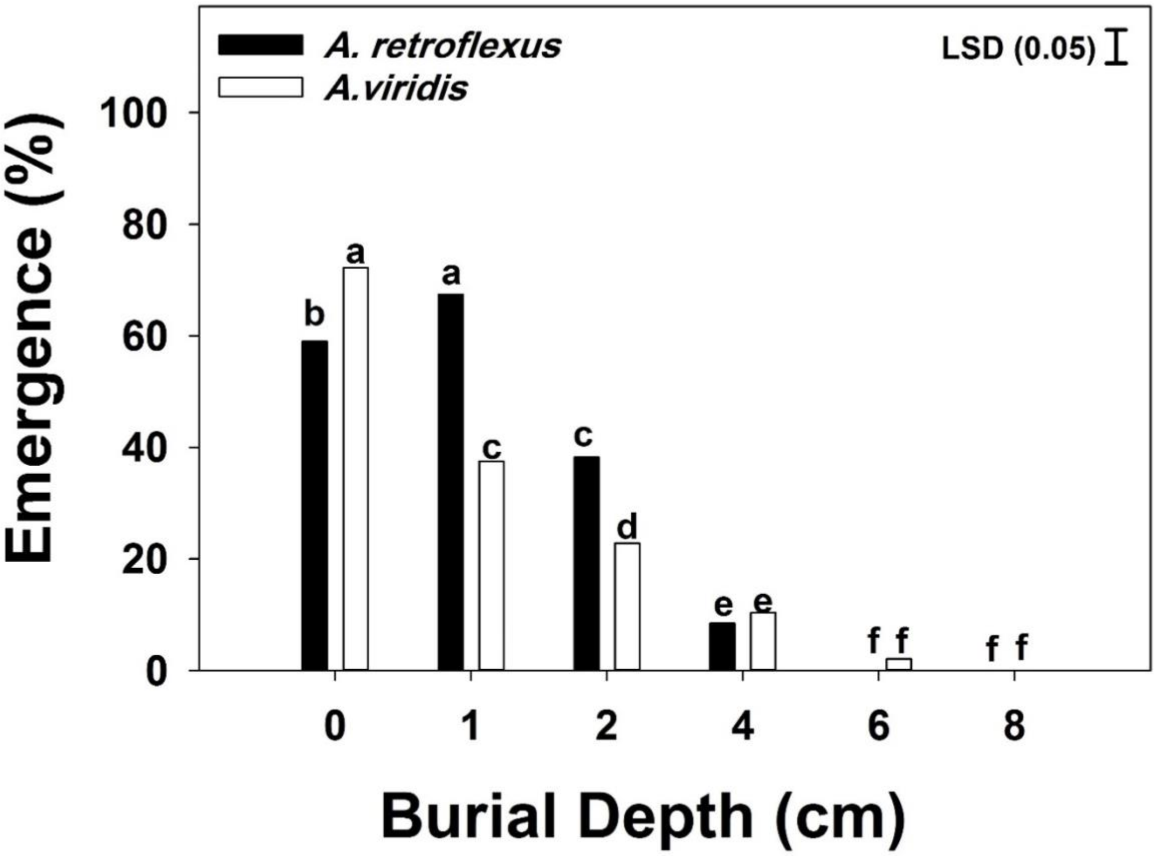


Figure 3.5 Effect of burial depth on seedling emergence *A. retroflexus* and *A. viridis*. Each bar of the graph shows the mean value of data [pooled across biotypes (Gatton and Goondiwindi) and experimental repeats, n=12]. Vertical bars depict the least significant difference (LSD) values at the 5% level of probability and the letters above bars show group differences between means (study 1).

Study 2: Seedbank persistence of *A. retroflexus* and *A. viridis*

Effect of seed burial depth and burial duration on seed viability of A. retroflexus and A. viridis

The effects of weed species, burial depth and burial duration and their interactions on the seed viability was significant ($p < 0.05$). In both species, regardless of the spatial position of seeds, the viability of the seeds was reduced over time and was lowest at 24-mo after seed placement (Figure 3.6 and Table 3.2). Compared to the seeds retrieved from the soil surface, the burial of *A. retroflexus* and *A. viridis* seeds at 10 cm increased seed viability by 317% and 235%, respectively, at 24-mo after seed placement. *A. retroflexus* seeds buried at 0, 5, and 10 cm lost 50% of their maximum viability (T_{50} parameter) at 9, 10, and 15-mo after seed placement, respectively. Similarly, the maximum seed viability of *A. viridis* was reduced by 50% at 6, 8, and 9-mo after seed placement, respectively.

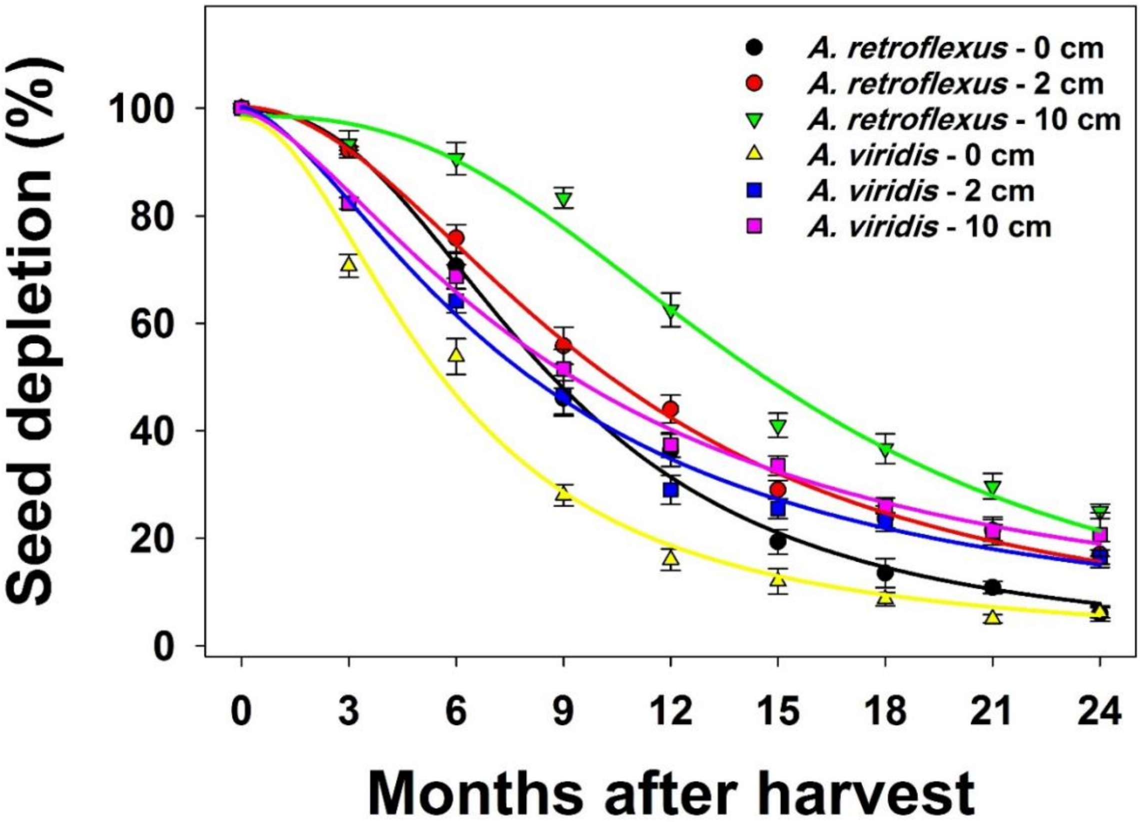


Figure 3.6 Effect of burial depth and burial duration on germination of *A. retroflexus* and *A. viridis*. Each point of the graph shows the mean value of data [pooled across biotypes (Gatton and Goondiwindi) and experimental repeats, n=12]. A three-logistic model was fitted to data. Vertical bars depict the standard error of means. The estimated parameters are given in Table 3.2 (study 2).

Not all viable seeds germinate due to dormancy and unfavourable conditions. However, as long as seeds are viable in a seed bank, there is a risk of weed emergence and reproduction (Baskin and Baskin 1998). Korres *et al.* (2018) reported that regardless of burial depth, only 4-5% of Palmer amaranth (*Amaranthus palmeri*) and waterhemp (*Amaranthus tuberculatus*) seeds were viable after 3-years. Although seed viability is largely related to plant species and environmental conditions, our results suggest that the inclusion of fallow conditions in crop rotation could be beneficial as up to 50% seed loss per year was observed.

Table 3.2 A three-parameter logistic model fitted to viability (%) of *A. retroflexus* and *A. viridis* at different burial depths and burial duration. (study 2)

Species and burial depth	Parameters			
	E_{max}	T_{50}	E_{rate}	R^2
Amaranthus retroflexus				
0	99.6 (1.09)	8.7 (0.29)	2.4 (0.14)	0.99
5	98.6 (1.66)	10.2 (0.29)	1.9 (0.09)	0.99
10	98.5 (1.18)	14.7 (0.67)	2.6 (0.32)	0.98
Amaranthus viridis				
0	98.1 (1.99)	5.7 (0.44)	1.9 (0.19)	0.99
5	97.1 (2.96)	8.0 (0.49)	1.5 (0.12)	0.99
10	99.4 (1.01)	9.3 (0.39)	1.5 (0.08)	0.99

Values presented in the parentheses are standard error of means (±SE).

$G(\%) = G_{max} /(1+ (T/T_{50})^{b})$, $G(\%)$ is viability percentage at given months after harvest T, $Gmax$ is the maximum vability; T_{50} is the months after harvest, which the maximum vability (%) reduced by 50% and b is the slope.

The results showed that the burial of seeds inhibited the emergence of both species, but it resulted in increased seed persistence. Steckel *et al.* (2007) reported that tillage increased seed persistence of common waterhemp (*Amaranthus rudis*). The results of the current study showed that the emergence of *A. retroflexus* and *A. viridis* occurred close to the soil surface. In the no-tillage systems, seeds remain on the soil surface, and it has been reported that initially, the emergence of weed species was higher in such systems in comparison to tillage systems (Steckel *et al.* 2007). It could be concluded that in the no-tillage systems, farmers may face more infestation compared to tillage systems initially but adopting the no-tillage systems could be contributing to better weed seed bank management.

Effect of seed burial depth and burial duration on seed depletion of *A. retroflexus* and *A. viridis*

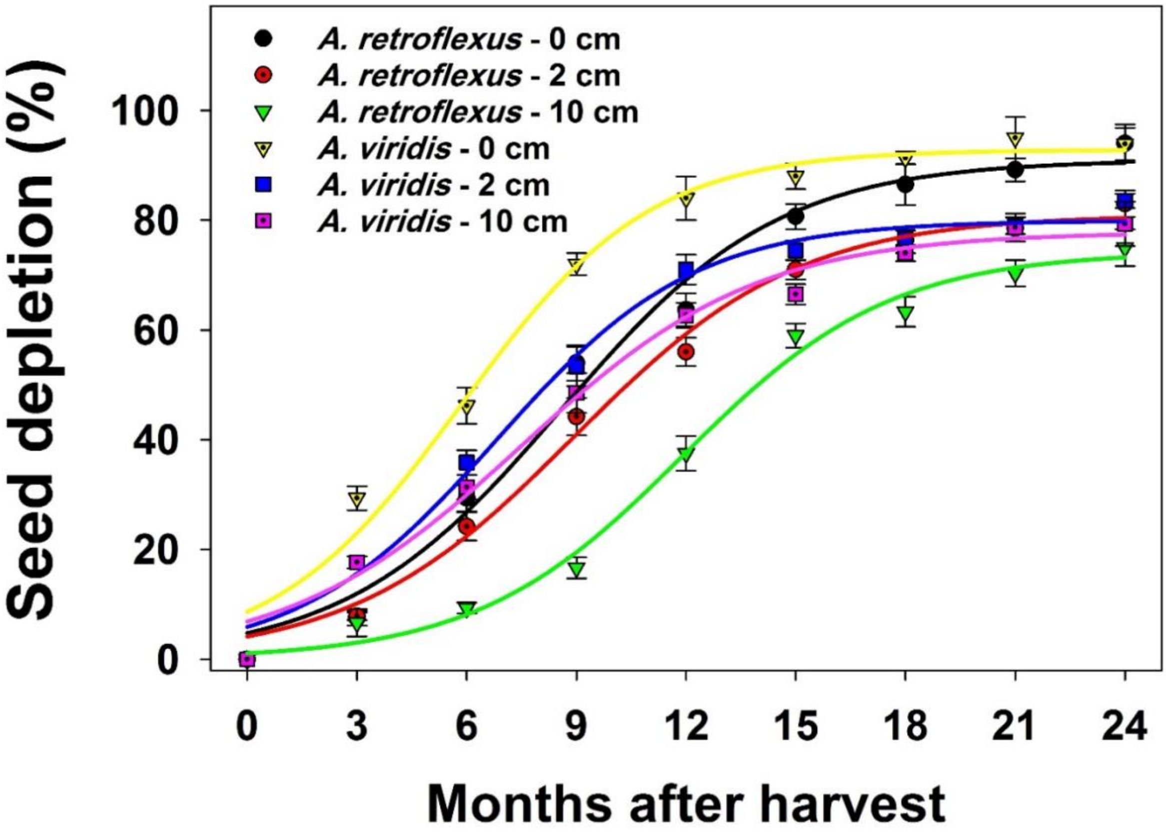


Figure 3.7 Effect of burial depth and burial duration on seed depletion of *A.retroflexus* and *A. viridis*. Each point of the graph shows the mean value of data [pooled across biotypes (Gatton and Goondiwindi) and experimental repeats, n=12].

