PARAQUAT AND GLYPHOSATE RESISTANCE IN *CONYZA BONARIENSIS* IN THE WESTERN CAPE IN THE REPUBLIC OF SOUTH AFRICA

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DECLARATION

I, the undersigned, do hereby declare that the work contained in this thesis is my own original work and has not previously in its entirety or in part been submitted at any university for a degree.

H. de Wet

Abstract

Conyza bonariensis (flaxleaf fleabane) was reported for the first time, as a weed in California in 1893—1896. The first report of the occurrence of this weed in South Africa was made in May 1895 in Franschoek, South Africa. Paraquat resistance in *C. bonariensis* was first reported in the 1970s and early 1980s when resistance was observed from vineyards and citrus plantations in Egypt. More recently a report of herbicide resistance in South Africa was made in January 2003 when resistance occurred in *C. bonariensis* in the Breede Valley, South Africa. The resistance was to glyphosate, but recently reports of resistance to glyphosate and paraquat were received.

C. bonariensis seeds were found to be positively photoblastic and germinated only under unfiltered white light and red light whilst no germination occurred under far-red light and in the dark. The optimum temperature range for *C. bonariensis* seed was found to be between 15 and 30°C, with no germination occurring at 0-5°C and at 35-40°C. Optimum germination occurred at the soil surface. No germination occurred at depths of 2 cm and deeper. Although the optimum temperature range was found to be the same for the different biotypes tested. However, germination was highest in the susceptible biotype.

Since farmers first reported paraquat and glyphosate resistance in *C. bonariensis* in the Breede Valley, South Africa, reports of resistance increase every year. Seed collected from populations suspected of being resistant to paraquat and glyphosate were obtained from the Breede Valley and screened for resistance. To determine the easiest, quickest, and most effective method to screen for paraquat and glyphosate resistance, two tests were evaluated viz. the petri dish assay method and the whole-plant dose-response method. Both screening methods identified paraquat and glyphosate resistant biotypes. The petri dish assay method was found to be a more rapid method of screening for resistance in *C. bonariensis*. During this study it was found that both paraquat and glyphosate resistance does occur in the Breede Valley.

The effect of growth stage on the level of herbicide resistance in *C. bonariensis* was tested. Herbicides other than paraquat and glyphosate were tested to determine if they could be used to control resistant *C. bonariensis* seedlings. The alternative herbicides tested included MCPA and Sorgomil Gold 600. The four herbicides were sprayed at different leaf stages. During the study it was found that growth stage does play an important role in the level of herbicide resistance. It was found that the control of different herbicides decreased with an increase in growth stage. The different herbicides showed varying levels of control depending on growth stage and resistant profile. Overall MCPA gave the best control at all leaf stages tested. What is gratifying was the finding that every biotype tested could be controlled by at least one of the treatments applied. This means that the producer using the most appropriate herbicide applied at the optimum application stage will be able to control most if not all the resistant populations of *C. bonariensis* that occur in the Western Cape.

Uittreksel

Conyza bonariensis (Kleinskraalhans) is vir die eerste keer as 'n onkruid gerapporteer in Kalifornia in 1893-1896. Die eerste waarneming van hierdie onkruid in Suid-Afrika is gemaak in Mei 1895 in Franschoek. Parakwat weerstandbiedendheid in *C. bonariensis* is die eerste maal in die 1970s en vroeë 1980s waargeneem, toe weerstandbiedendheid opgemerk is in wingerde en sitrus plantasies in Egipte. Meer onlangs is 'n geval van onkruiddoder weerstandbiedendheid in Suid-Afrika aangemeld in Januarie 2003, toe 'n biotipe van *C. bonariensis* in die Breede Vallei weerstand-biedendheid getoon het teen 'n onkruiddoder. Die weerstand was teen glifosaat, maar onlangse berigte van weerstandbiedendheid teen glifosaat sowel as parakwat is ontvang.

Daar is gevind dat die saadjies van *C. bonariensis* positief fotoblasties is en slegs ontkiem onder ongefiltreerde wit- en rooi lig, terwyl geen ontkieming voorkom onder ver-rooi lig en in die donker nie. Die optimum temperatuurreeks vir *C. bonariensis* saad is tussen 15 en 30°C, met geen ontkieming wat by 0-5°C en by 35-40°C voorkom nie. Optimum ontkieming kom voor op die grondoppervlak. Geen ontkieming kom by dieptes van 2 cm of dieper voor nie. Alhoewel die optimum temperatuurreeks dieselfde is vir die verskillende biotipes wat getoets is, is daar tog 'n verskil in die persentasie ontkieming tussen die biotipes met die beste ontieming by die sensitiewe biotipe.

Sedert boere die eerste geval van parakwat en glifosaat weerstandbiedendheid in *C. bonariensis* in die Breede Vallei, Suid-Afrika gerapporteer het, word meer gevalle van weerstandbiedendheid jaarliks aangemeld. Saad van populasies wat vermoedelik parakwat en glifosaat weerstandbiedend is, is in die Breede Vallei versamel en getoets vir weerstandbiedendheid. Om die maklikste, vinnigste en mees effektiewe metode van weerstandbiedendheidstoetsing te vind, is twee verskillende metodes van toetsing, naamlik die petribakkietoets en die heel plant dosis respons metode gebruik. Beide metodes van toetsing het parakwat en glifosaat weerstandbiedende biotipes geïdentifiseer. Daar is gevind dat die petri bakkie metode 'n vinniger manier van

toetsing vir weerstandbiedendheid is. Die studie het ook bewys dat parakwat en glifosaat weerstandbiedendheid wel in die Breede Vallei, Suid-Afrika voorkom.

Die effek van groeistadium op die vlak van onkruiddoder weerstandbiedendheid in *C. bonariensis* is ook tydens die studie getoets. Ander onkruiddoders buiten parakwat en glifosaat is getoets om te bepaal of hulle gebruik kan word vir die effektiewe beheer van weerstandbiedende *C. bonariensis* saailinge. Die alternatiewe onkruiddoders wat getoets is, was MCPA en Sorgomil Gold 600. Die vier onkruiddoders is gespuit by verskillende blaarstadiums. Gedurende die studie is daar gevind dat groeistadium wel 'n belangrike rol speel in die vlak van onkruiddoder weerstandbiedendheid. Die persentasie beheer van verskillende onkruiddoders neem af met 'n toename in die groeistadium. Die verskillende onkruiddoders se beheer het gewissel afhangend van weerstandbiedendheid en groeistadium. MCPA het die beste beheer by alle blaarstadiums wat getoets is getoon. Daar is ook gevind dat een of die ander van die onkruiddoders wat getoets is, gebruik kan word vir die suksesvolle beheer van onkruiddoder weerstandbiedendheid in elke biotipe wat getoets is. Dit beteken dat 'n produsent wat die korrekte onkruiddoder op die korrekte groeistadium toedien, in staat sal wees om die meeste, indien nie alle weestandbiedende *C. bonariensis* populasies wat in die Wes Kaap voorkom, te beheer.

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INTRODUCTION

Conyza bonariensis (flaxleaf fleabane) is a cosmopolitan annual weed that occurs throughout the world. It is believed that the weed is indigenous to South America and Eastern North America. *C. bonariensis* was reported for the first time, as a weed, in California in 1893-1896. It was reported in another part of Southern California in 1940. Natural populations of *C. bonariensis* occur in all the Canal Islands, except San Miguel and were reported in most states west of the Sierra Nevada (Wilken & Hannah, 1998). The first report of the occurrence of this weed in South Africa was made in May in 1895 in Franschoek, South Africa (Roux, May 2005, Kirstenbosch Herbarium, pers. comm.).

Since the first reports of the occurrence of *C. canadensis* as a weed in the world, this weed had developed resistance to paraquat and glyphosate throughout the world. Paraquat resistance in *C. bonariensis* was first reported in the late 1970s and early 1980s when resistance were observed from vineyards and citrus plantations in Egypt. Resistance was also found in *C. canadensis* from vineyards in Japan and in Hungary (Heap, 2004). Populations of *C. canadensis* resistant to paraquat were found in 1994 in several fruit orchards in Essex County, Ontario, Canada (Smisek, Doucet, Jones & Weaver, 1998; Weaver, 2001). Resistant *C. canadensis* has been documented in Tennessee, Missouri and Delaware in reduced tillage production systems (Van Gessel, 2001).

C. canadensis suspected of being resistant to glyphosate was first noted in a farm field in Logan County, Kentucky during the year 2000 (Rogers, 2003). One accession of *C. canadensis* in Delaware has been mentioned as resistant to glyphosate (Heap, 2004). Another case of glyphosate-resistance in *C. canadensis* was reported by a producer in Lauderdale County, Tennessee (Hayes, Mueller, Willis & Montgomery, 2002). More recently a report of herbicide resistance in South Africa was made in January 2003 when resistance occurred in *C. bonariensis* in the Breede Valley, South Africa. The resistance was to glyphosate, but recently reports of resistance to glyphosate and paraquat were received (Heap, 2004).

Although *C. bonariensis* does not cause problems in annual crops, where minimum till is practiced e.g. winter cereals, the weed is a pioneer plant and will colonize disturbed surfaces very quickly. This will occur where a new vineyard or orchard is laid out. About 80-90% of producers of perennial crops use glyphosate and/or paraquat several times, year in and year out, to control this weed (A.L.P. Cairns, June 2005, Department of Agronomy, University of Stellenbosch, pers. comm.). This has led to the development of resistance to these two herbicides. Anecdotal evidence shows that this weed quickly becomes resistant, especially in new plantings, e.g. *C. bonariensis* developed resistance in a newly planted orchard in the Breede Valley area of Southern Africa within four years after being planted (P.E. De Wet, June 2005, PO BOX 102, Worcester, pers. comm.).

The aim of this study is i) to identify populations of *C. bonariensis* which are resistant to glyphosate and/or paraquat in the Western Cape; ii) to investigate the effect of stage of application on herbicide activity; iii) to lay down a protocol for the screening of populations to identify resistant populations; iv) to establish the most effective treatments for the control of glyphosate and paraquat-resistant populations and v) to determine the effect of light, depth of sowing and temperature on the germination of resistant and susceptible biotypes of the weed.

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CHAPTER 1

HERBICIDE RESISTANCE

1.1 *Conyza bonariensis* (Flaxleaf fleabane)

Flaxleaf fleabane (*C. bonariensis* L. Cronq.) and horseweed (*C. canadensis*) are generally believed native to South America and eastern North America. *C. bonariensis* was first reported from California in 1893-1896. It was known to occur elsewhere in southern California by 1940. Naturalized populations of *C. bonariensis* (Plate 1.1) occur on all the Channel Islands except for San Miguel and have been reported from most states west of the Sierra Nevada (Wilken & Hannah, 1998).

Plate 1.1 *C. bonariensis* in its natural habitat (www.weedscience.org)

1.1.1 Taxonomy and Nomenclature

Kingdom:	Plantae
Taxonomic Rank:	Species
Synonym(s):	*Erigeron bonariensis* L.
	Erigeron linifolius Willd.
	Leptilon bonariense (L.) Small
	Leptilon linifolium (Willd.) Small
Common Name(s):	asthmaweed
	Flaxleaved fleabane
	Hairy fleabane

1.1.2 Taxonomic Hierarchy

Kingdom	Plantae --- Planta, plantes, plants, Vegetal
Subkingdom	Tracheobionta --- vascular plants
Division	Magnoliophyta --- angiospermes, angiosperms, Flowering plants, phanerogames
Class	Magnoliopsida --- dicots, dicotyledons
Subclass	Asteridae
Order	Asterales
Family	Asteraceae --- sunflowers, tournesols
Genus	*Conyza* Less. --- horseweed
Species	*Conyza bonariensis* (L.) Cronq. --- Asthmaweed, Flaxleaved fleabane, hairy fleabane (www.itis.usda.gov)

C. bonariensis is an annual or short-lived perennial, taprooted plant in the Asteraceae family (Wu & Walker, 2004). The stems can grow as large as 100 cm tall and is stiffly erect and moderately pubescent. The lateral branches of the plant are elongated. In most cases the lateral branches overtop the central axis. The leaves alternate and can vary from 5-10 cm in length. The leaves are cauline, oblanceolate to linear or oblong. The leaves are also short-petioled or tapered at the base and are sparsely to moderately pubescent. The heads are arranged in a panicle. The phyllaries are imbricated and is glabrous to short-pubescent. It is also narrowly lanceolate to linear-oblong. The flowers are mostly tubular (Wilken & Hannah, 1998).

C. bonariensis is self-compatible and is apparently not actively pollinated by insects. This suggests either autogamy or wind-pollination. The composite flowers of *C. bonariensis* exist of an inflorescence with female flowers on the outside and hermaphroditic flowers in the middle (Shaaltiel, Chua, Gepstein & Gressel, 1988). The reproductive capacity is high relative to total plant biomass and the small, light seeds and a relatively large pappus confer a relatively high level of dispersability. It was found that *C. bonariensis* can produce as many as 470 000 seeds per adult plant. Most species of *Conyza* produce basal rosettes prior to bolting and flowering, although the rosettes of *C. bonariensis* are relatively short-lived compared to those in the perennial taxa (Wilken & Hannah, 1998). The weed forms a rosette when the plant is exposed to humid short days and flowers in periods of dry long days and, thus,

needs plasticity to photooxidant stresses (Amsellem, Jansen, Driesenaar & Gressel, 1993).

In Australia, *C. bonariensis* represents a significant threat to dryland farming systems. The main reasons for the development of resistance is (a) a change in cropping systems over the last 10-15 years. The trend is towards adoption of a no-tillage system and reliance on glyphosate in fallow systems. (b) Increased acceptance of break crops as a permanent part of rotations also leads to resistance. (c) El Nino's influence in recent times. Drier periods leads to longer spray intervals and increased likelihood of treating larger isolated weeds and (d) the inability to understand individual paddock risks. More intensive planning and interaction from outside sources are required (Thorn, 2004).