The weed species, seed burial depth, burial duration, and their interactions had a significant ($p <$ 0.05) effect on the seedbank depletion. Irrespective of seed spatial position, seed depletion of both species was increased over time (Figure 7 and Table 3.3). At 24-months after seed placement, seed depletion of *A. retroflexus* ranged from 75% (10 cm depth) to 94% (soil surface). Similarly, seed depletion of *A. viridis* ranged from 79% to 94% at this time. Compared to seeds that were buried at 10 cm, the seed depletion of both species was higher on the soil surface. *A. retroflexus* seeds that were buried at 0, 5, and 10 cm showed a 50% depletion of seeds from the seedbank at 9, 9, and 12-months after seed placement, respectively. Similarly, 50% of *A. viridis* seeds were depleted from the seedbank at 6, 7, and 8-months after seed placement, respectively.

Avoiding seed bank replenishment and declining seed density is one of the most effective weed management strategies (Baskin and Baskin 1998, Lutman *et al.* 2002). The results of the current study showed that seed depletion (germinated or destroyed) of both species was higher on the soil surface compared to buried seeds. On the soil surface, seeds experience greater temperature fluctuation, seed predation, and microbial degradation (Long *et al.* 2015). In this study, the factors that influenced seed depletion were not studied; therefore, more studies are required to maximize the seed depletion of these species.

Table 3.3 A three-parameter sigmoid model fitted to seed depletion (%) of *Amaranthus retroflexus* and *A. viridis* at different burial depths and burial duration (study 2)

Species and burial depth	Parameters			
	X_{max}	X_{50}	b	R^2
Amaranthus retroflexus				
0	90.9 (3.09)	8.5 (0.47)	2.9 (0.40)	0.99
5	80.9 (2.18)	8.9 (0.37)	3.0 (0.31)	0.99
10	74.2 (2.51)	11.9 (0.40)	2.8 (0.32)	0.99
Amaranthus viridis				
0	92.8 (2.54)	5.8 (0.40)	2.5 (0.36)	0.99
5	79.9 (1.99)	6.8 (0.36)	2.6 (0.31)	0.99
10	77.8 (2.53)	7.5 (0.47)	3.2 (0.42)	0.99

Values presented in the parentheses are standard error of means (±SE).

$F = Xmax/(1+\exp(-(X-X_{50})/b))$, F is seed depletion (%) at given months after seed harvest X, $Xmax$ is the maximum seed depletion (%); X_{50} is months after after placement, which 50% of seed depletes and b is the slope.

In the present study, the biotypes of these species responded similarly to the tested environmental conditions. Genetic diversity and diverse maternal conditions during seed maturity could be responsible for the different responses of *Amaranthus* species biotypes to environmental conditions (Karimmojeni *et al.* 2014). In the current study, the maternal effect was removed by re-growing seeds in the same environment. It could be concluded that the ability to germinate in a wide spectrum of environmental conditions and persistence over time could be an innate characteristic of *Amaranthus* species. Hao *et al.* (2017) claimed that the germination potential of seeds may not make any difference in the invasiveness of *Amaranthus* species, but germination performances of these species may enhance the worldwide distribution of these species.

This study adds valuable information to our understanding of the germination biology of these species, which will be helpful in the development of suitable management practices and modelling the control strategies for the potential spread of *A. retroflexus* and *A. viridis* before these weeds become problematic across Australia. The study results showed that *A. retroflexus* and *A. viridis* germinate over a wide variety of environmental conditions. The results showed that germination of both species could be restricted under high osmotic and salt stress. A mix of deep and shallow tillage together can be an effective management practice to diminish the soil seedbank in arable lands, but the seed burial depth increases the seedbank persistence. The findings of this study will help agronomists working on cotton to build sustainable integrated weed management practices for effective management and control of *A. retroflexus* and *A. viridis*.

CHAPTER 4: Effect of different soil moisture regimes on the growth and fecundity of *Amaranthus retroflexus* and *Amaranthus viridis*

Publications included in this chapter

Asad MK, Mobli A. Jeff AW, Chauhan BS (2020) Effect of soil moisture regimes on the growth and fecundity of redroot pigweed (*Amaranthus retroflexus*) and slender amaranth (*Amaranthus viridis*) Weed Science, 1-22. doi:10.1017/wsc.2020.89

Introduction

Amaranthus viridis and *Amaranthus retroflexus* are broadleaf annual weeds naturalized in temperate and warm temperate countries of the world, including countries in Africa, Asia-Pacific, and Europe (Holm *et al.* 1997, Uva *et al.* 1997, Waselkov and Olsen 2014). *A. viridis* and *A. retroflexus* are C_4 weeds with aggressive growth habits and are considered the most complex and widespread species of the genus *Amaranthus*, and each species causes monetary losses to crop production globally (Ward *et al.* 2013). Both weeds have high potential to compete with horticultural and field crops for resources such as light, water, and nutrients (Carvalho and Christoffoleti 2008). A significant yield reduction in many crops has been reported. For example, *A. retroflexus* infestations can reduce soybean (*Glycine max)* yield by 38%, depending on its density and time of emergence (Bensch *et al.* 2003). Multiple resistance in these weeds to acetolactate synthase and photosystem II inhibitor herbicides has been reported in many countries; however, there are no reports of the presence of herbicide-resistant biotypes of these weeds in Australia (Heap 2020).

In Australia, crop production highly depends on rainfall events, and the climatic models have anticipated higher water deficiency in the future (DEE 2017). In the wake of changing climate, limited availability of water has become the major limiting factor to crop production and food security. Weeds respond to changes in climate and cultural practices efficiently, which leads to the dominance of weeds in agricultural ecosystems (Mahajan *et al.* 2012). Bajwa *et al.* (2017) stated that drought-tolerant weeds, such as *Parthenium hysterophorus*, grew vigorously and produced a significant number of seeds even at 50% field capacity (FC). The greater plasticity of weeds enables them to develop well in comparison to the crops in a water-restricted environment (Chauhan and Mahajan 2014, Crusciol *et al.* 2001, Mahajan *et al.* 2015). Therefore, studying the effect of water stress on weeds could help in formulating better weed management strategies.

Water stress hinders growth, nutrient assimilation and photosynthesis, which ultimately causes a significant reduction in biomass production. Water stress destroys the thylakoid membrane and photosynthetic pigments along with a reduction in chlorophyll contents (Anjum *et al.* 2011). In the presence of water stress, a plant activates its resistance mechanism, such as drought avoidance, drought tolerance, drought recovery, or drought escape (Fang and Xiong 2015). The mechanisms are stronger in C_4 plants compared to C_3 plants (Lawlor 2013, McLachlan and Swanton 1993, Stoller and Myers 1989). However, the resistance mechanism of *A. viridis* and *A. retroflexus* to cope with water stress conditions is not fully understood. C_4 plants maintain a high level of photosynthetic and osmotic modification to boost the concentration of leaf solute, causing the stomata to remain open for a long period during water stress conditions. This mechanism enables C_4 plants to maintain CO_2 diffusion to the chloroplast (Ehleringer 1983, Forseth and Ehleringer 1982).

Different weed species react to soil moisture stress differently; however, some weeds grow very well, complete their life cycle and produce considerable amounts of seeds in water stress conditions (Chauhan and Johnson 2010, Kaur *et al.* 2016). *A. viridis* and *A. retroflexus* are common weeds of Australia and the interaction of these weeds with environmental changes has not been studied extensively. Consequently, it will be essential to explore the impact of various soil moisture levels on *A. viridis* and *A. retroflexus* growth and reproductive potential. Such parameters can also be used to assess and differentiate the invasive potential of different Australian biotypes of *A. viridis* and *A. retroflexus*. Therefore, a study was conducted to evaluate the impact of varied moisture regimes on the growth and reproductive behavior of two Australian biotypes of *A. viridis* and *A. retroflexus*. Such research could guide us in evaluating and comparing the invasiveness of these weeds under future water-limiting climate scenarios.

Materials and Methods

Plant Material

In 2016, seeds of two biotypes of *A. viridis* and *A. retroflexus* were collected from Goondiwindi (28.41°S, 150.23°E; altitude 210 m) and Gatton (27.45°S, 152.21°E, altitude 90 m), Queensland, Australia, cleaned thoroughly and stored separately at room temperature. Seeds from both biotypes of each species were re-grown at Gatton to remove the maternal conditions (Mobli *et al.* 2019) and seeds collected from these plants were used in the present study. No seed dormancy breaking treatment was used before planting. Seeds were germinated in trays containing commercial Platinum Potting Mix (Centenary Landscaping, NSW, Australia). The potting mix contained biological organic-based products and had a pH of 5.6 and an electrical conductivity of 1.6 dS m^{-1}. Seedlings were transplanted at the 5-leaf stage (4-6 cm in height) into black, free-draining pots of a size of 25 cm in diameter and 30 cm in height filled with soil collected from the Gatton farm of the University of Queensland. The soil was air-dried and passed through a 3 mm sieve to establish uniform consistency. Soil texture was heavy clay loam with a pH of 6.7, the electrical conductivity of 0.14 dS m^{-1} and organic matter content of 2.8%. Each pot was filled with 12 kg of oven-dried soil (90°C for 72 hours). All pots were supplied with adequate water until the experiment commenced.

Soil Moisture Adjustment

Ten pots containing dry soil were weighed, watered until saturation, covered with a plastic sheet and left in a shade house. After 24 hours, the pots were weighed again to calculate the pot water contents, i.e. 100% field capacity (FC). The 75%, 50% and 25% FC were determined based on the fraction of the 100% FC. To re-establish FC in the pots during the study, an appropriate amount of tap water was added to the pots after every 4 days (Bajwa *et al.* 2017, Chauhan and Johnson 2010). The equation used for the calculation of pot water content is given below (Equation 1):

$$\text{Water holding capacity} = (W_w - W_d) \times \frac{100}{W_d} \qquad \text{(equation 1)}$$

where, W_w= weight of wet soil; W_d= weight of dry soil.

Experimentation

In the summer of 2017-2018, a pot study was carried out in a naturally ventilated shade house (mean temperature= 28°C) at the University of Queensland, Gatton, Australia. The experiment was a 2 X 2 X 4 factorial arrangement of species (*A. retroflexus* and *A. viridis*), biotype (Gatton and Goondiwindi), and moisture regime (25, 50, 75 and 100% FC) arranged in a randomized complete block design with three replications. Both *Amaranthus* species (Gatton and Goondiwindi biotype) were grown at the four different soil moisture levels. The experiment ran for 84 d until the plants were fully matured, and seed production had ceased. Plant height and number of leaves, biomass (shoot and root), inflorescence number per plant and seed number per plant were recorded at maturity. The shoot and root parts (washed to remove the soil particles) were bagged separately and oven-dried for 3 days at 70^{o}C. Afterwards, the weight was determined to record shoot and root biomass, and shoot:root biomass ratio. The number of seeds per plant was calculated by taking a sample of 1 g seed from each plant. The number of seeds in 1 g was counted and multiplied by the total seed weight of each plant. In the summer of 2018-2019, the experiment was repeated in the same conditions.

Data Analysis

Data from both species, biotypes and experimental runs were subjected to analysis of variance (GENSTAT 16th edition; VSN International, Hemel Hempstead, United Kingdom). Both biotypes of *A. retroflexus* and *A. viridis* (Gatton and Goondiwindi) responded similarly (p>0.05) to the degree of moisture stress. The treatment by experimental run interaction was also not significant in either study; therefore, data from both biotypes and repeats were combined (n=12 for each treatment). After combining data, again the data was subjected to analysis of variance and means was compared. Fisher's protected Least Significant Differences (LSD) mean comparison test at probability 0.05 was used. Figures were plotted using SigmaPlot 14 (Systat Software, San Jose, CA, United States).