In Australia, *C. bonariensis* are mainly weeds of roadsides, wastelands, watercourses and lawns. In grazed areas they rarely survive or become an economic problem. However, they are emerging as a significant summer weed of winter minimum tillage cropping systems especially where stock numbers have been reduced. It is common for them to emerge in spring and summer and many fields are now sprayed in summer and autumn (Moore, 2004).

Shaaltiel *et al.* (1988) prepared reciprocal crosses between paraquat resistant and sensitive individuals. The enzymes of the pathway that detoxifies superoxide to innocuous oxygen species that are involved in resistance, were evaluated in the F_1 and F_2 generations. All F_1 plants were as resistant as the resistant parent, irrespective of parental sex, demonstrating dominance and excluding maternal inheritance. Resistance in the F_2 generation was contributed in a 3:1 ratio (resistant to sensitive). They concluded that one dominant nuclear gene controls resistance by pleiotropically controlling the levels of enzymes of the active-oxygen detoxification pathway. Paraquat resistance was also found to be due to a single dominant gene in *C. philadelphicus* (Itoh & Miyahara, 1984). Resistance to paraquat in *Conyza* is not polygenic, as was found in evolved resistance in *Lolium* (Faulkner, 1974).

C. bonariensis is widespread in the South West of Western Australia. There are four common species *C. albida, C. bonariensis, C. canadensis* and *C. parva.* They

commonly grow together. They appear to have somewhat overlapping morphological characters, so very few field workers can identify with which species they are dealing (Moore, 2004). *C. bonariensis* is the most common specie occurring in northern NSW and southern QLD (Wu & Walker, 2004). In India, *C. bonariensis* is distributed in many parts of the Punjab province along the edges of roads, gardens and maize lands (Chaudhry, Janbaz, Uzair & Ejaz, 2001). In South Africa *C. bonariensis* mainly occur in the Western Cape. *C. canadensis* predominantly occur to the northern and eastern parts of the Western Cape (A.L.P. Cairns, June 2005, Department of Agronomy, University of Stellenbosch, pers. comm.).

1.2. Herbicides most commonly used on *Conyza bonariensis*

1.2.1 Paraquat

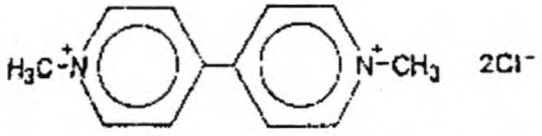

Figure 1.1 General structure of paraquat
(1,1' dimethyl-4, 4'-bipyridinium ion) (Cobb, 1992).

Extensive use of the bipyridilium herbicide paraquat has led to incidents of resistance in 12 different plant species within eight different genera worldwide (Fuerst & Vaughn, 1990). Paraquat was first used in the mid-1950s (Crafts, 1975). Paraquat (1,1' dimethyl-4, 4'-bipyridinium ion) is a nonselective herbicide and crop dessicant (Lehoczki, Laskay, Gaal & Szigeti, 1992). Paraquat is primarily used for the post-emergence control of terrestrial plants. Paraquat is used to kill annual grass and broadleaved weeds and to top-kill and suppress the growth of perennials. Plant selectivity is achieved with paraquat by applying it in croplands so as not to contact the crop plants, for example, pre-plant, pre-emergence and directed application to the crop plants, but post-emergence (foliar) to the emerged weeds. It is necessary to use a non-ionic surfactant in an aqueous spray mixture when paraquat is applied as a foliar treatment. It enhances the herbicidal effectiveness (Anderson, 1983).

Paraquat exerts its phytotoxic action by forming free radicals. By means of the intervention of photosystem I (PSI), dicationic paraquat is reduced to the monocation,

which in turn reduces molecular oxygen (O^2) to the anionic superoxide radical (O^{2-}), thus regenerating the dicationic paraquat. The radical O^{2-} in turn produces hydrogen peroxide (H_2O_2) and the hydroxyl free radical (OH^-). This is known as the Fenton reaction. The superoxide and hydroxyl radicals destroy cellular membranes by peroxidizing fatty acids (Valverde, 1991).

The mode of action of contact herbicides is, in general, the weakening and disorganization of cellular membranes accompanied by increased membrane permeability, resulting in the loss of cellular contents by leakage. Contact herbicides kill by acute toxicity. Acute toxicity infers rapid kill, usually within minutes or a few hours after contact. Little or no translocation occurs via the symplast because these herbicides kill living cells and tissues upon contact. They may, however, be transported to some extent via the transpiration stream, since this movement is through nonliving cells of the xylem. Where the phytotoxicity of a contact herbicide is dependant on light, such a herbicide will translocate in darkness via the symplast if the treated plants are not exposed to light following herbicide application; after the treated plants are exposed to light, herbicide translocation does not take place (Anderson, 1983).

1.2.2 Glyphosate

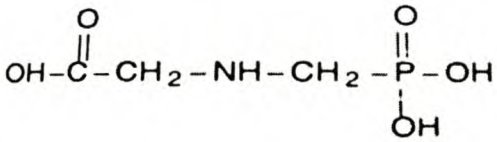

Figure 1.2 General structure of glyphosate
N- (phosphonomethyl) glycine (Cobb, 1992).

Glyphosate, N- (phosphonomethyl) glycine, is formulated as the isopropylamine salt of glyphosate. It was introduced in 1974 (Gressel, 2002). Glyphosate is the world's most important and widely used herbicide (Woodburn, 2000). Glyphosate is a relatively non-selective, broad-spectrum, systemic herbicide (Hartzler, 2001; www.greenpeaceusa.org). It is applied as a post-emergence, foliar treatment and it readily translocates throughout aerial and underground plant parts. It is phytotoxic to

most annual, biennial and perennial herbaceous plant species and certain woody species. Glyphosate is strongly adsorbed by soil colloids and it has little or no phytotoxicity following soil application. The principal use for glyphosate is for the post-emergence control of certain perennial weed species. Selective weed control may be achieved with glyphosate by the use of directed or shielded applications, by which the herbicide is prevented from contacting the foliage and green stems of desired plants and from applications to emerged weeds made preplant or pre-emergence to the crop (Anderson, 1983). The foliage and other portions of the plants readily absorb glyphosate. Once absorbed into the plant, glyphosate readily and extensively translocate symplastically. Once in the phloem, it generally follows the source-sink photosynthate movement pattern in the plant and accumulates in areas of active growth.

Glyphosate inhibits the shikimate pathway enzyme 5-enol-pyruvylshikamate-3-phosphate synthase (EPSPase) and enzymes that act late in that pathway. The EPSP synthase enzyme catalyses the conversion of shikimate-3-phosphate and phosphoenolpyruvate to yield 5-enolpyruvylshikimate-3-phosphate and inorganic phosphate in the shikimate pathway (Ng, Wickneswari, Salmijah, Teng & Ismail, 2003). The pathway is responsible for, among other things, the biosynthesis of aromatic amino acids: phenylalanine, tyrosine and tryptophane. The pathway is also responsible for the biosynthesis of such diverse plant compounds such as phytoalexins, plastoquinone, alkaloids, cinnamate, coumarin and flavonoids. The pathway produces many compounds with allelopathic activity (Cobb, 1992; www.agron.iastate.edu).

The modes of action of systemic herbicides are generally associated with disruption of the normal functioning of one or more physiological or metabolic plant processes. Systemic herbicides, in general, kill by chronic toxicity. Chronic toxicity infers "slow acting," with death of the plant occurring after a prolonged period of time following absorption of the herbicide by the plant. Systemic herbicides are translocated from their sites of entry to their sites of phytoxicity within the plant via the (1) transpiration stream or (2) photosynthate stream or both. Systemic herbicides move from living cell to living cell via plasmodesmata. They penetrate cell membranes and accumulate at their site of action in toxic amounts without disrupting the living system while in

transit.

Systemic herbicides probably exert their toxic action at multiple sites within the plant via one or more mechanisms and the most herbicide sensitive of these sites probably differs among plant species (Anderson, 1983). Glyphosate however, rapidly moves to the apical areas of the plant and inhibits protein synthesis. The plants stop growing and many plant tissues and parts slowly degrade due to lack of proteins. Death of the plants ultimately results from dehydration and desiccation (www.agron.iastate.edu).

1.3 Types of Resistance

The development of herbicide resistance in weed populations under herbicide selection is an evolutionary phenomenon. Herbicides are very intense selective agents and where genetic variability for herbicide response exists in weed populations, evolution of herbicide resistance can be rapid (Diggle, Neve & Smith, 2003). The probability and rate of herbicide resistance evolution depends on the interplay between the population dynamics and population genetics of weed populations (Maxwell & Mortimer, 1994). Important evolutionary factors include the intensity of selection (degree of discrimination between genotypes); the frequency of resistance traits in natural (unselected) populations; the mode of inheritance of resistance; the relative fitness of susceptible and resistant biotypes in the presence and absence of herbicide and gene flow within and between populations. The intrinsic population dynamics of weed populations is also important (Mortimer, Ulf-Hansen & Putwain, 1993).

Evolved resistance is the ability of a plant population to avoid being affected by a herbicide as a result of continuous or frequent application of the herbicide for long periods. This type of resistance differs from that observed when a species is not controlled by an herbicide to which it has never been exposed. This is known as natural resistance (Valverde, 1991). Herbicide resistance is thus the inherent ability of a species to survive and reproduce following exposure to a dose of herbicide normally lethal to its wild type.

1.3.1 Cross-resistance

Cross-resistance occurs where one resistance mechanism brings about resistance to several herbicidal compounds as a result of a single selector. There are two forms of cross-resistance, depending upon the mechanism of action. The term cross-resistance is used typically for an evolutionary event caused by a single selection (Powles & Preston, 2004).

1.3.1.1 Target site cross-resistance

Target site cross-resistance is the result of a modification of the herbicide-binding site, which precludes the various herbicides from binding to the target. This type of cross-resistance will occur to herbicides with the same target site of action that bind to the same domain on the target. Some target sites can have more than one separate domain, overlapping domains, or different binding sites in the same binding pocket. Target site cross resistance does not necessarily result in resistance to all herbicide classes with a similar mode of action or indeed all herbicides within a given herbicide class.

1.3.1.2 Non-target site resistance and nontarget-site cross-resistance

Nontarget-site resistance is resistance due to a mechanism(s) other than a target-site modification. Nontarget-site resistance can be endowed by mechanisms such as enhanced metabolism, reduced rates of herbicide translocation, sequestration, etc. Such mechanisms reduce the amount of herbicide reaching the target site. Nontarget-site cross-resistance occurs when a single mechanism endows resistance across herbicides with different modes of action. Such mechanisms are usually unrelated to the herbicide target site. Examples are cytochrome P450-based nontarget-site cross-resistance and glutathione transferase-based resistances, which degrade a spectrum of herbicides that have different sites of action (Powles & Shaner, 2001)

1.3.1.3 Intergroup cross-resistance

Intergroup cross-resistance occurs when a single evolutionary pressure selects resistance to herbicides with different known or unknown target sites. This can occur by co-ordinately upregulating a single enzyme or group of enzymes that degrade different herbicides, as well as by undefined mechanisms.

1.3.2 Multiple-resistance

Multiple-resistance implies that several resistance mechanisms evolved separately in time, due to separate selections by unrelated herbicides and then co-exist in the same individuals. Multiple-resistance is used to describe the result of multiple evolutionary events due to sequential selections. Multiple resistance is defined as the expression (within individuals or populations) of more than one resistance mechanism. Multiple resistant plants may possess from two to many distinct resistance mechanisms and may exhibit resistance to a few or many herbicides. The simplest cases are where an individual plant possesses two or more different resistance mechanisms, which provide resistance to a single herbicide, or class of herbicides. More complicated are situations where two or more distinct resistance mechanisms have been selected either sequentially or concurrently by different herbicides and endow resistance to the classes of herbicide to which they had been exposed (Powles & Preston, 2004).

1.3.3 Creeping multi-factorial resistance

This type of resistance is typified by a slow, incremental, creeping increase in the LD_{50} of the whole population as a function of repeated treatments. Various researchers have modelled such creeping resistance due to the slow accumulation of genes, alleles or duplications that each gives a small increase in resistance. This was well documented for diclofop-methyl resistance in field populations of *Lolium rigidum* in Australia where low rates are typically used and no target site resistance was initially found. Creeping resistances have been found earlier but the target site resistances have overshadowed their incidences, until the rampant creeping resistances covered much of Australian wheat fields. There can be a segregation of the cross-resistances when the primary resistance is controlled by more than one gene or multiple-resistance (Powles & Preston, 2004).

1.3.4 Resistance in *Conyza bonariensis*

Table 1.1 The occurrence of herbicide resistant *C. bonariensis* in the world (adapted out of www.weedscience.org)

	Location	Herbicide	Group	Year	Sites	Acres infested
1	Egypt	Paraquat	Bipyridiliums	1989	---	---
2	Israel	Atrazine & simazine	PS II inhibitors	1993	2-5	101-500
3	Israel	Chlorsulfuron	ALS inhibitors	1993	2-5	101-500
4	Japan	Diquat & paraquat	Bipyridiliums	1989	6-10	1-5
5	South Africa	Paraquat	Bipyridiliums	2003	6-10	101-500
6	South Africa	Glyphosate	Glycines	2003	2-5	11-50
7	Spain	Simazine	PS II inhibitors	1987	2-5	11-50
8	Spain	Glyphosate	Glycines	2004	6-10	1001-10000

Resistance in *C. bonariensis* occurs in 4 different herbicide groups within 5 locations throughout the world (Table 1). *C. bonariensis* is responsible for the infestation of approximately 1327 to 11 605 acres of land. Resistance was first reported in 1987, but recently herbicide resistance in *C. bonariensis* became a major problem, especially in South Africa and Spain. Glyphosate resistance in *C. bonariensis* is currently the biggest resistance problem with approximately 1012 to 10 050 infested acres of land in South Africa and Spain (Table 1.1).

1.4. Possible explanations for resistance in *C. bonariensis*

1.4.1 Paraquat resistance

There are five hypotheses that has been proposed as plausible mechanisms of paraquat resistance in plants:

1. With respect to *C. bonariensis* (L.) Cronq., restricted cuticular penetration has been eliminated as a potential mechanism of paraquat resistance
2. Detoxification of paraquat.
3. An altered site of action.
4. Enzymatic detoxification of paraquat generated noxious oxygen species.
5. A sequestration mechanism that would prevent paraquat from diffusing to the site of action (PS I).

There are several mechanisms whereby the herbicide could be prevented from reaching its site of action. These include reduced absorption of the herbicide into the leaves, reduced translocation of the herbicide, increased binding of the herbicide to the cell wall and reduced movement of the herbicide across cell membranes to the active site.

The first resistance mechanism proposed is related to the presence in some species and in resistant biotypes, of increased levels of enzymes capable of detoxifying free radicals produced by paraquat in the presence of light (Shaaltiel & Gressel, 1986). In the resistant biotypes of some species, including *Hordeum glaucum* and *C. bonariensis*, paraquat seems to have been excluded from its site of activity in the chloroplast, even if its final location has not been elucidated yet (Feurst, Nakatani, Dodge, Penner & Arntzen, 1985; Powles & Cornic, 1987; Feurst, 1989). In *H. glaucum* no differences in the activity of the enzymes superoxide dismutase, catalase and peroxidase, between resistant and susceptible biotypes, were observed. Both biotypes were also similar in the permeability to paraquat of their plasmatic membrane or of the membranes of the chloroplast envelope (Powles & Cornic, 1987). The penetration of the cuticle by paraquat is similar in both biotypes; however, translocation of the herbicide from the surface of the leaf is insignificant in the resistant biotype compared to a certain translocation degree present in the susceptible biotype. Based on these results, it was proposed (Bishop, Powles & Cornic, 1987) that the resistance mechanism is based on the exclusion of the paraquat from the

cytoplasm by means of its retention in the apoplast xylem, cell walls and intercellular space.

Strains of *C. bonariensis* contain a complex of enzymes capable of detoxifying the reactive oxygen species generated by the photosystem I blocker paraquat and keeping the plants alive until the paraquat is dissipated (Ye, Faltin, Ben-Hayyim, Eshdat & Gressel, 2000). In the resistant biotypes of some species, like *C. bonariensis*, paraquat seems to have been excluded from its site of activity in the chloroplast, even if its final location has not been elucidated yet (Feurst, *et al.*, 1985; Powles & Cornic, 1987; Feurst, 1989).

According to Norman, Feurst, Smeda and Vaughn (1993), paraquat is not altered in leaves of resistant or sensitive biotypes whether plants were incubated with light or complete darkness. This conclusion is consistent with a previous report that paraquat is not prone to biodegradation in susceptible or other resistant weed species (Summers, 1981). Thus, the metabolic detoxification of paraquat does not appear to account for resistance in *C. bonariensis*.

The results of a MDA production, O_2 consumption and EPR studies, done by Norman *et al.* (1993), indicate that paraquat resistance in *Conyza* is not due to an alteration of the PsaC protein and associated clusters of PSI. Other reports have also indicated that an altered site of action did not account for the paraquat resistance observed in *C. bonariensis* and other species (Harvey, Muldoon & Harper, 1978; Fuerst, *et al.*, 1985; Powles & Cornic, 1987; Vaughn, Vaughan & Camilleri, 1989). According to Shaaltiel and Gressel (1986, 1987a, 1987b), Ye & Gressel (1994) and Furasawa, Tanaka, Thanutong, Mizugushi, Yazaki and Asada (1984), the constitutive activities of SOD, ascorbate peroxidase and glutathione reductase, are the primary basis for resistance. Norman *et al.* (1993) is convinced that the SOD activity is not elevated in the stroma of chloroplasts in resistant *Conyza* plants. They believe that enhanced detoxification of paraquat-generated superoxide radicals to hydrogen peroxide by SOD do not contribute to resistance, but is due to a sequestration mechanism. Feurst *et al.* (1985) and Norman and Feurst (1997) also proposed that the mechanism of resistance to paraquat was due to the exclusion of the herbicide from its site of action in the chloroplast by some rapid sequestration mechanism. Bourque, Chen, Heck, Hubmeier,

Reynolds, Tran, Ratliff and Sammons (2002) concluded that metabolism does not play a key role in the resistance mechanism of *C. canadensis*.

It was found that increased levels of superoxide dismutase and other active O_2 detoxifying enzymes could be induced by a number of environmental stresses (Schöner & Krause, 1990). The increase in the level of these enzymes is putatively a response to increased levels of superoxide in the chloroplast induced by the stresses. Jansen, Shaaltiel, Kazzes, Canaani, Malkin and Gressel (1989) reported that a paraquat-resistant *C. bonariensis* biotype is more resistant to photoinhibition than the paraquat-susceptible biotype. Preston, Holtum and Powles (1991) were unable to demonstrate any difference in sensitivity to photoinhibition between the paraquat-resistant and paraquat-susceptible biotypes of *C. bonariensis*. They concluded that there is no difference in sensitivity with the paraquat-susceptible biotype.

Tanaka, Chisaka and Saka (1986), Bishop *et al.* (1987) and Feurst and Vaughn (1990) also suggested that the primary mode of paraquat resistance is due to the different mechanism of paraquat sequestration occurring before the paraquat reaches the chloroplast. The sequestration mechanism appears to require a structurally intact cell wall to be functional. Vaughn *et al.* (1989) are convinced that the major factor in resistance is due to compartmentalization and not due to enzymatic protection. Norman, Smeda, Vaughn and Feurst (1994) supported the hypothesis that paraquat resistance in *Conyza* is correlated with restricted movement (sequestration) in the resistant biotype.

Szigeti and Lehoczki (2003) suggested that a paraquat-inducible nuclear-coded protein is supposed to play a role in the resistance mechanism. It presumably carries paraquat to a metabolically inactive cell compartment, probably to the vacuole. They also found that the rise in the putrescine and total polyamine levels in resistant horseweed plants treated with paraquat appeared to be a general stress response, rather than a specific reason for or symptom of resistance. Paraquat enters the cell with the aid of transporter molecules localized in the plasmalemma. In maize seedlings, putrescine competitively inhibits the uptake, which could indicate that a polyamine transporter was responsible for the uptake of paraquat (Hart, DiTomaso, Linscott & Kochian, 1992). It was also suggested that the inducible protein in the paraquat

resistant plants presumably functions by carrying paraquat to a metabolically inactive compartment (Halász, Soós, Jóri, Rácz, Lásztity & Szigeti, 2002).

Szigeti, Rácz, Darko, Lásztity and Lehoczki (1996) also concluded that polyamines may play a role in the paraquat resistance of *C. canadensis* after they excluded that elevated levels of SOD was involved in the resistance mechanism. Ye, Muller, Zhang and Gressel (1997) confirmed the findings of Szigeti *et al.* (1996) by showing that putrescine levels are also constitutively elevated in a drought-resistant *C. bonariensis* biotype. They also found that constitutively elevated putrescine levels were correlated with elevated levels of putrescine-generating enzymes. Their activities are specifically elevated in the resistant plants. Exogenous putrescine application provided direct evidence that putrescine can increase oxidant resistance in *C. bonariensis*. Uemura, Tachihara, Tomitori, Kashiwagi and Igarashi (2005) also confirmed the existence of a polyamine transporter in yeast. The report also confirmed that the polyamine transporter might play a role in paraquat-resistance. It was also shown that polyamine content in the cytoplasm of yeast is elaborately regulated by several polamine transport systems in vacuoles (Tomitori, Kashiwagi, Asakawa, Kakinuma, Michael & Igarashi, 2001). According to Table 1.2 the mode of action of paraquat is a photosystem-I-electron diversion that results in a rapid disruption of cell membranes. The herbicide then penetrates into the cytoplasm and cause formation of peroxides and free electrons, which destroy the cell membranes. This destruction prevents translocation.

Table 1.2 Mode of action of non-translocated herbicides (Retzinger & Mallory-Smith, 1997)

Mode of Action	Chemical Group	Active Ingredient	Characteristics
Photosystem-I-electron diversion	Bipyridylium	Paraquat, diquat	Result in rapid disruption of cell membranes. Herbicide penetrates into cytoplasm, cause formation of peroxides and free electrons, which destroy cell membranes. Destruction prevents translocation. Postemergence use only, can expect only shoot kill.
Inhibition of glutamine syntheses	Unclassified	Glyfosinate-ammonium	
Inhibition of protopophyrinogen oxydase	Diphenyl esters	Bifenox, fluroglycofen, fomesafen, oxyfluorfen	Have foliar and soil activity. Control broadleaf weeds. Relatively unaffected by soil texture and organic matter

Mechanisms of resistance in other weeds include enhanced activity of protective enzymes associated with paraquat resistance in *Lolium perenne* L. (Harper & Harvey, 1978; Shaaltiel, *et al.* 1988) and in *Nicotiana tabacum* L. (Furasawa, *et al.*, 1984). Harvey *et al.* (1978) concluded that paraquat tolerance in perennial ryegrass is unlikely to depend upon reduced uptake, enhanced metabolism or altered translocation of the herbicide. It remains possible that tolerance is due to reduced herbicide penetration into the chloroplasts *in situ*, or to enzymological changes alleviating the toxic effects consequent on the presence of the free radical of reduced paraquat.

In paraquat-resistant *Hordeum glaucum* Steud. from Australia (Powles, 1986), resistance has been proposed to be due to herbicide sequestration which prevents not only transport to younger unexposed tissues, but also paraquat accumulation at the site of action in the chloroplast (Bishop, *et al.*, 1987; Powles & Cornic, 1987; Preston, Holtum & Powles, 1992). Paraquat translocation was reduced in the leaves of a resistant *H. glaucum* biotype. Lasat, DiTomaso, Hart & Kochian (1997) and Bishop *et al.* (1987) suggested that paraquat resistance in *H. glaucum* may be due to the herbicide sequestration in the vacuole and later they proposed that resistance in *H. glaucum* involves a temperature-dependant (Purba, Preston & Powles, 1995) alteration in paraquat transport across the plasma membrane or a biotype-specific enhancement in intracellular compartmentation of the herbicides (Lasat, DiTomaso, Hart & Kochian, 1996). Preston, Holtum and Powles (1992) reported that paraquat resistance in biotypes of *H. glaucum* and *H. leporinum* were a consequence of reduced movement of the herbicide in the resistant plants, but the mechanism involved is not the same in *H. glaucum* as in *H. leporinum*.

The mechanism of resistance to paraquat in a biotype of *Arctotheca calendula* L. is not a result of changes at the active site, decreased herbicide absorption or decreased translocation, but appears to be due to reduced penetration to the active site (Preston, Balachandran & Powles, 1994). Purba, Preston and Powles (1993) showed that a single incompletely dominant gene conferred resistance in *A.calendula*. Soar, Karotam, Preston and Powles (2003) found that resistance in *A. calendula* is correlated with reduced long distance movement of paraquat. Paraquat translocation in *A. calendula* is correlated with paraquat-induced injury and is reduced in paraquat-

resistant *A. calendula*. Some polyamines can reduce the toxic effects of paraquat (Soar, Preston, Karotam & Powles, 2004).

The mechanism of resistance in a paraquat-resistant population of *Lolium rigidum* from South Africa, is suggested to be primarily due to the sequestration of paraquat, limiting its translocation within the plants (Yu, Cairns & Powles, 2004). According to Cairns, (A.L.P. Cairns, June 2005, Department of Agronomy, University of Stellenbosch) experiments strongly indicates that paraquat resistance in *L. rigidum* is not related to cell wall binding/plasma membrane extrusion mechanism but associated with a cytoplasmic mechanism, most likely the greater rate of vacuolar sequestration. The mechanism of resistance to paraquat was not due to a change at the PSI target site within the chloroplast, as paraquat was equally effective as an electron acceptor in both resistant and sensitive populations. Preston, Tardif, Christopher and Powles (1996) reported that the possible mechanisms that are known to mediate resistance in *L. rigidum* are metabolism and resistant target-site enzymes. Alternative resistance mechanisms may include poor foliar uptake and translocation within the plant or within cells (Pratley, Urwin, Stanton, Baines, Broster, Cullis, Schafer, Bohn & Krueger, 1999).

In reality there is an absence of consensus as to the mechanism of resistance to paraquat in plants. In fact, it may be inappropriate to assume that there is a single mechanism of resistance in all tolerant plants described. It is conceivable that various species and perhaps different biotypes within a single species may be rendered resistant to paraquat via different mechanisms. This possibility may help explain the diverse and sometimes contradictory data generated in support of differing hypotheses for a mechanism of paraquat resistance (Hart & DiTomaso, 1994).