Results and Discussion

Water stress had a significant effect ($p<0.001$) on the growth and seed production of *A. viridis* and *A. retroflexus* (Table 3.3), and the highest effect on the plant's growth and seed production was observed at 25% FC (Figures 4.1-4.6)

Plant height

The effect of species, water treatments, and their interaction was significant ($p<0.001$) on plant height of *A. viridis* and *A. retroflexus* (Table 4.1). Maximum height achieved by *A. viridis* and *A. retroflexus* plants was 83 and 106 cm, respectively (Figure 8). Compared with the no water stress treatment (100% FC), plant height of *A. viridis* was reduced by 14%, 37% and 50% at 75%, 50% and 25% of FC, respectively. The corresponding reductions in plant height for *A. retroflexus* were 14%, 38% and 54%, respectively.

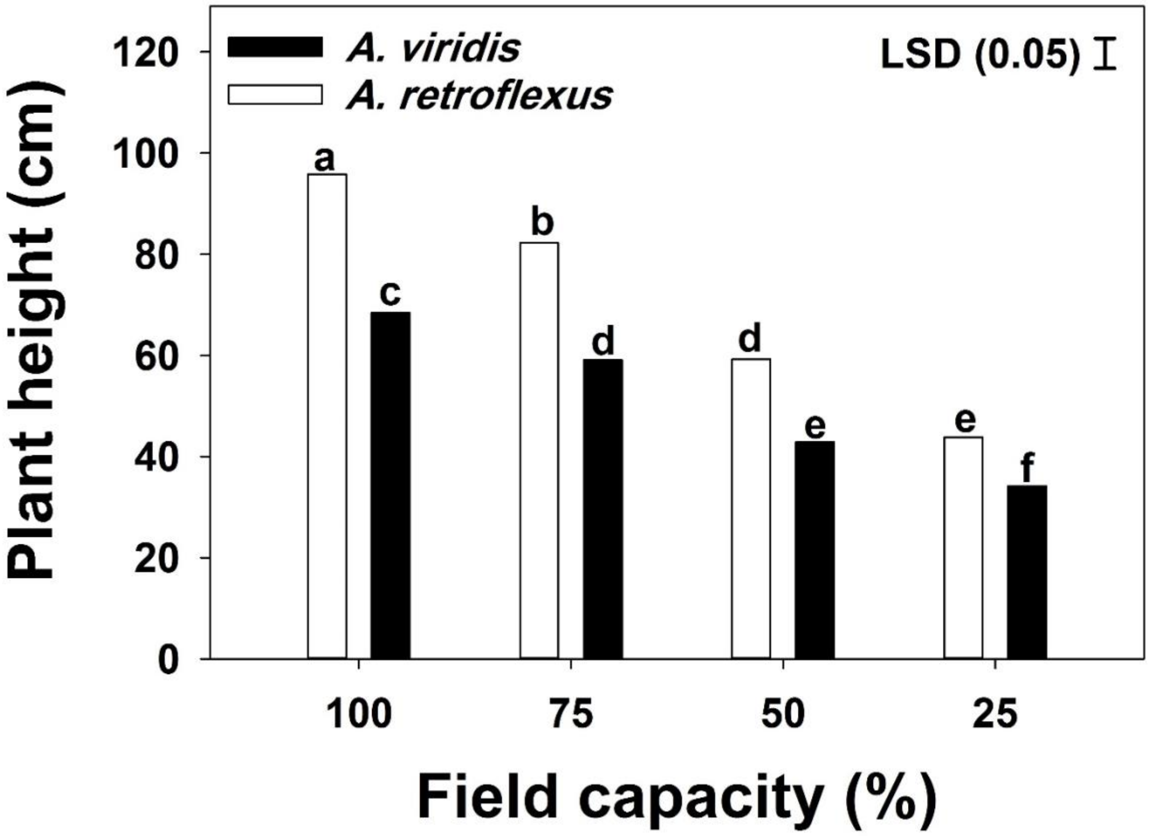


Figure 4.1 The effect of soil moisture on plant height of *A. retroflexus* and *A. viridis*. The moisture content was kept at 100, 75, 50, and 25% of field capacity (FC). Vertical bar represents LSD at probability 5%. Data was pooled over biotype and experimental runs (n=12).

Number of leaves per plant

Species, water treatments, and their interaction significantly affected the number of leaves (Table 4.2). In both weed species, the maximum number of leaves was produced in the treatment of 100% FC (Figure 9). Leaf production by *A. viridis* and *A. retroflexus* plants was inversely proportional to the water stress applied to the plant. The maximum number of leaves produced by *A. viridis* and *A.*

retroflexus plants at 25% of FC was 53% and 54% fewer than that of plants at 100% of FC, respectively.

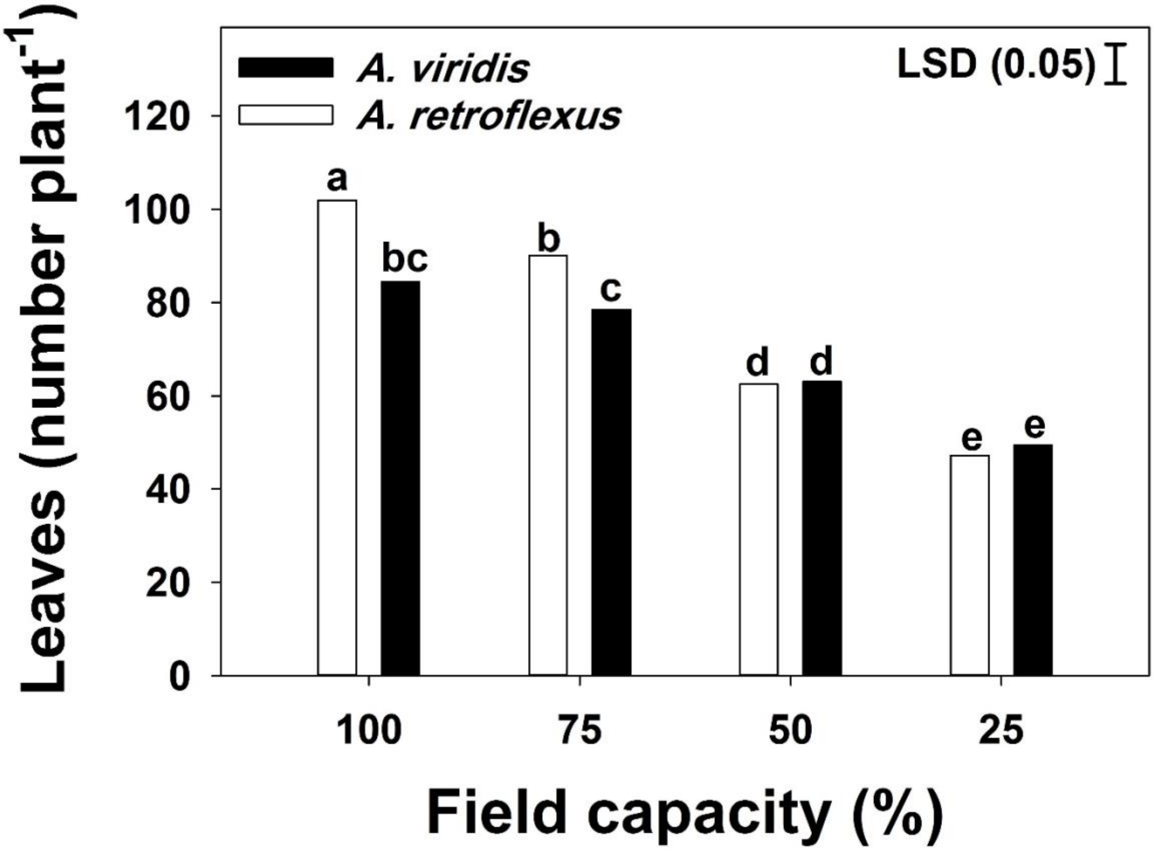


Figure 4.2 The effect of soil moisture on number of leaves of *A. retroflexus* and *A. viridis*. The moisture content was kept at 100, 75, 50, and 25% of field capacity (FC). Vertical bar represents LSD at probability 5%. Data was pooled over biotype and experimental runs (n=12).

Biomass

Although species, water treatments, and their interaction significantly ($p<0.05$) affected shoot biomass, only the effect of water treatment was significant ($p<0.001$) on root biomass (Table 4.1).

Table 4.1 Analyses of variance for various plant parameters of two biotypes of *Amaranthus viridis* and *A. retroflexus* at maturity.

Source	Degree of freedom (df)	Plant height (cm)	leaves (number plant^{-1})	Inflorecences (number plant^{-1})	Seed (number plant^{-1})	Shoot biomass (g plant^{-1})	Root biomass (g plant^{-1})	Shoot:root ratio
		P value						
Replication	11	0.26	0.30	0.35	0.60	0.16	0.50	0.21
Species	1	<0.001	<0.001	<0.001	<0.001	<0.001	0.37	0.57
Water treatment	3	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Species × Water treatment	3	<0.001	<0.001	<0.001	<0.001	0.01	0.52	0.15
Error	77							

Both biotypes of *A. viridis* and *A. retroflexus* from Gatton and Goondiwindi responded similarly (p<0.001) to water stress levels. The treatment by experimental run interaction was also not significant in either study; therefore, data from both biotypes and runs were combined.

Shoot and root biomass of both species declined with increasing water stress (Figure 4.3 and 4.4).

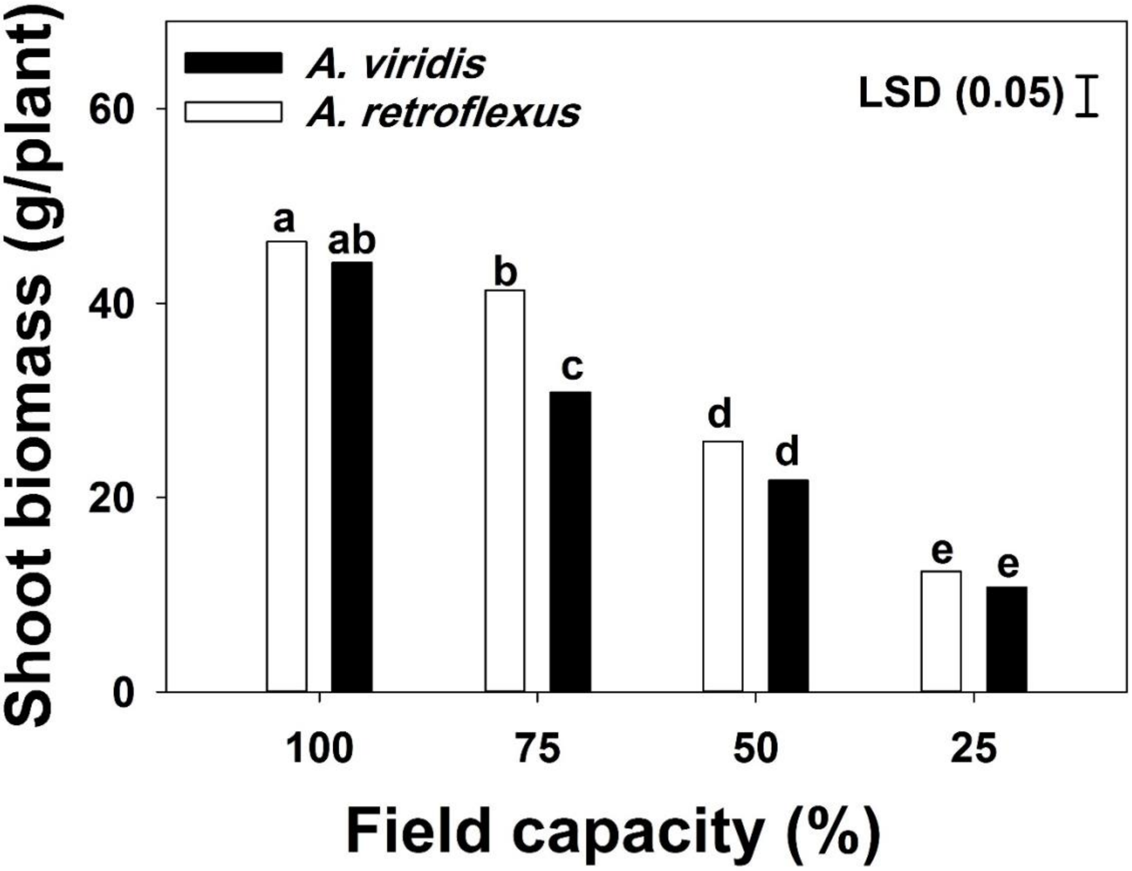


Figure 4.3 The effect of soil moisture on shoot biomass of *A. retroflexus* and *A. viridis*. The moisture content was kept at 100, 75, 50, and 25% of field capacity (FC). Vertical bar represents LSD at probability 5%. Data was pooled over biotype and experimental runs (n=12).

The highest shoot biomass of *A. viridis* (44 g plant^{-1}) and *A. retroflexus* (46 g plant^{-1}) was observed at 100% FC. At 25% of FC, the shoot biomass of *A. viridis* and *A. retroflexus* plants were reduced by 76% and 73%, respectively, compared with the shoot biomass at 100% FC. No differences were observed between root biomass at 100% and 75% FC. At 50% of FC, the root biomass of both species was decreased by 22% in comparison with 100% FC, however, no differences were observed between root biomass at 50% and 25% of FC.

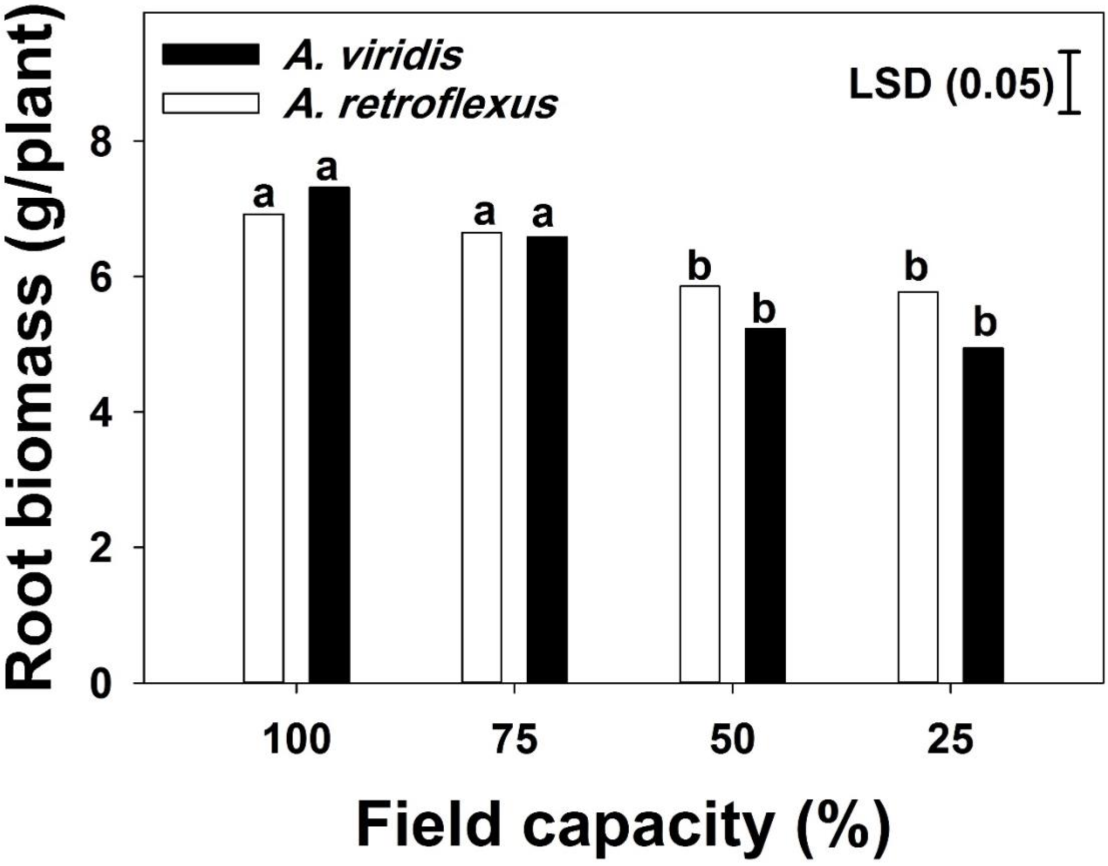


Figure 4.4 The effect of soil moisture on root biomass of *A. retroflexus* and *A. viridis* as described by a linear model. The moisture content was kept at 100, 75, 50, and 25% of field capacity (FC). Vertical bar represents LSD at probability 5%. Data was pooled over biotype and experimental runs (n=12).

In the rainfed Australian agriculture system, plants may experience severe water deficit conditions with changes in rainfall patterns (longer dry conditions and sporadic distribution). Soil moisture plays a key role in weed establishment, growth, and regeneration (Chauhan and Johnson 2010). Weed species respond phenologically and physiologically to different levels of water availability (Bajwa *et al.* 2017; Chauhan and Johnson 2010). However, the impact of drought conditions on plants depends largely upon plant species, timing, extent and duration of drought (Stout and Simpson 1978). In the current study, although growth and seed production of *A. viridis* was lower than *A. retroflexus,* both weed species responded similarly to moisture stress.

In the current study at 25% FC, a sharp decline in *A. viridis* and *A. retroflexus* plant height, number of leaves, and biomass production was observed in comparison to 100% of FC. It seems that severe water stress resulted in the reduction of their fitness as both weeds produced lower biomass but could not completely inhibit the growth and seed production of these species. Sarangi *et al.* (2016) reported that high water stress (25% FC) could reduce the growth index of common waterhemp (*Amaranthus rudis*) by 43% in comparison with no water stress conditions (100% FC). Studies on other *Amaranthus* species palmer amaranth (*A. palmeri*), grain amaranth (*A. rudis*) showed that severe moisture stress hindered their growth, but could not inhibit their vegetative growth completely (Moore and Franklin 2011, Moran and Showler 2005, Olufolaji and Ojo 2010, Sarangi *et al.* 2016).

Shoot:root biomass ratio

Only the effect of soil moisture was significant ($p<0.001$) on shoot:root ratio of *A. viridis* and *A. retroflexus* (Table 4.1). The shoot:root biomass ratio was declined with increasing water stress levels (Figure 4.5). The shoot:root biomass ratio of both species was similar between 75% and 100% of FC. In both species, the highest decline in the shoot:root biomass ratio was observed at 25% of FC. At 25% of FC, the shoot:root biomass ratio of both species was reduced by 67% in comparison with 100% of FC.

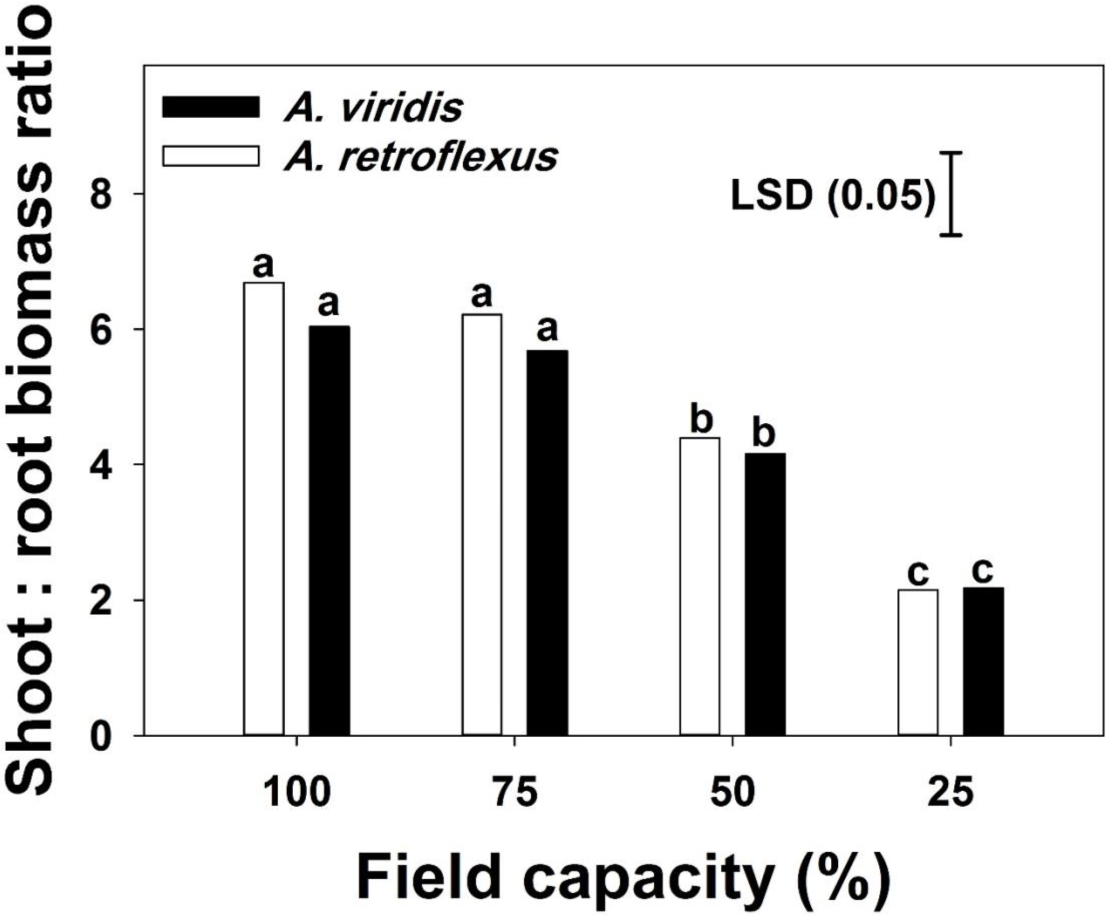


Figure 4.5 The effect of soil moisture on shoot: root biomass ratio of *A. retroflexus* and *A. viridis*. The moisture content was kept at 100, 75, 50, and 25% of field capacity (FC). Vertical bar represents LSD at probability 5%. Data was pooled over biotype and experimental runs (n=12).

Water stress had a significant impact on the shoot and root biomass of both weed species, and consequently, the shoot:root biomass declined. Likewise, in *A. rudis* and junglerice (*Echinochloa colona*) (C_4 plants), the lowest shoot:root biomass ratio was observed at a high water stress condition (Mahajan *et al.* 2019; Sarangi *et al.* 2016). Studies showed that some drought-tolerant plants respond to drought conditions by reducing aboveground vegetation to avoid shoot dehydration and to increase water use efficiency (Ogburn and Edwards 2010, Tardieu 2013). Although changes in the shoot:root biomass ratio could be a criterion for explaining a plant's response to drought conditions, biochemical and molecular studies are required to elucidate the mechanism of drought tolerance in the plant (Ali *et al.* 2009, Fang and Xiong 2015).

Inflorescences and number of seeds per plant

The effect of species, water treatments, and their interaction was significant ($p<0.001$) on the number of inflorescences and seed production of *A. viridis* and *A. retroflexus* (Table 4.1). The number of inflorescences and seeds per plant of both species declined with increasing water stress (Figure 4.6). Compared to 100% FC, a 52% and 45% reduction of the maximum number of inflorescences of *A. viridis* and *A. retroflexus* was observed at 50% of FC, respectively. Similarly, the maximum number of seeds of *A. viridis* and *A. retroflexus* was reduced by 66% and 70% at 50% FC in comparison with 100% FC, respectively. At 100% of FC, *A. viridis* and *A. retroflexus* produced 3070 and 1740 seeds plant^{-1}, respectively. Although seed production was strongly affected by soil moisture, even at 25% FC, *A. viridis* and *A. retroflexus* plants produced 290 and 370 seeds plant^{-1}, respectively.