1.4.2 Glyphosate resistance

Table 1.3 Mode of action of foliar applied herbicides that is symplastically translocated (Retzinger & Mallory-Smith, 1997)

Mode of Action	Chemical Group	Active Ingredient	Characteristics
Auxin Growth Regulators	Aryloxyalkanoic acids	2,4-D; 2,4-DB; MCPA; Triclopyr; Fluroxypyr	Moving from leaves with sugar to sites of metabolic activity. Potential to kill perennial weeds. Symptoms are pigment loss, growth stoppage and malformed new growth. Most injury appears after several days.
	Benzoic acids	Dicamba; chlorthal-dimethyl	
Microtuble assembly inhibition	Pyridinecarbolic acids	Clopyralid; Picloram; Thiazopyr	
Inhibition of EPSP synthase	Unclassified	Glyphosate; Sulfosate	Usage is limited for foliar use, because chemicals are rapidly inactivated in soil. Symptoms are yellowing of new growth. It is a non-selective herbicide.
Inhibition of acetolactate synthase	Imidazoliones	Imazapyr; Imazethapyr; Imazamox	Shoot meristems cease growth, Yellow to purple symptoms appear, roots develop poorly. Symptom development requires up to three weeks
	Triazolopyrimides	Metosulam	
	Sulfonylureas	Chlorsulfuron, chlorimuron-ethyl, ethoxsulfuron, metsulfuron-methyl, nicosulfuron, primisulfuron, prosulfuron, sulfosulfuron, rimsulfuron, thifensulfuron, triasulfuron, tribenuron	
Inhibition of carotenoid biosynthesis	Triazoles	Amitrole	
	Unclassified	Clomazone	
	Pyridazinones	Norflurazon	
Inhibition of acetyl CoA carboxylase	Aryloxyalkanoic acids	Diclofop-methyl, fluazifop-P-butyl, Quiazlofop-P-butyl, fenoxaprop-P-ethyl, propaquizafop, clodinafop-propargyl, haloxyfop	Symptoms are discoloration and disintegration of meristematic tissue. Leaves turn yellow and red. Usage for selective grass control. Use early postemergence on seedling grasses.
	Cyclohexanedione	Tralkoxydim, sethoxydim, cycloxydim	

The mode of action of glyphosate is the inhibition of the EPSP synthase enzyme (Table 1.3). Feng, Pratley and Bohn (1999) concluded that neither uptake, translocation nor metabolism plays a major role in glyphosate resistance in *Lolium rigidum*. Wakelin, Lorraine-Colwill and Preston (2004) found different translocation

the ability of glyphosate to accumulate in the shoot meristem. Glyphosate resistance in *L. rigidum* is not due to reduced sensitivity of EPSP synthase to glyphosate (Gruys, Biest-Taylor, Feng, Baerson, Rodriguez, You, Tran, Feng, Krueger, Pratley, Urwin & Stanton, 1999; Lorraine-Colwill, Hawkes, Williams, Warner, Sutton, Powles & Preston, 1999). One resistant *L. rigidum* population (Echuca) had a higher level of EPSP synthase activity than a susceptible population (Gruys, *et al.*, 1999); however, the other glyphosate resistant population (Orange) did not have increased EPSP activity. Reduced movement of glyphosate to its site of action in the plastid was proposed as a possible mechanism of resistance in the Orange population (Lorraine-Colwill, *et al.*, 1999). Pratley *et al.* (1999) reported increased levels of resistance in subsequent generations of the Echuca population that had been selected with glyphosate and suggested the involvement of more than one gene. The number of genes involved in resistance has not been determined. The basis of glyphosate resistance in *L. rigidum* from Australia is not clearly understood. Uptake, translocation and metabolism were not different between resistant and susceptible biotypes (Simarmata, Kaufmann & Penner, 2003). Lorraine-Colwill *et al.* (1999) proposed that possible differences in glyphosate transport into or accumulation of glyphosate in the chloroplast might explain the differential basis of sensitivity.

Feng, Tran, Chiu, Sammons, Heck and CaJacob (2004) reported that resistance in *Conyza canadensis* is likely due to an altered cellular distribution that impaired phloem loading and plastidic import of glyphosate resulting in reduced overall translocation as well as inhibition of EPSPS. Reduced translocation of glyphosate was correlated with a glyphosate-resistant horseweed phenotype and transmitted genetically in crosses between susceptible and resistant biotypes (VanGessel, 2001). Preliminary studies in horseweed suggested that resistance was not due to uptake or metabolism, but due to reduced root translocation (Bourque, *et al.* 2002); furthermore, the EPSPS remained sensitive to glyphosate on the basis of accumulation of tissue shikimate (Mueller, Massey, Hayes, Main & Stewart, 2003). Koger and Reddy (2005) reported that reduced translocation of glyphosate plays a major role in glyphosate resistance in resistant biotypes of *C. canadensis*.

The Malaysian *Eleusine indica* (goosegrass) resistant to glyphosate has been reported to have target-site resistance due to a mutation in the EPSP-synthase gene (Tran,

Baerson, Brinker, Casagrande, Faletti, Feng, Nemeth, Reynolds, Rodriguez, Schafer, Stalker, Taylor, Teng & Dill, 1999). *E. indica* contains an EPSP-synthase enzyme with reduced sensitivity to glyphosate (Lee & Ngim, 2000). *E. indica* resistance was not attributed to alterations in uptake, translocation or metabolism (Tran, *et al.*, 1999), but appears to be due to an EPSP target site mutation of proline to serine at position 106 (Baerson, Rodriguez, Tran, Feng, Biest & Dill, 2002; Ng, Wickneswary, Salmijah, Teng and Ismail, 2003). It is expected that resistance to glyphosate will appear less frequently than for most herbicide modes of action, following a pattern similar to that observed for phenoxy herbicides (Powles & Shaner, 2001). Ng, Wickneswary, Salmijah and Ismail (2004) reported that glyphosate resistance in *E. indica* is inherited as a single, nuclear and incompletely dominant gene. The demonstration of monogenic inheritance for glyphosate resistance suggests that the resistant EPSPS could be the sole determinant responsible for the resistance mechanism in all resistant populations of *E. indica.*

Studies have indicated that the mechanism of resistance in *Convolvulvus arvensis* is due to gene amplification, which leads to greater copies of the numbers of genes coding for EPSPase. It results in greater EPSPase activity in the presence of glyphosate, enough for both inhibition and continued enzymatic activity (Tran, *et al.* 1999). Several populations of *C. arvensis* with differences in sensitivity to glyphosate were identified in 1984 (DeGennaro & Weller, 1984). Resistance has not evolved in the populations in response to herbicide selection pressure, but elucidation of the biochemical mechanisms of resistance may prove illuminating. Increased activity of the shikimate pathway has been suggested as the mechanism that provides glyphosate resistance in one *C. arvensis* population (Westwood & Weller, 1997). The resistant population contains a higher level of 3-deoxy-D-*arabino*-heptulosonate-7-phosphate synthase, the first enzyme in the shikimate pathway, than the susceptible population. The authors suggest there is higher activity of the shikimate pathway and that greater carbon flow through the pathway along with a greater phenylalanine pool in the resistant population allows this population to be less affected by glyphosate. The authors also hypothesize that multiple mechanisms were responsible for the resistance.

1.5 Resistance in the World

Although herbicide resistance was reported as early as 1957 to 2,4-D in Hawaii, the first real report of herbicide resistance was to triazine herbicide in common groundsel (*Senecio vulgaris*) and was reported in 1968 from the U.S.A. (Tharayil-Santhakumar, Undated). One of the first reports on herbicide resistance dates from 1970, when certain populations of *S. vulgaris* in the state of Washington, U.S.A., could no longer be controlled even with massive doses of simazine (Valverde, 1991). According to the U.S. Environmental Protection Agency, there are at least 270 weed species resistant to herbicides worldwide (www.panna.org). Till recently, 254 biotypes belonging to 155 species have reported resistance to various herbicides (Heap, 2004).

Table 1.4 Worldwide glyphosate resistance (A.L.P. Cairns, June 2005, Department of Agronomy, University of Stellenbosch, pers. comm.)

Country	**Glyphosate Resistant Weeds**	
	No.	**Weed Species**
South Africa (Western Cape)	4	*Conyza bonariensis*
		Lolium multiflorum
		Lolium rigidum
		Plantago lanceolata
United States	3	*Conyza canadensis*
		Lolium rigidum
		Lolium multiflorum
Australia	1	*Lolium rigidum*
Chile	1	*Lolium multiflorum*
Malaysia	1	*Eleusine indica*

Paraquat resistance was first reported in the late 1970s and early 1980s when resistance was observed in *C. bonariensis* from vineyards and citrus plantations in Egypt, *Erigeron philadelphicus* from mulberry fields (Watanabe, Honma, Ito & Miyrahara, 1982), *E. canadensis* from vineyards in Japan, *Hordeum glaucum* from alfalfa fields in Australia. In total, resistance to paraquat or diquat has been reported in the field for 4 grass species and 12 broadleaf weeds. Tolerance to paraquat was reported in *Lolium perenne* (Faulkner, 1982). Resistance was spotted in cases where

paraquat had been applied 2-3 times for 5-11 years (Polos, Mikulas, Szigeti, Matkovics, Hai, Parducz & Lehoczki, 1988). Till recently 21 weed species have reported resistance to bipyridiliums (Heap, 2000).

Paraquat resistance has also been observed in species like: *Amarantus lividus* (livid amaranth), *Bidens pilosa* (blackjack), *Eleusine indica* (goosegrass) and *Solanum nigrum* (black nightshade). Paraquat resistance was also found in *Conyza bonariensis* in Egypt, *C. canadensis* (Canadian fleabane) in Hungary and Japan, *C. philadelphicus* (Philadelphia fleabane) in Japan, *Poa annua* (annual meadow grass) in the U.K., *Lolium perenne* (perennial ryegrass) in Northern Ireland and *Hordeum glaucum* in Australia (Powles, 1986; Cobb, 1992; Alizadeh, Preston & Powles, 1998). A biotype of *Vulpia bromoides* from a lucerne field in Australia was also shown to be resistant to paraquat (Purba, *et al.*, 1993). Populations of *C. canadensis* and Virginia pepperweed (*Lepidium virginicum*) resistant to paraquat were found in 1994 in several fruit orchards in Essex County, Ontario, Canada (Smisek, Doucet, Jones & Weaver, 1998; Weaver, 2001).

Lolium rigidum is the most widespread and troublesome weed of Australian agriculture. Its wide distribution, frequent high densities and high degree of genetic variability, together with a long history of intense selection with herbicides, have resulted in evolution of resistance to herbicides with nine different modes of action in Australia (Heap, 2001). *L. rigidum* was found to be resistant to diclofop-methyl in 1982 (Heap & Knight, 1982).

Resistance to chlorotoluron by populations of blackgrass (*Alopecurus myosuroides*) was first observed in 1982 in Essex and has since been detected in six southern counties of England. The first case of sulfonylurea resistance was reported by Mallory-Smith, Thill and Dial (1990) and Primiani, Cotterman and Saari (1990) and involved weeds such as *Kochia scoparia* L. and *Lactuca serriola* L. The first report on triazine-resistance populations was found in common groundsel by Ryan (1970). Triazine-resistant biotypes of common lambsquarters, yellow foxtail and *C. canadensis* were found in Southern Spain by De Prado, Dominquez and Tena (1989). In Hungary, triazine-resistant broadleaf weeds such as *Amaranthus bouchonii*, *A. hybridus*, *A. retroflexus*, *Chenopodium album* L. and *C. canadensis* have developed

cross-resistance to some phenylureas, carbamates, uracils and also to chloridazon and pyridate (Szigeti & Lehoczki, 2003).

Resistance to glyphosate in weed species has evolved comparatively slowly and remains rare. Steve Weller at Purdue University, found glyphosate resistance in field bindweed (*Convolvulvus arvensis*) (www.agron.iastate.edu). Glyphosate resistance in *C. canadensis* has been confirmed in Ohio, Indiana, Tennessee, Kentucky, Missouri, Maryland, Delaware and New Jersey, primarily on continuous soybeans (Brunoehler, 2003). Resistant *C. canadensis* has been documented in Tennessee, Missouri and Delaware in reduced tillage production systems (VanGessel, 2001). *Conyza canadensis* suspected of being resistant to glyphosate were first noted in a farm field in Logan County, Kentucky during the year 2000 (Rogers, 2003).

In 1996 an Australian farmer found Roundup-resistant ryegrass weeds in a field that had been sprayed with glyphosate 10 times in the previous 15 years (www.panna.org). Other weeds that are resistant to glyphosate are tropical spiderwort (*Commelina benghalensis*) and morning glories (*Ipomoea purpurea*). Tropical spiderwort has spread in alarming proportions in fields in Georgia, Florida and North Carolina. It was first detected in the United States in the 1930s. In 1999, it was found in five counties in southern Georgia. By 2002, 41 Georgian counties reported tropical spiderwort and 17 listed it as moderate to severe. A 2003 survey revealed that tropical spiderwort was entrenched in Georgia, affecting 52 counties, with 29 counties listing the weed as moderate to severe (www.gmwatch.org).

Evolved resistance to glyphosate has been identified in two accessions of *Lolium rigidum* Gaudin (rigid ryegrass) in Australia (Powles, Lorraine-Colwill, Dellow & Preston, 1998; Pratley, *et al.*, 1999; Neve, Sadler & Powles, 2004) and one accession of *Eleusine indica* L. Gaertn. (goosegrass) was found in Malaysia (Lee & Ngim, 2000). Also one accession of rigid ryegrass in South Africa, one accession of rigid ryegrass in California and one accession of *C. canadensis* in Delaware has been mentioned as resistant to glyphosate (Heap, 2004). In late 1999, growers from central Chile reported poor control of *L. multiflorum* Lam. (Italian ryegrass) in fruit orchards after glyphosate application (Perez & Kogan, 2003). Another case of glyphosate-

resistance in *C. canadensis* was reported by a producer in Lauderdale County, Tennessee (Hayes, Mueller, Willis & Montgomery, 2002).

1.6 Resistance in South Africa

The first confirmed case of herbicide resistance in South Africa occurred in *Avena fatua*. This biotype was resistant to diclofop-methyl (Cairns & Hugo, 1986). Resistance to triazine occurred in *Amaranthus hybridus* (smooth pigweed) (Sereda, Erasmus & Coetzer, 1996). *Raphanus raphanistrum* (wild radish) showed indications of resistance to chlorsulfuron (Smit & Cairns, 2001; Smit, 2001). Cairns and Eksteen (2001) reported paraquat resistant *L. rigidum* in a vineyard in South Africa.

More recently a report of herbicide resistance in South Africa was made in January 2003 when resistance occurred in *C. bonariensis* in the Breede Valley, South Africa. The resistance was to glyphosate, but initial findings report resistance to glyphosate and paraquat (Heap, 2003). Field evolved paraquat resistance in a population of *L. rigidum* in the Western Cape, South Africa was reported by Yu *et al.* (2004). Resistance in *Plantago lanceolata* (buckhorn plantain) was found in May 2003 in the Breede Valley, South Africa. Resistance was to glyphosate (Heap, 2003).