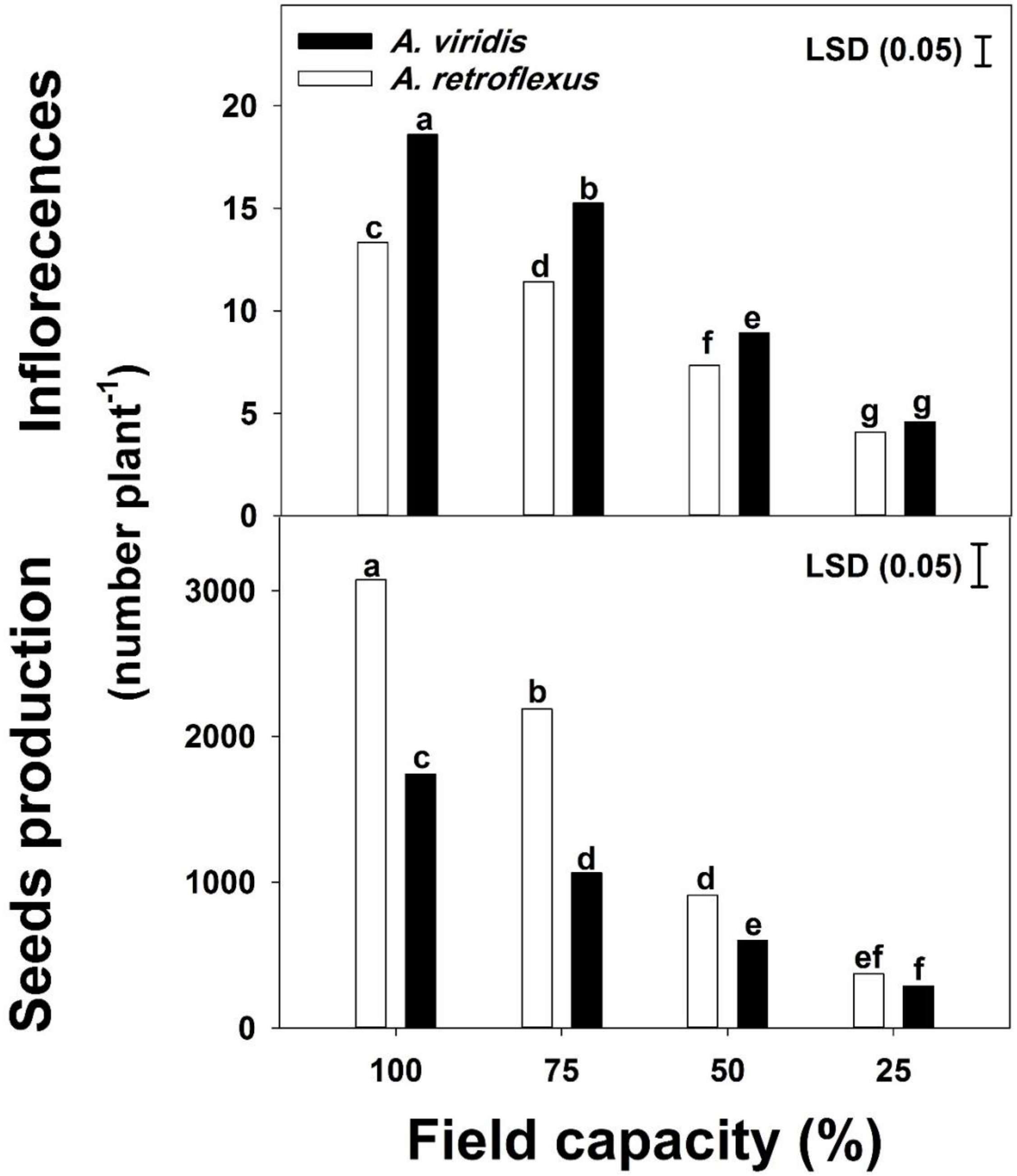


Figure 4.6 The effect of soil moisture on number of inflorescences and seeds of *A. retroflexus* and *A. viridis*. The moisture content was kept at 100, 75, 50, and 25% of field capacity (FC). Vertical bars represent LSD at probability 5%. Data was pooled over biotype and experimental runs (n=12).

Seed production is the major contributor in weed infestations as even a small number of seeds per plant can cause a major infestation in the subsequent crop season. In the current study, despite a high biomass reduction in *A. viridis* and *A. retroflexus*, both weeds produced a significant number of seeds per plant even at 25% FC. A similar reduction in seed production of *E. colona* and *A. rudis* has been reported as a result of increased water stress levels (Mahajan *et al.* 2019, Sarangi *et al.* 2016). It could be concluded that drought-tolerant species can sustain their reproductive growth even at a high-level of soil moisture stress. Despite high seed retention of *Amaranthus* species at crop maturity and a high potential for control of their seed by harvest weed seed control strategies, these weeds should be managed at the earliest stage due to high competition for resources and their large seed production in drought conditions (Sarangi *et al.* 2016, Schwartz *et al.* 2016, 2017).

In the present study, severe water stress after *A. viridis* and *A. retroflexus* establishment reduced the growth and seed production of these weeds, but both weeds completed their life cycle. Both biotypes of *A. viridis* and *A. retroflexus* from Gatton and Goondiwindi responded similarly to water stress. Similarly, Sarangi *et al.* (2016) observed no significant differences between growth and seed production of *A. rudis* biotypes under different water stress levels. Gioria and Pyšek (2017) and Bajwa *et al.* (2018) have claimed that different responses of weed biotypes to the environmental stress could be attributed to maternal conditions during plant development and genetic diversity between biotypes. In the current study, the effect of maternal conditions on seeds was removed by growing both biotypes in the same environment, and it could be concluded that a similar response of these biotypes to moisture stress was an innate trait.

Both weeds have an aggressive growth habit and high biomass production ability which impacts crop growth and yield due to severe competition for resources in their critical weed-free period (Horak and Loughin 2000). Patterson (1995) reported that water stress has a significant impact on the critical weed-free period in different crop species. Furthermore, it has been reported that post-emergence herbicide efficacy in *Amaranthus* species may be influenced by water stress conditions (Slabbert and Krüger 2011). Although our results showed that these weeds could be troublesome in water stress conditions, the competitiveness, fitness, and responses of these weeds to management strategies under drought conditions should be assessed. Therefore, the comprehensive knowledge of the response of these *Amaranthus* species under water-stress conditions is essential to develop integrated weed management tactics for these species.

CHAPTER 5: Effect of emergence time on growth and fecundity of *Amaranthus retroflexus* and *Amaranthus viridis:* emerging problem weeds of cotton systems in Australia

Publications included in this chapter

Asad MK, Mobli A, Jeff AW, Chauhan BS (2020) Effect of emergence time on growth and fecundity of *Amaranthus retroflexus* and *Amaranthus viridis:* emerging problem weeds of cotton systems in Australia. Weed Science (In Review)

Introduction

Amaranthus retroflexus and *Amaranthus viridis* are monoecious summer annuals of Amaranthaceae family. Both species are widely distributed temperate and warm temperate countries of the world, including countries in Africa, Asia, and Europe and United states (Holm *et al.* 1997, Uva *et al.* 1997, Waselkov and Olsen 2014). These species are also becoming emerging problematic weeds, which are infesting many cropping systems across the New South Wales, South Australia, and Tasmania regions of Australia (Manalil *et al.* 2017, Osten *et al.* 2007, Walker *et al.* 2005).

Amaranthus retroflexus and *A. viridis* are common C_4 weed species of cultivated land which causes losses to crop production globally because of their wide distribution, aggressive growth habits, and prolific seed production (Ward *et al.* 2013). A single *A. retroflexus* plant can produce up to 300,000 seeds while an *A. viridis* (self-fertile) can produce up to 7000 seed throughout the life cycle of the plant (Costea *et al.* 2004; Holm *et al.* 1997; McLachlan *et al.* 1995).The spread of seeds of these species mainly occurs by rain water, air, farm equipment, cotton gin trash, livestock and cover crop seeds (Farmer *et al.* 2017, Holm *et al.* 1997).

The high competitiveness of *A. retroflexus* and *A. viridis* for resources results in rapid growth and a significant yield reduction in several crops (Carvalho and Christoffoleti 2008, Rajagopalan *et al.* 1993). For example, the presence of 12 plants m^{-2} of *A. viridis* caused a significant reduction in leaf area (25%), leaf biomass (72%), and stem biomass (74%) of red pepper (*Capsicum baccatum*) (Barbasso *et al.* 2018). Bensch *et al.* (2003) reported that soybean (Glycine max) yield could reduce by 38% depending on *A. retroflexus* density and its emergence date. The over reliance of farmers on chemical weed management strategies in America has led to evolution of many resistant biotypes to acetolactate synthase and photosystem II inhibitor herbicides (Heap 2020). Although the presence of these species has been reported in Australia, there are no official reports on the level of infestation, economic losses, herbicide-resistant biotypes, and other eco-biological weed management information these species in Australia. Therefore, comprehensive studies on Australian biotypes of these weeds are required before they become problematic weeds across the Australia.

Weed density, weed interference duration, and weed emergence timing in comparison to the crop are major factors causing significant yield losses (Estorninos *et al.* 2005, Hussain *et al.* 2015, Lindström and Kokko 2002). Emergence time has significant effect on growth and fecundity of weeds. Previous research showed that potential peak growth and seed production of turnipweed (*Rapistrum rugosum*) and mustard (*Brassica tournefortii*) occurred when both weeds experienced a favourable environmental condition in terms of temperature, photoperiod (Mobli *et al.* 2020). It also observed that when the emergence of these weeds was delayed from April to July, their biomass and seed production was reduced by more than 75%. Weed phenology, periodic event of stages of weed lifecycle influenced by change in temperature and light, is the major contributor to weed-crop competition (Ghersa and Holt 1995). Temperature and photoperiod are the most influential factors impacting the phenological development of weeds (Hodges, 1991, Major and Kiniry 1991, Patterson 1992). The knowledge of weed phenology is very critical in accessing the weed growth, seed production; biomass production and level of competition with the crops (Grant 1989). Therefore, the studies on growth and seed production of weeds sown at different time may provide primary information for more effective management strategies. The main objectives of this study were toi evaluate of the growth and seed production of two biotypes of *A. retroflexus* and *A. viridis* in response to different panting dates.

Material and Methods

Weed seeds details

Two biotypes of *A. retroflexus* and *A. viridis* were collected in 2016 from Goondiwindi (28.41°S, 150.23°E; altitude 210 m) and Gatton (27.45°S, 152.21°E, altitude 90 m), located in the northern region of Queensland, Australia. The maternal conditions effect on collected seeds from the different regions was taken out with re-growing both biotypes of these weeds at Gatton, and the matured seed from these plants was used in the study (Mobli *et al.* 2019a). Seed of both weeds had good germination, and no seed breaking dormancy treatment was used.

Experimentation

An outdoor pot experiment was conducted at the research facility of the University of Queensland, Gatton, during the summer seasons of 2017 to evaluate the effects of planting date on two biotypes of *A. retroflexus* and *A. viridis*. The mean thermal fluctuation and daylight hours of research site are provided in Figure 14.

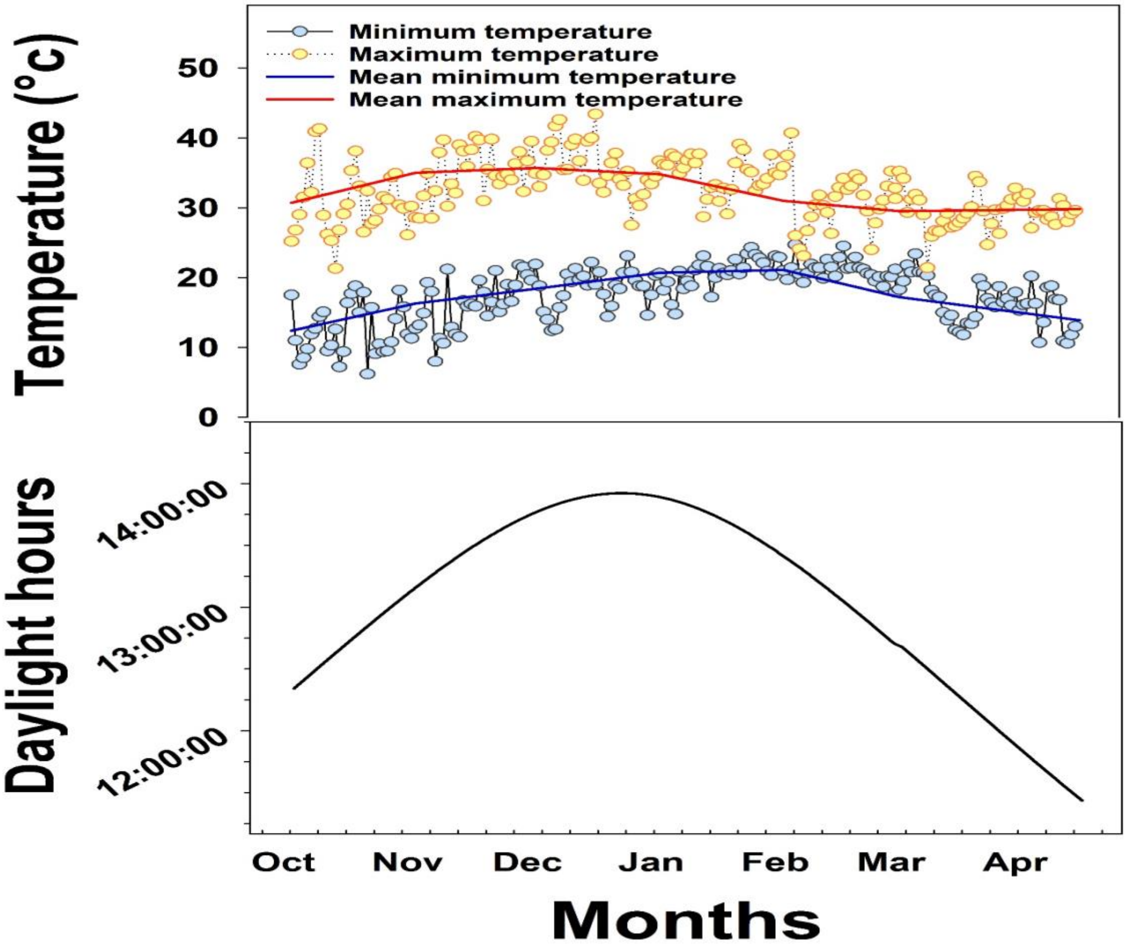


Figure 5.1 The mean thermal fluctuation and daylight hours of research site (Gatton) during growth period in 2017-2018.