Table 1.5 Occurrence of glyphosate and paraquat resistant weeds in the Western Cape and elsewhere (A.L.P. Cairns, June, 2005, Department of Agronomy, University of Stellenbosch, pers. comm.)

Weed	Glyphosate resistance in the Western Cape	Glyphosate resistance reported in other countries	Paraquat resistance in the Western Cape	Paraquat resistance reported in other countries
Conyza bonariensis	Yes	None	Yes	Egypt, Japan
C. canadensis	No	US	No	US, Belgium, Canada, Japan
Eleusine indica	No	Malaysia	No	Malaysia, US
Lolium multiflorum	Yes	Chile, US	Yes	None
L. rigidum	Yes	Australia, California	Yes	None
Plantago lanceolata	Yes	None	No	None

Plate 1.2 A map showing the distribution of glyphosate and paraquat resistant *C. bonariensis* in the Western Cape, South Africa (Cairns, unpublished).
(●) Glyphosate resistant, (●) Paraquat resistant.

From Plate 1.2 and Tables 1.3, 1.4 and 1.5 it is evident that the Western Cape has more weed species resistant to glyphosate, paraquat and both glyphosate and paraquat than any other area in the world. Until recently when glyphosate resistance was reported from Spain (Heap, 2004) the Western Cape was the only area to have reported glyphosate resistance in *C. bonariensis*. The Western Cape is also unique in that it is the only place in the world where paraquat and paraquat and glyphosate resistant ryegrass occurs (Heap, 2003). In addition to this, *P. lanceolata* has been shown to be resistant to glyphosate (Heap, 2004). This is the first report of herbicide resistance of any kind in this weed.

1.7 References

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CHAPTER II

THE EFFECT OF LIGHT QUALITY, TEMPERATURE AND DEPTH OF BURIAL ON THE GERMINATION OF *CONYZA BONARIENSIS*

2.1 Abstract.

The effect of light quality, temperature and depth of burial on the germination of *Conyza bonariensis* seed was studied. The seeds were found to be positively photoblastic and germinated only under unfiltered white light and red light. No germination occurred under far-red light and in the dark. The optimum temperature range for *C. bonariensis* seed was found to be between 15 and 30°C. No germination occurred at 0-5°C and at 35-40°C. Optimum germination occurred at the soil surface, with no germination at depths of 2 cm and deeper. Three different biotypes were tested and the optimum temperature range was the same for the three biotypes, but percentage germination showed a difference.

Keywords: *Conyza bonariensis*, germination, light, soil depth, temperature

2.2 Introduction

Flaxleaf fleabane (*Conyza bonariensis* L. Cronq.) and horseweed (*C. canadensis*) are generally believed native to South America and eastern North America. *C. bonariensis* was first reported from California in 1893-1896. It was known to occur elsewhere in southern California by 1940. Naturalized populations of *C. bonariensis* occur on all the Channel Islands except for San Miguel and have been reported from most states west of the Sierra Nevada (Wilken & Hannah, 1998).

C. bonariensis is self-compatible and is apparently not actively pollinated by insects. This suggests either autogamy or wind-pollination. The composite flowers of *C. bonariensis* consist of an inflorescence with female flowers on the outside and hermaphroditic flowers in the middle (Shaaltiel & Gressel, 1987). The reproductive capacity is high relative to total plant biomass and the small, light seeds and a relatively large pappus confer a relatively high level of dispersability.

The flower head (capitulum) of *C. canadensis* is 3 to 5 mm in diameter and is composed of many outer, white pistillate ray florets and central yellowish-green perfect disk florets (Yamasue, Kamiyama, Hanioka & Kusanagi, 1992). Each flower head of *C. bonariensis* consists of very small seeds that are wind dispersed. The seeds of *C. bonariensis* are tiny, flattened oval seeds with a beakless pappus. The seeds can be up to 2 mm in length. Seeds are light brown and the pappus is whitish-baige to straw colour.

According to a study done by Weaver (2001), up to 200 000 seeds can be formed by one adult plant of the closely related *C. canadensis*. The mean number of seeds per flower head of *C. canadensis* ranges from 60 to 70 (Smisek, 1995; Thebaud & Abbott, 1995). The number of flower heads per plant and therefore total seed production, is proportional to stem height (Regehr & Bazzaz, 1979). A plant 40 cm tall produces about 2 000 seeds, while a plant 1.5 metre tall produces about 230 000 seeds (Weaver, 2001). Bhowmik and Bekech (1993) reported that plants of *C. canadensis* grown at a density of 10 plants m^{-2} in a no-till field without a crop produced approximately 200 000 seeds per plant. According to Wilken and Hannah, (1998) one adult plant of *C. canadensis* produces only 25-40 pistillate flowers per head, whereas *C. bonariensis* produces 70-200 flower heads per plant. Wu and Walker (2004) found that a mature plant of *C. bonariensis* produces an average of 110 000 seeds. One single flower head contains 190 to 550 seeds, with an average of 400 seeds per capitulum. During this study it was found that an adult *C. bonariensis* plant of 1.2 metre tall produced close to 470 000 seeds. The number of seeds per flower head fluctuated between 350 and 400 seeds.

Seeds of *C. bonariensis* are ideally suited for wind dispersion. The distance a single seed can travel is difficult to determine but is more limited by the characteristics of the prevailing winds than on any other factor. Wind dispersal is one mechanism that allows these species to sample an array of heterogeneous environments. Wind dispersal is common in the composite (Asteraceae) family. Regehr and Bazzaz (1979) suggested that tall plants have a dispersal advantage, which may be more important for fitness than maximum reproductive effort. They reported seed deposition onto a corn field up to 122 m downwind from the edge of a dense stand of *C. canadensis*.

Factors that may have an influence on the germination of *C. bonariensis* seeds, is the type of light to which the seeds are exposed. It is said that germination of *C. bonariensis* seeds is light dependant (Michael, 1977; Zinzolker, Kigel & Rubin, 1985; Rollin & Tan, 2004). The depth, at which the seeds occur in the soil, may also play an important role in the percentage seeds that germinate each year. Soil depth plays an important role in the germination success of seeds of any given species. Because the seeds of *C. bonariensis* are so small, it seemed logical that seeds would not germinate from deep soil depths. According to Rollin and Tan (2004) the majority of *C. bonariensis* seedlings emerged from seed on the soil surface or from a depth of 0.5 cm. A very small number emerged from 1 cm while none emerged from depths of 2 cm. Therefore tillage can have a huge influence on the survival of *C. bonariensis* seeds. Some may be buried to stay dormant, but some may be exposed to light and stimulated to germinate. Whether they establish as seedlings or die after germination because they are buried too deeply, the seed bank will be reduced. *C. canadensis* was shown to be especially susceptible to tillage (Tremmel & Peterson, 1983) that reduces survival by more than 90%.

Optimum temperature range for successful germination is also an important factor, if not the most important factor. Each species of weed has an optimum temperature range for germination and it vary quite a lot between species. It is also possible that the optimum temperature range for one species can vary between biotypes. Seed longevity in the soil may also play a role in the successful germination of *Conyza* spp. Annual weeds rely on dispersal in time (dormancy) and space to avoid the selection pressures of tillage and herbicides and establish either through the seedbank or immigration of propagules (Hilgenfeld, 2001). Tsuyuzaki and Kanda (1996) reported finding viable seeds of *C. canadensis* in the seedbank of a 20-yr-old abandoned pasture despite its absence in the aboveground vegetation. *C. canadensis* was an important component of the seed bank beneath an abandoned agricultural field over a ten-year period, but not of the field vegetation (Leck & Leck, 1998). Under laboratory conditions, the longevity of *C. canadensis* seeds is only 2-3 year (Hayashi, 1979). Fresh seeds of *C. bonariensis* are not dormant at maturity and germinate when temperature and moisture conditions are favourable (Wu, 2004).

The aim of this study is to determine a) the influence of light quality on the germination of *C. bonariensis*, b) the effect of depth of burial on germination and c) the effect of temperature on germination to determine the optimum control strategy for this weed. These parameters will, to a large extent, determine the germination pattern of the species under local field conditions. This information will thus be critical in planning both herbicidal and cultural control methods.

2.3 Material and Methods

2.3.1 Plant Material

Seed from biotypes of *C. bonariensis* suspected of being resistant to either glyphosate or paraquat were collected from several farms in the Breede Valley (approximately 100km north east of Cape Town) in the Western Cape, South Africa. The seed were collected from farmers that reported that glyphosate and/or paraquat failed to control *C. bonariensis*. All seed was collected from vineyards. The seed were taken to Welgevallen Experimental Farm at the University of Stellenbosch where it was stored in brown paper bags at room temperature.

2.3.2 Light Quality

C. bonariensis seed was exposed to white, red and far-red light and darkness to determine the effect of light quality on germination. The different light qualities were obtained by using Perspex filters designed to give monochromatic light of the specific wavelength required. The filters were fitted into the lids of light proof wooden boxes. Six replicates of 50 seeds each in 9 cm petri dishes were exposed to each of the four light types. Each petri dish was lined with two Whatman no 1 filterpapers moistened with 5 mL of distilled water. To minimise moisture loss during the duration of the experiment the six petri dishes were sealed into a ziplock plastic bag. Germination was determined after 14 days and the results analysed using the Excel package.

2.3.3 Sowing depth

The effect of sowing depth of *C. bonariensis* seeds on seedling emergence was determined by burying the seeds at depths of 0, 0.5, 1, 1.5, 2, 2.5 and 3 cm in 60 cm^2 plastic pots. This experiment was conducted in a glasshouse at 25/18^{0}C day/night temperatures using soil from the Welgevallen Experimental Farm at Stellenbosch.

Fifty seeds were used per replicate and pots were irrigated daily by an automatic sprinkler system. The experimental design was a randomised complete block and 50 seeds were buried in each plastic pot. Treatment design was a 7x3 factorial with factors Sowing depth and days after sowing. For each treatment there were four replicates. Emergence counts were made twice a week for seedlings that had emerged beyond 1 mm past the soil surface (Grundy, Mead & Burston, 2003). The cumulative emergence counts at 20 days after sowing were logit transformed to achieve constant variance and analysed using Statistica.

2.3.4 Temperature

The effect of temperature on seed germination of glyphosate resistant, paraquat resistant and susceptible *C. bonariensis* biotypes was assessed in experiments conducted in insulated growth chambers with a photon flux density of 300 µmol quanta $m^{-2}s^{-1}$. Temperatures were constantly maintained at the desired temperatures ± 1°C by thermostats and mercury thermometers were also placed in each incubator as a check. Four replicates of 50 seeds each in 9cm petri dishes were exposed to constant temperatures of 0, 5, 10, 15, 20, 25, 30, 35 and 40°C. Each petri dish was lined with two Whatman no 1 filterpapers moistened with 5mL of distilled water. To minimise moisture loss during the duration of the experiment six petri dishes were sealed into ziplock plastic bags. The germination study used a randomised complete block design with four replicates and was conducted over a period of fourteen days. The treatment design was a 3x9 factorial with factors Biotype and Temperature.

Germination was recorded every second day. Germination was defined as the stage when the radicle or stem had extended more than 1 mm beyond the seed coat (Steinmaus, Prather & Holt, 2000). The germination count data were logit transformed ($Y = \log_e \{P/(1-P)\}$, where P is the probability of germination occurring to achieve constant variance. Data was analysed using the Bootstrap command of Statistica.

2.4 Results and Discussion

2.4.1 Light Quality

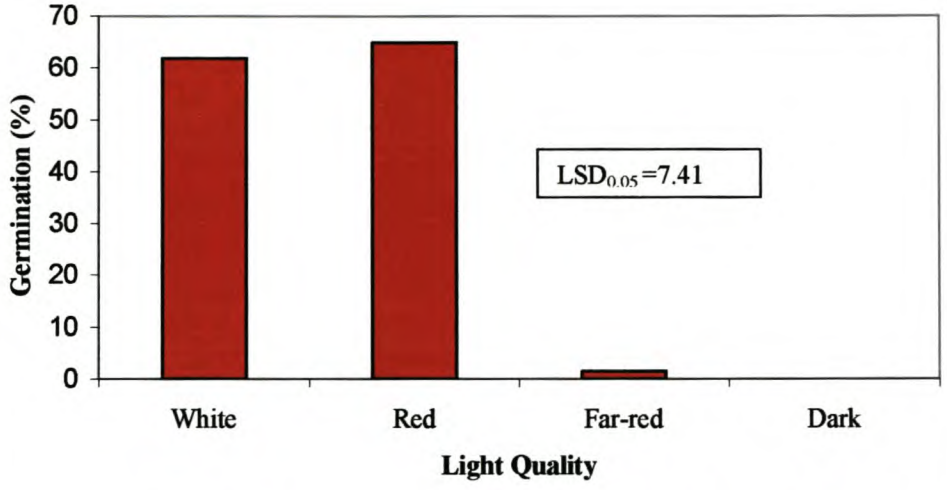

Figure 2.1 The germination of *Conyza bonariensis* seed in different light qualities

From Fig 2.1 it is clear that the highest percentage of germination occurred in red light (65%) and that no seeds germinated in the dark. Far-red had a germination percentage of 1.5%. White light had a germination percentage of 61.83%. A significant difference was found in the results of this experiment, with a p value of less than 0.0001 and it is obvious that light quality controls the germination of these seeds.

It is well known that red light (660 nm) promotes germination and far-red light (730 nm) inhibits germination (www.mrs.umn.edu; www.neuron.montana.edu). Normal daylight can be described as unfiltered white light. White light is a mixture of light of wavelengths between 400 and 800 nm where no single wavelength will characterize or specify white light (www.newton.dep.anl.gov). In the dark, there is no wavelength that can stimulate the germination of the seeds, or the wavelength is lower than the required wavelength for germination. Germination occurs best in the 400 – 700 nm wavelength range (www.generalhorticulture.tamu.edu). This can explain why no germination occurred in the far-red treatment, because the wavelength of far-red light falls outside the wavelength range of germination.