Seeds were sown on 3rd day of every month from October to January (four planting date of summer-cotton crop production season) in the trays containing the commercial Platinum Potting Mix (Centenary Landscaping, NSW, Australia). The potting mix contained biological organic-based products and had a pH of 5.6 and an electrical conductivity of 1.6 dS m^{-1}. When seedlings reached at the 5-leaf stage (4 to 6 cm height), the transplantation of uniform seedlings were made in free-draining plastic pots of size 30 cm in diameter and 40 cm in height. The pots were filled with clay loam soil having a pH of 6.7 and electrical conductivity of 0.14 dS m^{-1} and organic matter content of 2.8%. All the pots were supplied with adequate water through drip irrigation throughout the life cycle of plants. The research site was kept weed-free by hand-weeding. Plant height and number of leaves per plant were recorded at 14 days interval until plant maturity. Plant height was measured from shoot apex to tip of the shoot of reproductive structure. In each planting date, when plants were fully matured, and seed production has ceased, plants were harvested. Shoot and root dry biomass, number inflorescences per plant, and seed production were recorded at harvest. The shoot and root parts (washed to remove the soil particles) were bagged separately and oven-dried for 4 days at 70^{o}C. The number of seeds was calculated by taking the weight of 100 seeds from each plant and expanding it to total plant seeds. For each planting date, plants development was recorded by calculating cumulative growing degree days (GDDs) equation 1. The equation was of the form:

$$GDD_{10} = \sum(((\text{Maximum daily temperature} + \text{Minimum daily temperature})/2) -10) \quad [\text{ Equation 1}]$$

The number of days or GDDs required for 50% of flowering was considered as a time of 50% flowering. The experiment was repeated in the summer of 2018.

Data Analysis

The experiment (factorial arrangement of species × biotypes × planting date) was conducted in a randomized complete block design. In each planting date, ten replicates for each population were considered (40 plants for each population for total four planting dates). The Shapiro-Wilk and Breusch-Pagan test were performed to checking the normality and homogeneity of data, and original data was used for ANOVA (GENSTAT 16th edition; VSN International, Hemel Hempstead, United Kingdom). Data was pooled over the experimental runs as no significant differences was observed. When no significant difference was observed between biotypes or species, data was pooled. The Fisher's protected Least Significant Differences (LSD) mean comparison test at probability 0.05 was used. A three-parameter sigmoidal model was fitted to plant height and number of leaves data when grown at different planting date (Equation 2) using SigmaPlot 14 (Systat Software, San Jose, CA, United States). The equation was of the form:

$$F = \frac{X_{max}}{1 + e^{\frac{-(X - X_{50})}{b}}} \qquad \text{(equation 2)}$$

F is plant height or number of leaves per plant at GDD X, X_{max} is the maximum plant height or number of leaves per plant; X_{50} is the GDDs required to reach a 50% plant height or number of leaves per plant and b is the slope.

Results and Discussion

Effect of planting date on *A. retroflexus* and *A. viridis* flowering and growth period

The planting date had significant ($P<0.001$) effects on the required number of days to plant maturity and flowering of both weeds (Table 5.1).

Table 5.1 The effect of planting date on *A. retroflexus* and *A. viridis* flowering and growth period.

Planting date	Days after sowing	
	Flowering	Growth period
October	50 a	122 a
November	39 b	94 b
December	29 c	74 c
January	27 c	72 c
6.9LSD (0.05)	5.6	6.9
	Growing degree days	
October	653 a	1482
November	639 a	1492
December	494 b	1454
January	477 b	1488
LSD (0.05)	53.4	Ns

Data were pooled over the biotype, species, and experimental runs (n=80)
NS= non-significant

At each planting date, although both weeds matured at the same amount of GDDs, these weeds required a different number of days to complete their life cycle. The growth period and flowering of both weeds when planted in October was occurred at 122 and 50 days after planting, respectively. When planted in January the growth period and flowering was reduced to 27 and 72 days, respectively. Similarly, the amount of GDDs required for flowering reduced from 653 to 477 when these weeds planted in January. No significant differences in GDDs and the number of days required for growth period and flowering was observed when planted in December or January.

Emergence date strongly associated with growth and fecundity of weeds (Bosnic and Swanton 1997, Spaunhorst *et al.* 2018, Willenborg *et al.* 2005). In the current study was observed that the growth period and flowering duration of both species were reduced with a delay in the time of plantation. When the plantation was delayed, these species experienced lower temperatures and shorter daylight hours, which resulted in shortening the growth period and sooner flowering in comparison with the October planting date. A similar response of temperature and daylight hours (photoperiod) was also observed in Palmer amaranth (*Amaranthus palmeri*) and common waterhemp (*Amaranthus rudis*) (Spaunhorst *et al.* 2018, Wu and Owen 2014). In many weeds, temperature and photoperiod are the most influential factor in phenological stages (Hatfield *et al.* 2011, Hatfield and Prueger 2015). In the present study January plantation observed flowering sooner as it experiences shorter daylight hours and the growth period was significantly reduced. It has been reported that rapid flowering and shortening of the growth period is associated with photoperiod, and both these species are classified as a short-day species (Huang *et al.* 2000).

Effect of planting date on *A. retroflexus* and *A. viridis* height

The interaction of population and planting date had a significant ($P<0.001$) effect on the plant height of both species (Figure 5.2 and Table 5.2).

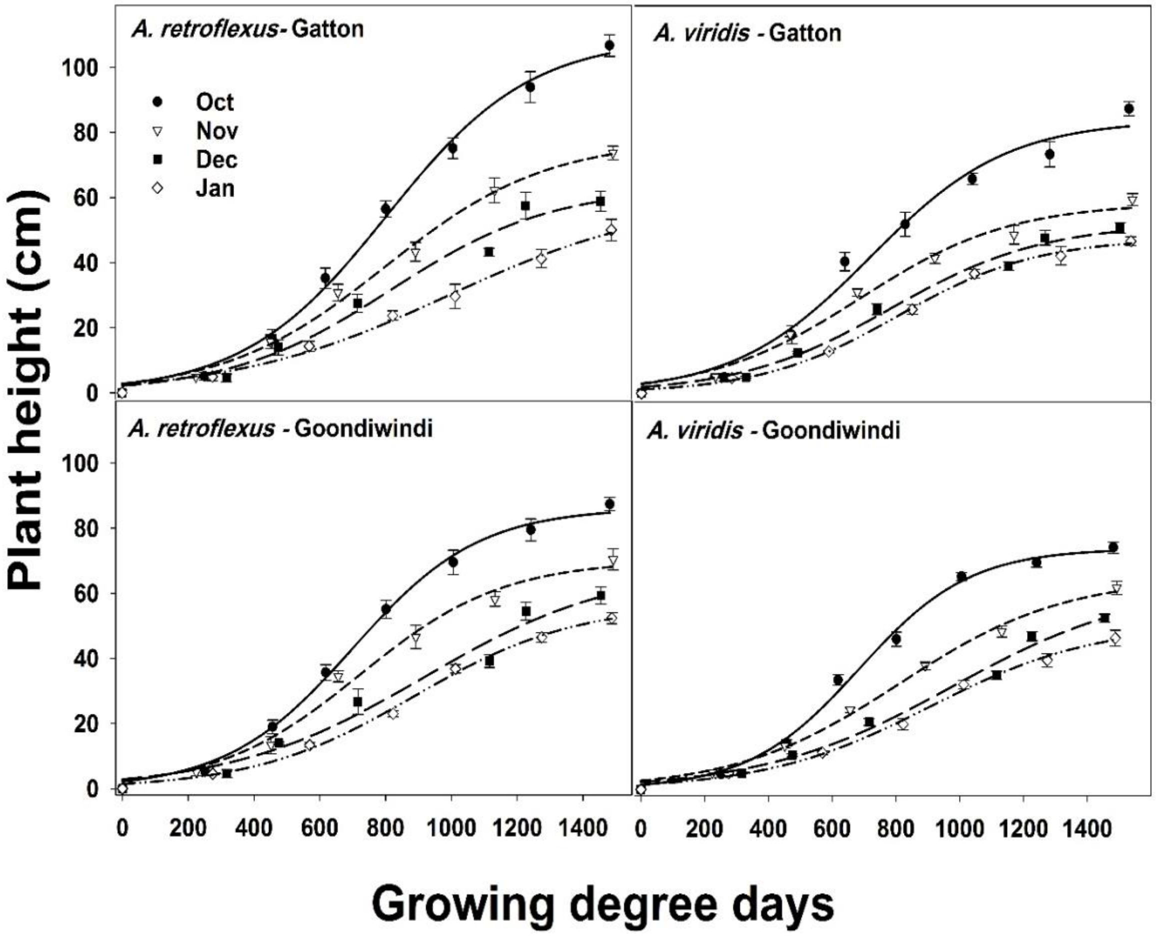


Figure 5.2 The effect of planting date on *A. retroflexus* and *A. viridis* height. Data were pooled over the experimental runs. Vertical bars show standard error of mean. Estimated parameters are presented in Table 5.2

When both species were planted in October, they grow taller in comparison with other planting dates, and a delay in planting date resulted in a reduction of plant height of both species. When Gatton and Goondiwindi biotypes of *A. retroflexus* were planted in October, their mean plant height was 106.7 and 87.4 cm. When planted in January, the plant height was reduced to 50 and 52 cm for the Gatton and Goondiwindi biotypes, respectively. Similarly, the plant height of Gatton and Goondiwindi biotypes of *A. viridis* was reduced from 87.4 to 46.7 cm and 74.3 to 46.5 cm, when it was planted in January, respectively. A three-parameter sigmoid model estimated that Gatton and Goondiwindi biotypes of *A. retroflexus* required 801 to 990 GDDs and 700 to 916 GDDs for producing 50% of their maximum plant height (X_{50} parameter) in different planting dates, respectively. Similarly, the

Gatton and Goondiwindi biotypes of *A. viridis* required 665 to 780 GDDs and 684 to 978 GDDs, in different planting dates to reach to 50% of their maximum plant height, respectively.

Table 5.2 A three-parameter sigmoidal model fitted to plant height of *A. retroflexus* and *A. viridis* when grown at different emerging date.

Species- biotypes-planting date			Model parameters			
			$Xmax$	b	X_{50}	R^2
			Plant height (cm)			
Amaranthus retroflexus	Gatton	October	109.1 (4.2)	218.3 (20.4)	805.3 (28.0)	0.99
		November	77.7 (4.1)	244.8 (28.4)	801.0 (41.3)	0.99
		December	63.2 (7.6)	245.3 (61.3)	816.4 (101.2)	0.98
		January	59.6 (6.8)	323.0 (50.0)	990.0 (95.0)	0.98
	Goondiwindi					
		October	86.2 (2.3)	192.0 (16.1)	699.9 (9.7)	0.99
		November	70.2 (4.2)	216.6 (34.4)	722.3 (46.1)	0.99
		December	68.4 (13.7)	293.5 (83.8)	916.8 (166.6)	0.97
		January	56.7 (2.7)	245.2 (24.5)	866.2 (34.61)	0.99
Amaranthus viridis	Gatton	October	83.9 (5.2)	207.9 (37.7)	696.5 (46.7)	0.98
		November	58.5 (3.7)	229.1 (38.4)	665.6 (50.3)	0.99
		December	52.2 (4.2)	232.0 (46.3)	758.7 (68.8)	0.99
		January	47.6 (1.3)	211.7 (17.1)	780.3 (21.0)	0.97
	Goondiwindi					
		October	74.00 (2.2)	173.6 (3.1)	684.3 (20.8)	0.99
		November	65.2 (3.3)	258.4 (26.9)	812.2 (40.4)	0.99
		December	63.4 (10.2)	298.2 (59.8)	978.5 (128.4)	0.98
		January	50.9 (3.2)	259.19 (31.7)	906.5 (47.4)	0.99

Values presented in the parentheses are standard error of means (±SE).
$F = X_{max} /[1+ exp (-(X-X_{50}/ b)]$, F is plant height at GDD X, X_{max} is the maximum plant height; X_{50} is the GDDs required to reach a 50% plant height and b is the slope.