It was found in another study that *C. bonariensis* is photoblastic and has an absolute requirement for light to germinate (Rollin & Tan, 2004). Michael (1977) and Zinzolker *et al.* (1985) found similar results when they did studies on *Conyza* spp. *C. bonariensis* shows a different response from other Asteraceae, such as *C. canadensis* and *Sonchus oleraceus*, that can germinate in the absence of light (Gorski, 1975; Widderick, 2002). Various authors have reported that light is required for germination of different weed seeds (Shontz & Oosting, 1970).

Far-red light allows photoblastic seeds to sense that light energy for photosynthesis is not adequate for seedling growth, consequently preventing germination (Toole, Toole, Borthwick & Hendricks, 1955; Cone, Jaspers & Kendrick, 1985). On the other hand, red light that is abundant in direct sunlight, promotes the germination of photoblastic seeds. The photo reversibility of seed germination under alternating irradiation of red and far-red light appears to exist as a survival mechanism of small-seeded plants; it induces seed germination only under the optimum light condition in which subsequent growth and development of the plants are most likely to succeed (Milberg, Andersson & Thompson, 2000).

2.4.2 Sowing depth (cm)

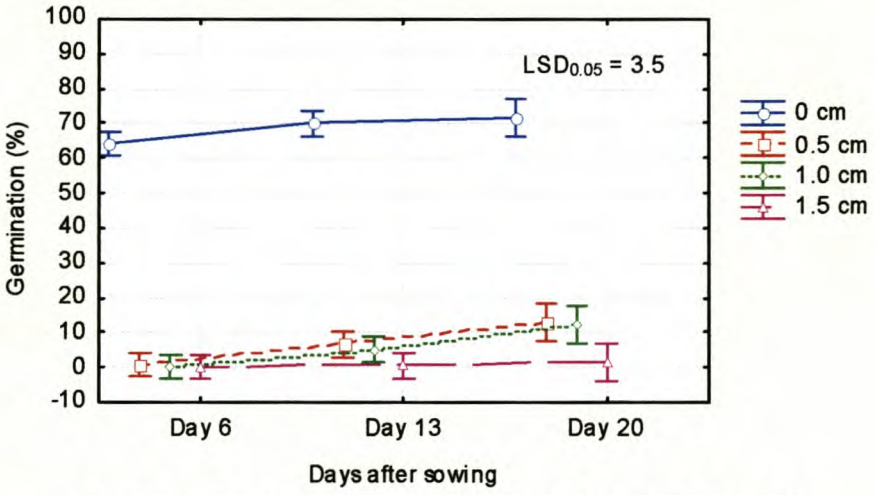

Figure 2.2 The influence of sowing depth on the germination of *Conyza bonariensis*

From Fig. 2.2, Plate 2.1 and Plate 2.2 it is clear that no germination of *C. bonariensis* seeds occurred at a soil depth deeper than 1 cm. Because no germination occurred

deeper than 1 cm, Fig 2.2 only shows results from sowing depths of 0, 0.5, 1.0 and 1.5 cm. There is a significant interaction between sowing depth and time after sowing ($p<0.0000$). A much higher germination percntage was obtained from the surface sown seeds and germination was also faster compared to the other sowing depths. The results of the experiment, clearly show, that sowing depth and time after sowing plays an important role in the percentage germination of *C. bonariensis* seeds.

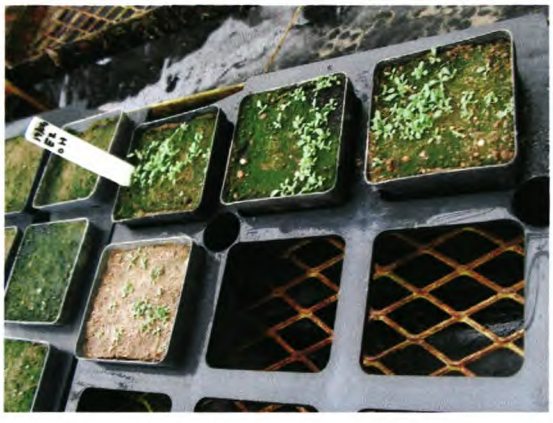

Plate 2.1 Germination of *Conyza bonariensis* seeds sown on the soil surface

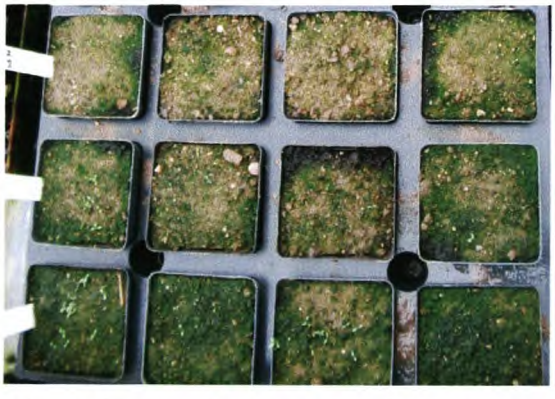

Plate 2.2 Germination of *Conyza bonariensis* seeds sown at 0.5 cm (bottom), 1 cm (middle) and 1.5 cm (top).

Rollin and Tan (2004) found that the percentages of seedlings that emerged from seed on the soil surface or buried to a depth of 0.5 cm were significantly higher than those buried to depth of 1 cm at 32 days after sowing. No seedlings emerged from seeds buried at depths of 2 cm and 5 cm. This agrees with a similar study, which showed that emergence was reduced by 90% when *C. canadensis* seeds were placed at a depth of 1 cm compared to surface grown seeds (Tremmel & Peterson, 1983). No germinable seeds of *C. canadensis* were found below 6 cm at a no-till site in

Massachusetts (Bhowmik & Bekech, 1993). *C. bonariensis* seeds are very small in size and the amount of substrate required for emergence by small seeds from burial depths > 1 cm is lacking (Grundy, *et al.*, 2003). If the weed cannot emerge from a certain depth, once the weed seed bank on the surface has been exhausted and no cultivation takes place establishment of weed seedlings will stop.

2.4.3 Temperature

Temperature plays an important role in the germination process of seeds of any given species. For any species there is an optimum, minimum and maximum temperature range for the germination of the seeds. According to Rollin and Tan (2004) the optimum temperature for the germination of *Conyza bonariensis* seeds is 20°C. They recorded that optimum germination occurred close to 20°C and no germination occurred at 35°C.

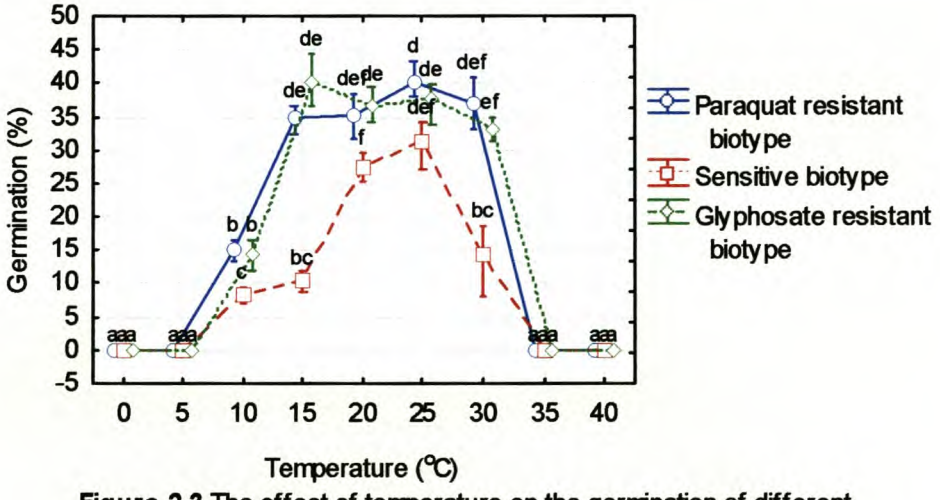

Figure 2.3 The effect of temperature on the germination of different biotypes of *Conyza bonariensis*

Fig 2.3 shows that the sensitive biotype has a lower mean germination percentage than the two resistant biotypes. No germination occurred at 0, 5, 35 and 40°C. There was a significant interaction ($p<0.0000$) between Biotype and Temperature. The interaction indicates that the glyphosate- and paraquat-resistant biotypes and the sensitive biotype responded differently to the same temperature range. According to Fig 2.3 the two resistant biotypes seem to have germinated better over a wider range of temperatures.

The two resistant biotypes germinated optimally between 15 – 30°C, whereas the sensitive biotype germinated optimally between 20 – 25°C. This study also agrees with similar studies done by Zinzolker *et al.* (1985) that *Conyza* spp. can germinate at temperatures between 10 and 25°C. Rollin and Tan (2004) found that the base 4.2°C germination temperature is lower than that of the closely related *C. canadensis* (14.2°C) (Steinmaus, *et al.*, 2000). The germination temperature response in the study of Rollin and Tan (2004) explain the germination of *C. bonariensis* in the early autumn and early spring in Australia, where temperatures close to 20°C are experienced in the north-east grain region. The fact that no germination was recorded at 5 and 35°C suggests that these temperatures are close to the base and maximum temperatures, respectively for seed germination. Buhler and Owen (1997) and Buhler and Hoffman (1999) reported that seeds of *C. canadensis* are not dormant at maturity and germinate readily at day/night temperatures of 22/16°C. The low temperature threshold (base temperature) for germination of *C. canadensis* populations in California was estimated as approximately 13°C (Steinmaus, *et al.*, 2000). If the temperature at a certain time of the year is above or below minimum and maximum, the weed seed will not germinate.

2.5 Conclusions

These results explain why seed of this weed germinates throughout the year in a Mediterranean-type climate. *C. bonariensis* seed has no dormancy (Buhler & Owen, 1997; Buhler & Hoffman, 1999) and can germinate immediately when the seed is shed from the mother plant, provided that sufficient moisture is available. The autumn and early winter temperatures in the Western Cape are mild enough to allow germination and thus it is not surprising that the main flush of germination of this species occurs after the first autumn rains. If this flush of surface-germinating seedlings is destroyed by herbicide and the soil is not disturbed again it is likely that a very high proportion of the surface seed bank will be destroyed. Mulching the soil surface with a material that prevents light from reaching the soil so as to inhibit germination may also be a viable option for the cultural control of this weed. Where a mould board plough can be used ploughing the seed under it would also prevent germination providing that the buried seed is not brought to the surface in the same operation. The sowing of a dense cover crop is also likely to form a viable control

strategy especially in situations where the population has developed resistance to herbicides.

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CHAPTER III

CONFIRMATION OF PARAQUAT AND GLYPHOSATE RESISTANCE IN *CONYZA BONARIENSIS*

3.1 Abstract

Farmers of the Breede Valley in the Western Cape, South Africa reported that *Conyza bonariensis* biotypes were resistant to either glyphosate or paraquat. Seed that was suspected of being paraquat and glyphosate resistant were obtained from the Breede Valley in the Western Cape and screened for resistance. Two different methods of testing were used to screen for paraquat and glyphosate resistance. In the petri dish assay for resistance seeds of suspected resistant and susceptible *C. bonariensis* biotypes were incubated in petri dishes with concentrations of 0, 0.05, 0.5, 5, 50 and 500 ppm of the selected herbicide and the effect of the herbicides on seedling survival was evaluated. In the whole-plant dose-response method seedlings of suspected resistant and susceptible *C. bonariensis* biotypes were sprayed with doses of 0, 400, 800, 1200, 1600 and 2000 g a.i. ha^{-1} of product of glyphosate and paraquat respectively. Both methods of screening identified paraquat and glyphosate resistant biotypes. Resistance of *C. bonariensis* to paraquat and glyphosate in the Breede Valley was thus confirmed.

Keywords: *Conyza bonariensis*, glyphosate, herbicide resistance, paraquat, screening method

3.2 Introduction

Herbicide resistance has become one of the biggest problems for farmers in recent years. According to Valverde (1991) herbicide resistance is the inherent ability of a species to survive and reproduce following exposure to a dose of herbicide normally lethal to its wild type. The first authenticated report of herbicide resistance was in 1968 when common groundsel (*Senecio vulgaris*) growing in the USA, was found to be resistant to triazines (Tharayil-Santhakumar, Undated). Another early report of herbicide resistance dates from 1970, when certain populations of *Senecio vulgaris* in the state of Washington, U.S.A., could no longer be controlled even with massive doses of simazine (Valverde, 1991). Since these early reports of herbicide resistance

many more cases have come to light. According to the U.S. Environmental Protection Agency, there are at least 270 weed species resistant to herbicides worldwide (www.panna.org). Till recently, 254 biotypes belonging to 155 species have reported resistance to various herbicides (Heap, 2004).

Weed resistance to paraquat has been reported in annual bluegrass (*Poa annua* L.), Philadelphia fleabane (*Conyza philadelphicus* L.) and hairy fleabane (*Conyza bonariensis* L. Cronq.) in England, Japan and Egypt, respectively (Gressel, Ammon, Fogelfors, Gasquez, Kay & Kees, 1982). In every case paraquat was applied several times a year for more than 5 years. The resistant biotype of *C. bonariensis* originated in the Tahrir irrigation area in Egypt. An intensive paraquat spraying programme was undertaken in vine and citrus plantations in 1970 and difficulties in controlling this weed were first observed in the mid 1970s (Harvey & Harper, 1982). Resistance was also reported in *Conyza philadelphicus* from mulberry fields in Japan (Watanabe, Honma, Ito & Miyrahara, 1982).

In cropping areas, paraquat and diquat resistance has appeared in three annual weed species: capeweed (*Arctotheca calendula* (L.) Levyns) (Powles, Tucker & Morgan, 1989) and two barley grasses, *Hordeum glaucum* Steud. (Warner & Mackie, 1983; Powles, 1986) and *Hordeum leporinum* Link. (Tucker & Powles, 1988). Purba, Preston and Powles (1993) also found a paraquat resistant biotype of *Vulpia bromoides* (L.) in Australia in 1993. *C. canadensis* resistant to paraquat has been documented in Tennessee, Missouri and Delaware in reduced tillage production systems (VanGessel, 2001).