In the current study, species planted in October grows taller than those planted late in the growing season. This observation could be attributed to temperature, daylight hours, and growth period. Spaunhorst et al (2018) reported that the plant height of *A. palmeri* was reduced significantly as a result of the late emergence of this weed and they claimed that late emerged seedlings are less competitive.

Effect of planting date *on A. retroflexus* and *A. viridis* leaf number

The interaction effect of population and planting date was significant (P<0.001) on the number of leaves per plant of both species (Figure 5.3 and Table 5.3).

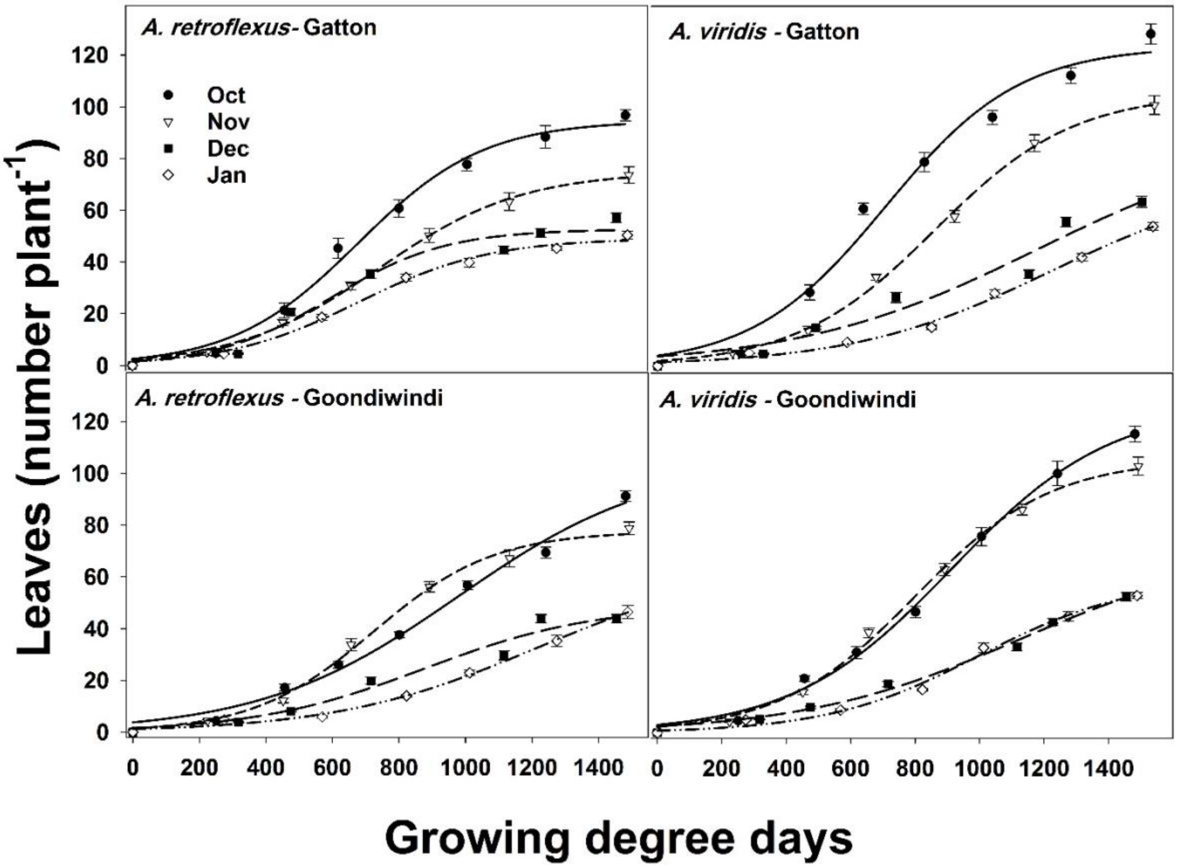


Figure 5.3 The effect of planting date on *A. retroflexus* and *A. viridis* number of leaves. Data were pooled over the experimental runs. Vertical bars show standard error of mean. Estimated parameters are presented in Table 5.3

The highest number of leaves of both species was produced in the October planting date, and the number of leaves of both species was reduced by a delay in planting date. When Gatton and Goondiwindi biotypes of *A. retroflexus* were planted in January, the number of leaves per plant was reduced by 48% and 49%, in comparison with the produced number of leaves in October, respectively. Similarly, the number of leaves of Gatton and Goondiwindi biotypes of *A. viridis* was reduced by 58% and 54%, respectively, as a result of a delay in planting date from October to January. A three-parameter sigmoid model showed that Gatton and Goondiwindi biotypes of *A. retroflexus* required 598 to 680 GDDs and 723 to 1216 GDDs for producing 50% of their leaves (X_{50} parameter) in different planting dates, respectively. Similarly, the Gatton and Goondiwindi biotypes of *A. viridis* required 658 to 1183 GDDs and 801 to 1120 GDDs, on different planting dates to produce 50% of their maximum leaves per plant, respectively.

Table 5.3 A three-parameter sigmoidal model fitted to number of leaves of *Amaranthus retroflexus* and *Amaranthus viridis* when grown at different emerging date.

Species- biotypes-planting date			Model parameters			
			Xmax	*b*	X_{50}	R^2
			Leaves (number plant^{-1})			
Amaranthus retroflexus	Gatton	October	94.6 (3.7)	188.9 (20.4)	678.4 (28.5)	0.99
		November	74.9 (2.0)	215.2 (28.4)	739.4 (19.9)	0.99
		December	52.7 (3.4)	172.0 (61.3)	598.2 (56.5)	0.97
		January	49.1 (1.8)	197.9 (50.0)	680.3 (29.5)	0.99
	Goondiwindi					
		October	105.2 (11.2)	297.4 (43.5)	978.5 (84.3)	0.99
		November	77.7 (2.7)	181.2 (18.7)	723.0 (24.2)	0.99
		December	49.2 (9.4)	258.2 (82.1)	786.6 (149.0)	0.97
		January	65.4 (9.1)	305.4 (38.0)	1216.2 (98.2)	0.99
Amaranthus viridis	Gatton	October	123.6 (7.3)	201.1 (36.4)	685.8 (44.4)	0.98
		November	105.4 (2.9)	209.3 (14.2)	833.6 (19.9)	0.99
		December	90.8 (13.0)	363.5 (38.8)	1145.9 (391.5)	0.96
		January	73.4 (10.3)	300.7 (40.9)	1183.0 (100.2)	0.99
	Goondiwindi					
		October	127.3 (5.9)	247.3 (20.9)	914.1 (33.9)	0.99
		November	105.8 (3.3)	210.9 (16.5)	801.1 (22.9)	0.99
		December	71.9 (13.3)	339.1 (55.2)	1120.6 (147.2)	0.99
		January	59.8 (5.1)	237.2 (36.5)	998.7 (57.9)	0.99

Values presented in the parentheses are standard error of means (±SE).
$F = X_{max} /[1+ \exp (-(X-X_{50}/ b)]$, *F* is number of leaves at GDD *X*, X_{max} is the maximum number of leaves; X_{50} is the GDDs required to reach a 50% number of leaves and *b* is the slope.

Similar to the plant height, the number of leaves per plant reduced with a delay in planting date. Huang *et al.* (2000) reported that the number of leaves of the main stem of *A. retroflexus* was significantly reduced as daylight hours decreased. In the current study, significant differences were observed between the biotypes of both species in plant height and number of leaves per plant. The differences in the response of weed species to environmental conditions are attributed to genetic diversity and maternal plant conditions during plant growth and seed production (Bajwa *et al.* 2018, Mobli *et al.* 2019b). In the present study, the effect of maternal conditions was remove by re-growing both population at the same condition in Gatton. Therefore, it could be concluded that observed differences is attributed to genetic diversity between these biotypes. Spaunhorst *et al.* (2018) reported that *A. palmeri* biotypes that originated from different locations exhibited different biological characteristics, and the environmental plasticity of this species contributes to their survival and further distribution in new locations.

Effect of planting date on *A. retroflexus* and *A. viridis* shoot and root biomass

The effect of the population was significant (P<0.001) on the *A. retroflexus* shoot biomass and *A. viridis* root biomass (Figure 5.4).

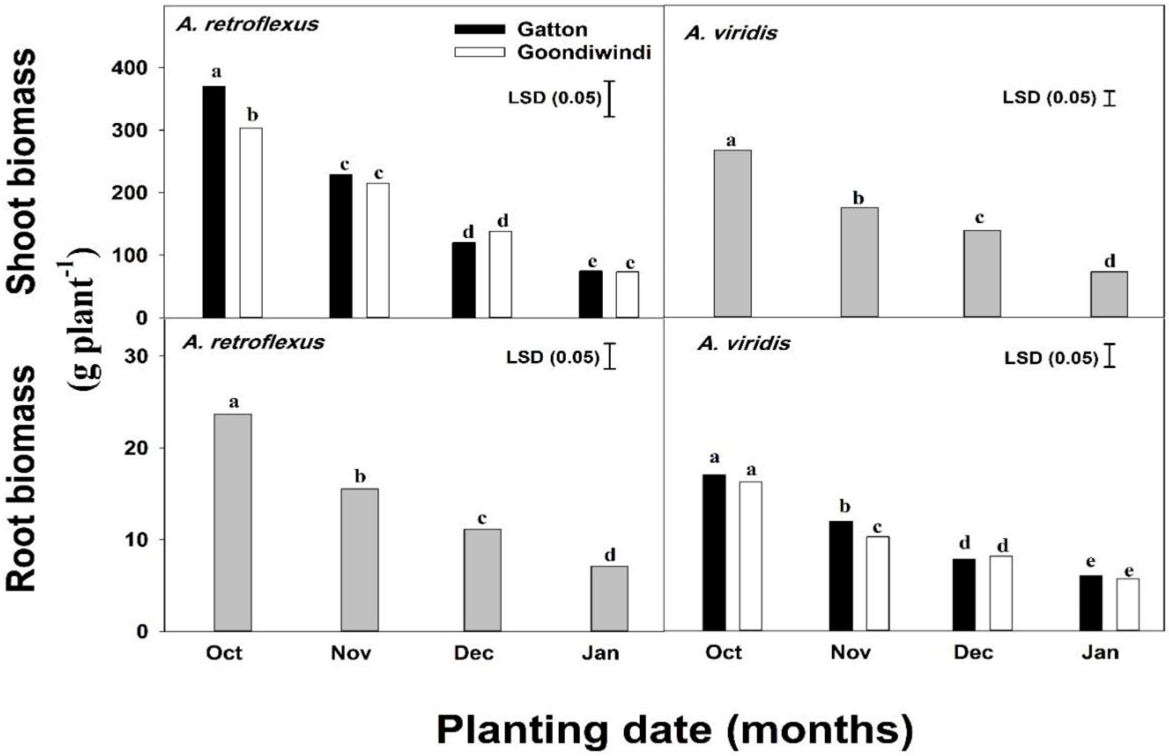


Figure 5.4 Effect of planting date on *A. retroflexus* and *A. viridis* shoot and root biomass. Data were pooled over the experimental runs and biotype. Vertical bars are least significant difference (LSD) values at the 5% level of probability and letters above bars show group differences between means.

Delayed in planting time resulted in shoot and root biomass reduction of both species, and the reduction was highest in January planting. Compered to planting in October the shoot biomass of Gatton and Goondiwindi biotypes of *A. retroflexus* was reduced by 80% and 76% in planting date January, respectively. Similarly, the shoot biomass of *A. viridis* was reduced by 73% as result of delay the planting time from October to January. When *A. retroflexus* was planted in October, the root biomass was reduced by 70%, in comparison with planting in April. Although Gatton and Goondiwindi biotypes of *A. viridis* produced different root biomass in November planting, the root biomass of both biotypes were reduced by 65% as a result of a delay in planting from October to January.

In the current study, shoot and root biomass of both species was sharply reduced, followed by a reduction of plant height and the number of leaves as a result of a delay in planting date. Heneghan and Johnson (2017) reported that when waterhemp (*Amaranthus tuberculatus*) was emerged in May, the mean biomass could reach to 1120g per plant, but when the planting date was delayed to July, the biomass was reduced to 266 g per plant.

Weed competitiveness is highly associated with weed biomass and their phenological characteristics (Huang *et al.* 2001). The prediction of phenological stages and potential competitiveness of weed in different conditions is an integral part of integrated weed management strategies (Ghersa and Holt, 1995, Deen *et al.* 1998).

<u>Effect of planting date on *A. retroflexus* and *A. viridis* number of influences and seed production</u>

Although the interaction effect of biotypes and emergence time had a significant (P<0.001) effect on the number of inflorescences of both species, the effect of biotypes on seed production was not significant (Figure 5.6).

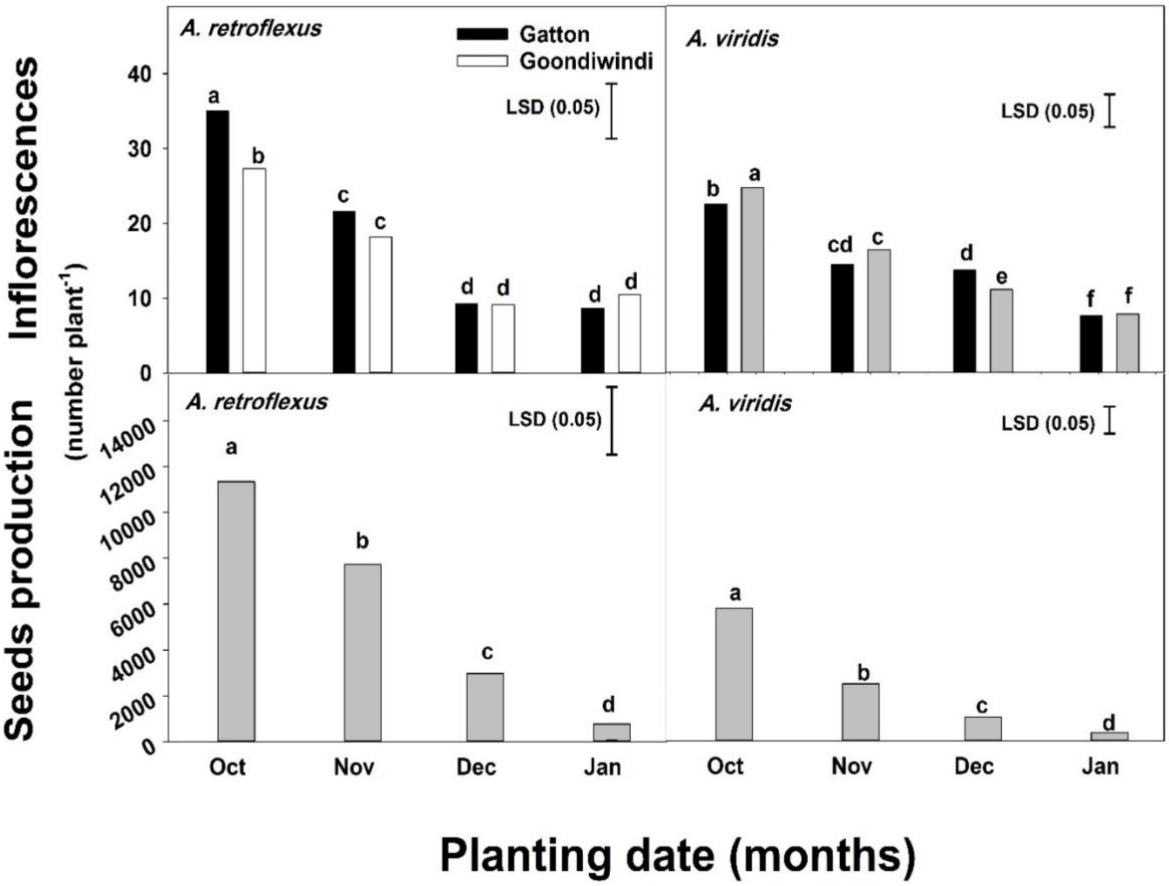


Figure 5.6 The effect of planting date on *A. retroflexus* and *A. viridis* number of inflorescence and seed production. Data were pooled over the experimental runs and biotype. Vertical bars are least significant difference (LSD) values at the 5% level of probability and letters above bars show group differences between means.

The number of inflorescences and seed production of both species was reduced by a delay in emergence time, and the reduction was highest in planting date January. When Gatton and Goondiwindi biotypes of *A. retroflexus* were planted in January, compared to produce the number of inflorescences in October, it reduced by 75% and 62%, respectively. Similarly, the number of inflorescences of Gatton and Goondiwindi biotypes of *A. viridis* also was reduced by 66% and 68% respectively, in comparison with October planting. In October planting time, *A. retroflexus* and *A. viridis* were produced 11350 and 5780 seed per plant, but the number of seed was reduced to 770 and 365 in planting date January, respectively.

It was observed in in current study that both species could germinate and completed their life cycle throughout the summer growing season (October to April). Although the growth and fecundity of these species depend on emergence time, however, these weeds could emerge throughout the summer growing season under irrigation or sufficient rainfall and produce a significant number of seeds. A similar result was observed for *A. palmeri*, and seed production was reduced as a result of late emergence (Spaunhorst *et al.* 2018). Heneghan and Johnson (2017) reported that when the emergence of *A. tuberculatus* was delayed from May to July, the seed production was reduced by 70%, but late-emerged plants of this weed could produce more than 276000 seeds per plant. It could be concluded that the weed seed control of these weeds plays an important role in their management as late cohorts of these species could produce a significant number of seeds and enrich the soil seed bank. Although it has been reported that weed seed harvest control strategies are effective method for managing *Amaranthus* species (Norsworthy *et al.* 2016, Schwartz *et al.* 2016), but late emerged plants of these weeds grow shorter and may escape from the control. Therefore, more studies and consideration should be taken on the control of late cohorts of these species.

CHAPTER 6: General Discussion

In Australian agro-ecosystems, *Amaranthus retroflexus* and *Amaranthus viridis* are two emerging problematic weeds as both can reduce the crops' productivity (Manalil *et al.* 2017, Osten *et al.* 2007, Walker *et al.* 2005), thereby impacting the welfare of both human beings as well as livestock. Studies were conducted on several aspects of *A. retroflexus* and *A. viridis* biology with a very specific aim of building understanding of germination, growth and seed bank dynamics of *A. retroflexus* and *A. viridis*. The key findings are highlighted in collocation with their implications for the understanding of the behavior of this weed as well as their management. The project also discusses the association between various aspects of their biology and their success when it comes to invasion.

Germination ecology and seedbank persistence

When it comes to invasion success, the germination ecology of *A. retroflexus* and *A. viridis* plays an integral role. According to several studies, *A. retroflexus* and *A. viridis* weeds are prolific producers of seeds and their seeds demonstrate very high germinability and dispersal ability (Stevens 1957, Ikenaga *et al.* 1975a).The current study has established that these species can foster in a variety of environmental conditions because of their superior germinability. This advance ability to germinate under an array of climatic and geographic conditions plays a major role in the successful introduction and establishment of these species in alien and diverse environmental conditions. Germination and emergence of *A. retroflexus* and *A. viridis* from seedbank depends largely depends on the availability of favorable temperature and light (Chauhan and Johnson 2010). The germination of *A. retroflexus* and *A. viridis* was found highly favorable at 25/35ºC and 20/30ºC, respectively. However, we observed substantial germination on 10/20ºC and 15/25ºC. The geographic as well as temporal constraints to invasion have also been conquered by these weeds due to their ability to germinate in a wide range of temperatures. This temperature range prevails in most of the summer season in the cotton growing areas of New South Wales (NSW) and Queensland (QLD) which allows *A.*

retroflexus and *A. viridis* emerge in flushes. The duration and scale of emergence largely depends on availability of moisture through rainfall distribution during the season.

Even under moisture stress, *A. retroflexus* and *A. viridis*, seeds can germinate, demonstrating how the species successfully takes over barren lands with inadequate moisture levels. Although the germination of these species may be inhibited under high moisture stress. However, the abundant summer rainfall in cotton-growing regions of Australia (NSW and QLD) helps these species to germinate in flushes along with the summer rainfall events.

Similarly, seed longevity studies have showcased the significance of seed burial in establishing the seedbank persistence of *A. retroflexus* and *A. viridis*. In both species, regardless of the spatial position of seeds, the viability of the seeds was reduced over time and was lowest at 24-months after seed placement. At 24-months after seed placement, seed depletion of *A. retroflexus* ranged from 75% (10 cm depth) to 94% (soil surface). In this experiment of seed burial a decline in persistence of seeds was observed over time at a constant rate. After 2-month storage, the germination of *A. retroflexus* and *A. viridis* reached 93% and 86%, respectively. After 2-months, a low variation (less than 5%) in seed dormancy was observed for both species. The results showed that both *Amaranthus* species exhibited primary dormancy, which eased with an after-ripening period.

Moisture Stress

Under no moisture stress conditions (100% of field capacity; FC), *A. retroflexus* and *A. viridis* demonstrated optimal weed growth and reproductive output. Therefore, these species are best established under tropical environments. But both weeds also can tolerate moderate drought (50% of FC). It is evident that the plants are equipped with this tolerance ability due to their resilient morphology and efficient growth characteristics. Further, for both weeds, it was observed in drought conditions, plants remained in vegetative growth stage for a long period prior to flowering. It is also believed that *A. retroflexus* and *A. viridis* undergo a C_4 photosynthesis mechanism, thereby enabling them to grow efficiently (Moore *et al.* 1987). At 25% of FC, the shoot: root biomass ratio of both species was reduced by 67% in comparison with 100% of FC. Both species responded to drought conditions by reducing of aboveground vegetation to avoid shoot dehydration and to increase water use efficiency. *A. retroflexus* and *A. viridis* weeds can also complete their life cycles even under drought conditions and are also capable of producing substantial amounts of viable seeds. Therefore, by observing these changes in reproductive biology, it can be presumed that seed production and dormancy in these species can also be adaptable in cases of future drought conditions. The ability of *A. retroflexus* and *A. viridis* weeds to establish themselves under diverse conditions is a result of their potential to establish under varied soil moisture levels as well as to tolerate substantial drought.

Growth and fecundity in response to emergence

Throughout the period of study (October to January, 2018-2019), *A. retroflexus and A. viridis* showed constant germination under field conditions. When the weeds were planted in October, optimal growth and reproductive potential of these weeds was observed; the weeds were taller and developed abundant number of leaves, biomass and seeds when compared to those planted on other planting dates. A decrease in daylight hours and temperature led to flowering, thereby demonstrating the association between parameters of temperature and photoperiod, and the phenological stage, thereby leading to a shorter vegetative phase. Because of rapid flowering due to the delay in planting, the growth period was decreased, and for both weeds, the growth and seed production were consequently reduced. To reduce weed control expenses, *A. retroflexus* and *A. viridis* must be controlled in early season, since weeds that grow early in the season tend to require substantial amount of resources and effort to control them. While it has been established, a low number of seeds are produced by late cohorts of the weeds, one must not sidestep the control of late-emerged weed plants, if they aim to attain proper long-term weed management.

CONCLUSIONS AND FUTURE RESEARCH DIRECTIONS

In the above discussion, it is evident that *A. retroflexus* and *A. viridis* are invasive in several countries around the globe and their spread is difficult to control. Information obtained from this study pertaining to the seed longevity, dormancy of seeds, seed production, germination, emergence rate, growth and phenology of both the weeds help fill in some of the information gaps regarding these in Australia. But it is important to state that there are many aspects of their biology that remain unknown. One such parameter is the reproductive biology (i.e. methods of pollen dispersal and fertilisation) of the various biotypes of the weeds prevalent in Australia.

This study further evidenced that although in cropping situations, mechanical and chemical control of *A. retroflexus* and *A. viridis* is effective, such methods do not perform well in frequently invaded areas as well as areas deemed to be of conservation value. In habitats such as these, these weeds has the capability to establish themselves again from the large and persistent seed bank, following disturbance or even immigration from seed sources present close by. It is important to biologically control these weeds, as it mitigates the size of the soil seed bank as well as their performance, in order to supress it in areas that experience a high frequency of disturbance, including regions that experience frequent floods. But there is a need to conduct an extensive study on this process.

Research has also established that during the process of evaluation of the potential weed threat in a region, it is important to take into account the existence of multiple biotypes. In this study, it was established that when different biotypes of a species demonstrating insignificant morphological differences are introduced in a foreign environment, they demonstrate various degrees of weediness. Therefore, although various species of weed are present in Australia, it is important to be vigilant and prevent even more introductions of the same species in the soil. That is because it could enhance the genetic variability in the population, while also introducing an array of aggressive biotypes.

Amaranthus retroflexus and *A. viridis* continue to pose challenges to several countries, with their invasive behaviour. The current studies have suggested that the species in question demonstrate productive biology, as they can evolve at a quick pace thereby making them very invasive. Although it is possible to manage these weeds, efforts must be directed towards various aspects such as raising social awareness, working on quarantine and preventive measures, paying attention towards policy-making and lastly, enabling integrated weed management

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