In almost 30 years of extensive and expanding usage, evolved resistance to glyphosate has been reported in four weed species worldwide (Feng, Tran, Chiu, Sammons, Heck & CaJacob, 2004). The first case was detected in rigid ryegrass (*Lolium rigidum*) (Powles, Lorraine-Colwill, Dellow & Preston, 1998; Pratley, Urwin, Stanton, Baines, Broster, Cullis, Schafer, Bohn & Kreuger, 1999), followed by goosegrass (*Eleusine indica*) in Malaysia (Lee & Ngim, 2000), horseweed (*C. canadensis*) in the United States (VanGessel, 2001) and Italian ryegrass (*L. multiflorum*) in Chile (Perez & Kogan, 2003). *C. canadensis* suspected of being resistant to glyphosate were first noted in a farm field in Logan County, Kentucky during the year 2000 (Rogers, 2003).

Glyphosate-resistance in *C. canadensis* was also reported by a producer in Lauderdale County, Tennessee (Hayes, Mueller, Willis & Montgomery, 2002). More recently glyphosate resistance in *C. bonariensis* in the Breede Valley, South Africa was reported in January 2003. The resistance was to glyphosate, but initial findings report resistance to glyphosate and paraquat (Heap, 2003).

The mechanism of paraquat action involves the PSI-mediated reduction of the paraquat di-cation. This results in the formation of the mono-cation radical. The mono-cation radical reduces O_2 to O_2^-, the superoxide anion radical, resulting in the regeneration of the paraquat di-cation. Subsequently, H_2O_2 and the hydroxyl radical (OH^-) may be produced by a variety of reactions (Dodge, 1982; Dodge, 1983). Hydroxyl radicals are known to cause peroxidation of fatty acids. This is apparently a cause of the observed loss of membrane integrity (Harris & Dodge, 1972; Hutchison, 1979; Dodge, 1983). The presence of paraquat causes the diversion of electrons, which normally reduce NADP and maintain the reduced state of α-tocopherol, glutathione and ascorbate, which function in cellular protection mechanisms. The action of superoxide dismutase, catalase and peroxidase would presumably remain unaffected by this electron diversion.

Glyphosate is a broad-spectrum, non-selective herbicide with limited or no soil activity (VanGessel, 2001). Glyphosate inhibits the shikimate pathway enzyme 5-enol-pyruvylshikamate-3-phosphate synthase (EPSPase) and enzymes that act late in that pathway. The EPSP synthase enzyme catalyses the conversion of shikimate-3-phosphate and phosphoenolpyruvate to yield 5-enolpyruvylshikimate-3-phosphate and inorganic phosphate in the shikimate pathway (Ng, Wickneswari, Salmijah, Teng & Ismail, 2003). The pathway is responsible for, among other things, the biosynthesis of aromatic amino acids: phenylalanine, tyrosine and tryptophane. The pathway is also responsible for the biosynthesis of such diverse plant compounds as phytoalexins, plastoquinone, alkaloids, cinnamate, coumarin and flavonoids. The pathway produces many compounds with allelopathic activity (Cobb, 1992).

Farmers of the Breede Valley in the Western Cape, South Africa became aware of the problem of herbicide resistance in *C. bonariensis* during the past five years and concerns were raised of how to control this weed. The farmers reported that the

biotypes were resistant to either glyphosate or paraquat. Due to the many and varied causes of poor weed control under field conditions, it is unacceptable to assume that resistance is responsible without confirmation. Confirmation is obtained by exposing the suspected biotypes to replicated dose response trials under controlled conditions.

The aim of this study is thus to confirm if poor control of some biotypes of *C. bonariensis* in the Western Cape is due to glyphosate and/or paraquat resistance. Secondly, two different methods of screening for herbicide resistance will be evaluated *viz* a seedling petri dish assay and a whole plant assay.

3.3 Material and Methods

3.3.1 Plant Material

Seed from biotypes of *C. bonariensis* suspected of being resistant to either glyphosate or paraquat were collected from several farms in the Breede Valley (approximately 100km north east of Cape Town) in the Western Cape, South Africa. The seed were collected from vineyards where farmers reported that glyphosate and/or paraquat failed to control *C. bonariensis*. All biotypes were screened for resistance to both herbicides in separate experiments. Seeds were also collected from biotypes that appeared to be sensitive to both types of herbicide. The control or baseline biotype that was found to be susceptible to both glyphosate and paraquat was collected from plants growing on uncultivated, but disturbed soil at Millers Point on the southern Cape Peninsula 21km from Cape Point. The probability that this population had ever been exposed to either glyphosate and paraquat applications in the past is virtually nil. All seed collected was stored at room temperature in brown paper bags at Welgevallen Experimental Farm at Stellenbosch.

3.3.2 Petri dish assay for resistance

Petri dish assays were conducted for *C. bonariensis* biotypes to confirm and determine the level of herbicide resistance in the laboratory. In the first experiment seed from the suspected glyphosate susceptible control biotype (MillerP) and two suspected glyphosate resistant biotypes from the Breede Valley, Heimat and Sonja was tested. In the second experiment seed from the suspected paraquat susceptible control biotype (MillerP) and suspected paraquat resistant biotype (Klopper) was

tested. This was done to establish whether the petri dish assay method is a reliable method to detect resistance to paraquat and glyphosate.

In a follow-up third experiment, two biotypes from the Breede Valley [Biotype 3A (Sonja) and Biotype 11A (Die Eike)] were tested to confirm or reject assumptions that they are resistant to glyphosate. Similarly a fourth experiment was carried out to confirm resistance to paraquat in Biotypes 6A (Ashton) and biotype 1A (Goudini). These specific biotypes were tested with the petri dish assay and the whole-plant dose-response to see if the same results were obtained. Sonja and Klopper were tested because farmers reported that double the recommend dosage did not kill the biotypes

Commercial formulations of Gramoxone® (paraquat dichloride, 200 g L^{-1}) and Roundup® (glyphosate-isopropylamine, 360 g L^{-1}) were used to prepare solutions of 0, 0.1, 1, 10, 100 and 1000 ppm of the two herbicides in distilled water. Two replicates of 40 seeds each for each biotype of each treatment were placed in 9cm petri dishes. The experimental design was a completely randomized design with factorial arrangement of treatments. Factors were biotype and concentration. Each petri dish was lined with two Whatman no. 1 filter papers moistened with 5mL of distilled water. Five mL of each treatment was added to the replicates to make solutions of 0, 0.05, 0.5, 5, 50 and 500 ppm for each herbicide.

To minimise moisture loss during the duration of the experiment the petri dishes were sealed into a ziplock plastic bag. The ziplock bags were placed in an insulated growth cabinet with a photon flux density of 300 µmol quanta $m^{-2}s^{-1}$. The temperature was maintained at 20°C ± 1°C. The experiment was performed in continuous light. Seedling counts were made every week and the final number of surviving seedlings was made after 21 days. Analysis of variance was performed on the data using the Statistica 7 package. Fisher's protected LSD at a 5% level of probability was used to identify significant differences.

3.3.3 Whole-plant dose-response

Seeds from six diferent *C. bonariensis* biotypes (Biotypes 1-6H) were collected from the Breede Valley. These six biotypes were collected from the following farms; 1H (Wysersdrift), 2H (Kleinbegin), 3H (Sandrivier), 4H (Kloppersbosch, 5H (Sonja) and 6H (Alartskraal). These six biotypes were tested for resistance to glyphosate and paraquat in different experiments. In a third experiment seed from seven other biotypes (Biotypes 1A, 3A, 5A, 6A, 10A, 11A and 12A) was tested for resistance to paraquat. These biotypes were obtained from the following farms: 1A (Goudini), 3A (Sonja), 5A (Die Wingerd), 6A (Ashton), 10A (Goree), 11A (Die Eike) and 12A (Klopper). Seeds from the different biotypes were planted in 16cm diameter plastic pots. This experiment was conducted in a greenhouse at a 12 hour 25/18°C day/night temperature using soil from Welgevallen Experimental Farm at Stellenbosch. Emerged seedlings were thinned to 4 plants per pot. Pots were watered daily by an automatic sprinkler system. Plants were approximately 10 weeks old at the time of spraying with rosettes 4 to 6 cm in diameter. When the plants were 10 weeks old, each biotype was divided into treatment groups with four replicate pots per treatment.

Commercial formulations of Gramoxone® (paraquat dichloride, 200 g L^{-1}) and Rondup® (glyphosate-isopropylamine, 360 g L^{-1}) were used in these experiments. Paraquat and glyphosate dosage rates were 2, 4, 6, 8 and 10 L ha^{-1} of the respective commercial formulations. The wetting agent Agral 90 (0.5% v/v) was used with paraquat at 0.04 mL per 100mL. Herbicides were applied to the plants within a spray chamber with a flat-fan nozzle that is calibrated to spray 100 L ha^{-1} at a pressure of 210 kPa. Paraquat was applied in a volume of 400 L ha^{-1} and glyphosate at a volume of 200 L ha^{-1}. Sprayed plants were left overnight in the spray room to maximize the effect of the herbicides (Brian & Headford, 1968) and returned to the greenhouse the following day. The plants were monitored weekly and the survival rate recorded 21 days after spraying.

The experimental design was a randomised complete block with a two-factor factorial arrangement of treatments (Biotype and Dose). Analysis of variance was performed on the data using the Statistica 7 package. Fisher's protected LSD at a 5% level of probability was used.

3.4 Results

3.4.1 Petri dish assay

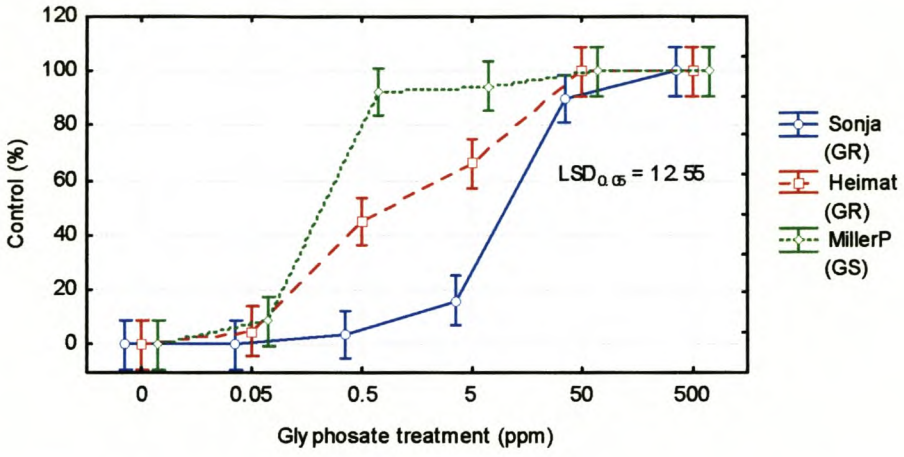

Figure 3.1 The effect of different glyphosate concentrations on the control of different *Conyza bonariensis* biotypes in a petri dish assay

The p value (0.0000) indicates that there is a significant interaction between the three biotypes and glyphosate concentrations (Fig 3.1). Sonja (Biotype 3A) was found to be the most resistant biotype with only a 90% control at 50 ppm. MillerP (the control biotype) had a 92.5% control percentage at 0.5 ppm. Heimat had a 66.25% control at 5 ppm, but was controlled 100% at 50 ppm. This indicates that there is a significant difference in resistance between the three biotypes, with Sonja the most resistant biotype to glyphosate and Heimat moderately resistant.

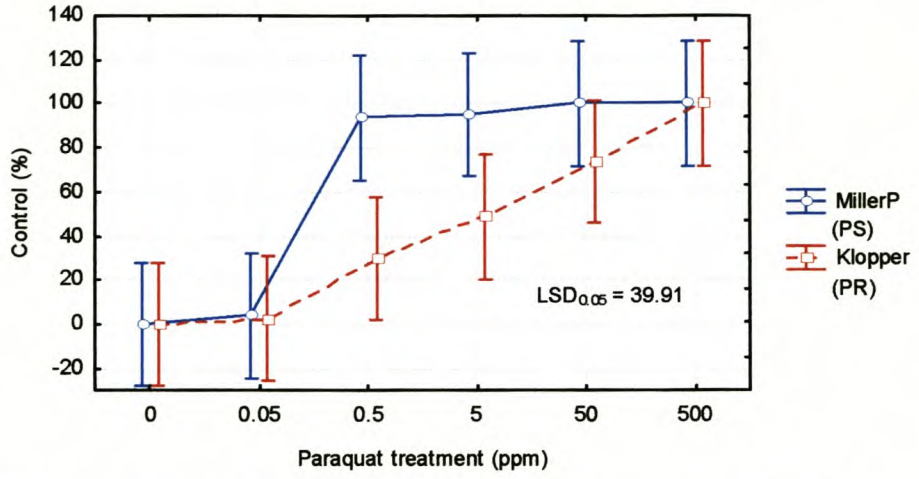

Figure 3.2 The effect of different paraquat concentrations on the control of different *Conyza bonariensis* biotypes in a petri dish assay

The interaction between biotype and paraquat concentration is not statistically significant due to the high variability (Annexure A, Table 2.2). However, from Fig 3.2 it is clear that biotype Klopper is far more resistant to paraquat than biotype Miller P.

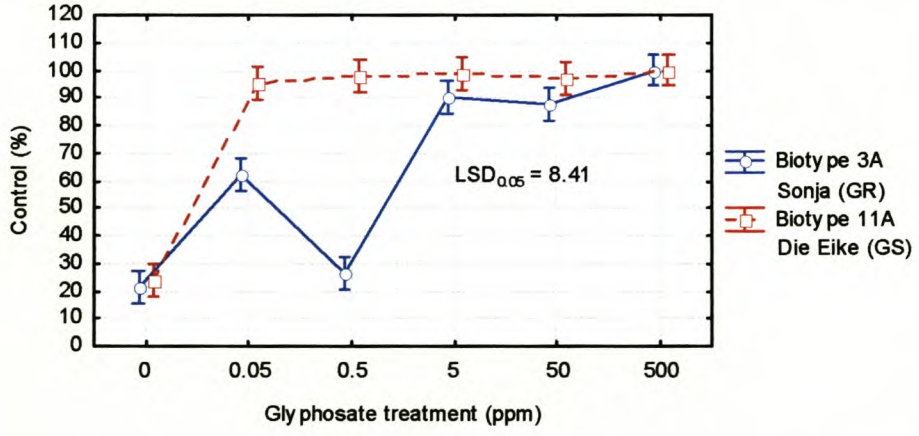

Figure 3.3 The effect of different glyphosate concentrations on the control of two *Conyza bonariensis* biotypes in a petri dish assay

There is a significant ($p = 0.0000$) interaction between the two biotypes and the glyphosate concentrations. Fig 3.3 shows different responses for biotypes 3A (Sonja) and 11A (Die Eike) at 0.05 and 0.5 ppm. Ninety five percent of Die Eike was controlled at only 0.05 ppm whereas the control of Sonja at this dosage rate was 62.5%. The difference in response of the two biotypes at 0.5ppm was even greater and

it is clear that the two biotypes responded significantly different to the glyphosate concentrations. Sonja is clearly a glyphosate-resistant biotype.

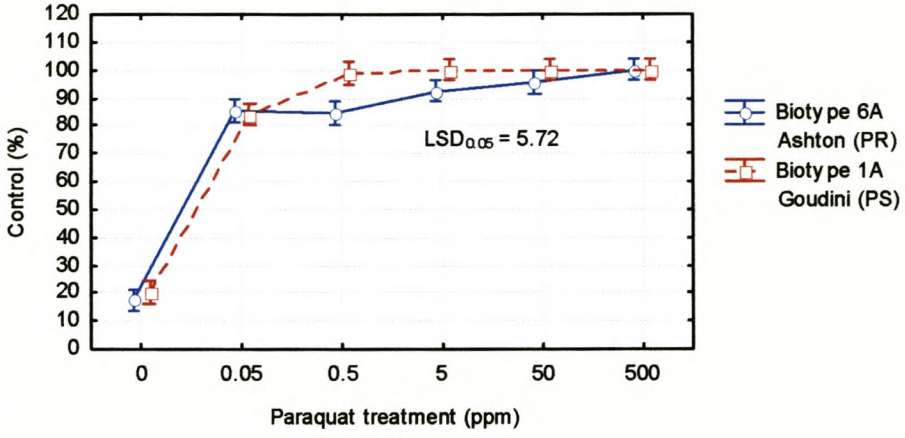

Figure 3.4 The effect of different paraquat concentrationson the control of two *Conyza bonariensis* biotypes in a petri dish assay

Fig 3.4 shows that there is a significant interaction ($p = 0.0118$) between the two biotypes tested for resistance and the paraquat concentrations. There are however not large differences in response to the paraquat concentrations.

3.4.2 Whole-plant dose-response

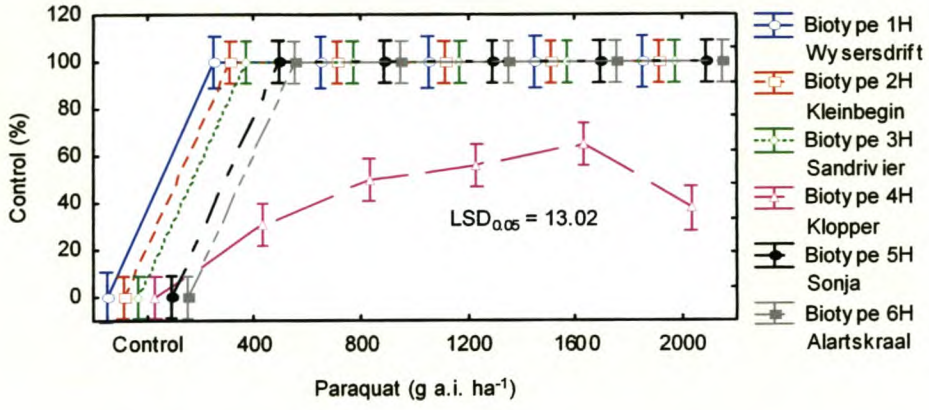

Figure 3.5 The effect of different paraquat doses applied to six different *Conyza bonariensis* biotypes

Plate 3.1 This plate shows that biotype Klopper was the only biotype to have survived 2000 g a.i. paraquat ha^{-1}

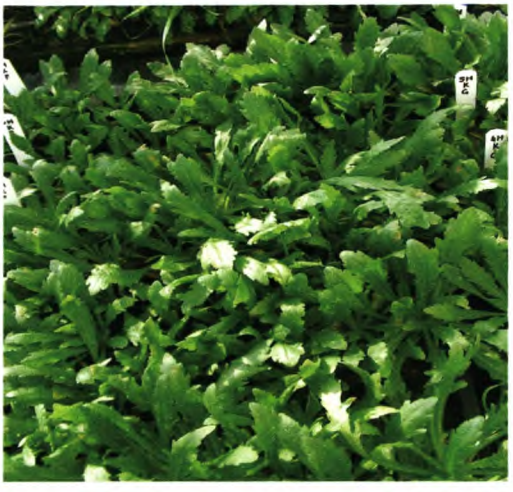

Plate 3.2 Unsprayed control of six biotypes of *C. bonariensis*

There is a significant (p = 0.0000) interaction between biotype and paraquat dose. Fig 3.5 and Plate 3.1 shows that biotype Klopper is the only biotype to survive all the paraquat treatments from 400 to 2000 g a.i. ha^{-1}. Klopper had a control percentage of 65% at 1600 g a.i. ha^{-1}, but only 37.5% at 2000 g a.i. ha^{-1}. Plate 3.2 shows the unsprayed control of the experiment and is a further indication of the occurrence of resistance in biotype Klopper. Fig 3.5 shows that all other biotypes were controlled by

400 g a.i. paraquat ha^{-1}. According to Fig 3.5 and Plate 3.1 it can be assumed that biotype Klopper is highly resistant to paraquat.

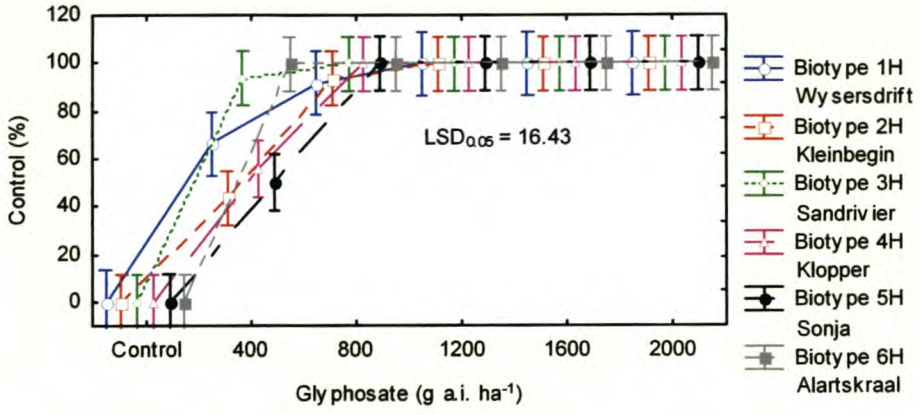

Figure 3.6 The effect of different glyphosate doses applied to six different *Conyza bonariensis* biotypes

Plate 3.3 This plate shows the biotypes that survived 800 g a.i. glyphosate ha^{-1}

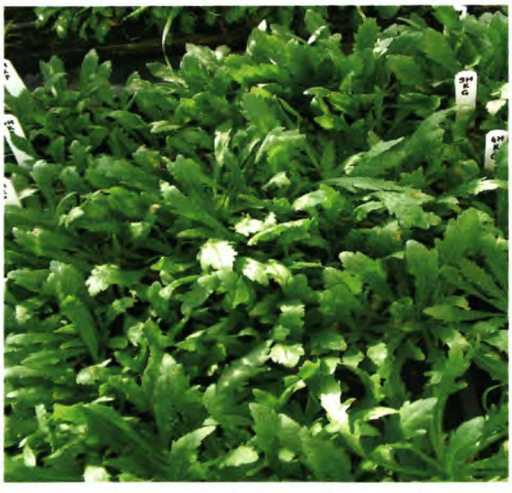

Plate 3.2 Unsprayed control of six biotypes of *C. bonariensis*

The p value of 0.000294 indicates that there are significant interactions between the biotypes and the glyphosate concentrations. Fig 3.6 and Plate 3.3 shows that none of the six biotypes is highly resistant to glyphosate. There were only one plant surviving 800 g a.i. ha^{-1} glyphosate in biotype 1H (Wysersdrift) and one surviving plant in biotype 2H (Kleinbegin). There were no plant survivors at treatments higher than 800 g a.i. ha^{-1}. Kleinbegin has the lowest control percentage (43.75) at 400 g a.i. ha^{-1}, but that does not clearly indicates resistance in this biotype. According to Fig 3.6 and Plate 3.3 it can be assumed that not one of the tested biotypes is highly resistant to

glyphosate, but there are differences in sensitivity to glyphosate at the dose of 400 g a.i. ha^{-1}, which might indicate the early stage of development of resistance.

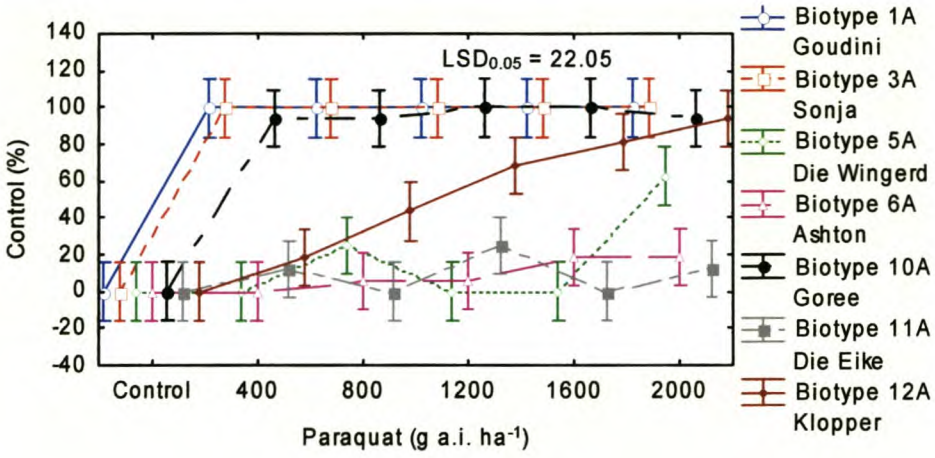

Figure 3.7 The effect of different paraquat doses applied to seven different *Conyza bonariensis* biotypes

Plate 3.5 This plate shows the biotypes that survived 2000 g a.i. paraquat ha^{-1}

Plate 3.6 Unsprayed control of six biotypes of *C. bonariensis*

There is a significant ($p < 0.00$) interaction between biotype and paraquat doses. Fig 3.7 and Plate 3.5 show that the percentage control of paraquat in this experiment varied a lot. Two biotypes (1A - Goudini and 3A - Sonja) were totally controlled with 400 g a.i. ha^{-1} and one biotype (10A – Goree) was completely controlled with 1200 g a.i. ha^{-1}. The control of biotype 12A (Klopper) increased with increasing dosage rate of paraquat and reached 93.75% at 2000 g a.i. ha^{-1}. Biotype 5A (Die Wingerd) also

shows poor control with paraquat and only 62.5% was controlled at 2000 g a.i. ha^{-1} paraquat. Biotype 6A (Ashton) and 11A (Die Eike) showed high levels of resistance to paraquat with control of only 18.75% and 12.5% at 10 L ha^{-1} paraquat. Die Wingerd, Ashton, Die Eike and Klopper can be described as being resistant to paraquat.

3.5 Discussion

The results of the petri assay show that the biotype Sonja is very resistant to glyphosate. In Fig 3.1 some of the plants survived up to 50 ppm and in Fig 3.3 there were also surviving plants at 50 ppm. In Fig 3.2 it was found that biotype Klopper is very resistant to paraquat with only 73.75% control at 50 ppm. In Fig 3.4 two different biotypes were tested for paraquat resistance after farmers noticed reduced control in these specific biotypes. Although there were not many surviving plants at the different concentrations, the two biotypes (Ashton and Goudini) showed a difference at 0.5 ppm where Goudini was better controlled with paraquat. Because Ashton was not completely controlled at 50 ppm, it can be described as resistant to paraquat.

The whole-plant dose-response experiments show that biotype Klopper is extremely resistant to paraquat (Fig 3.5). Although Fig 3.7 confirms that Klopper is resistant to paraquat, Die Eike, Die Wingerd and Ashton biotypes proved to be even more so. Fig 3.6 shows that Sonja, although not extremely so, is the most glyphosate resistant biotype. According to the results of the petri dish assays and the whole-plant dose-response experiments, it is clear that the petri dish assay can be used as a method to quickly and effectively identifies herbicide resistance in *C. bonariensis*.

A possible explanation for the weaker control of biotype 4H at 2000 g a.i. ha^{-1} (Fig 3.5) may be explained by the fact that weed populations are so diverse and when sampling takes place, it is possible to select only resistant plants, although there may be sensitive plants in the population as well (A.L.P. Cairns, May 2005, Department of Agronomy, University of Stellenbosch, pers. comm.). It is possible that the plants tested at 2000 g a.i. ha^{-1} are highly resistant to paraquat and thus the poor control at such a high herbicide rate. Llewellyn and Powles (2001) also reported that large

differences occur in the proportion of fields containing resistant populations between agronomic areas. It reflects different cropping and therefore herbicide use history. Gressel (2002) reported that it should be noted that resistant weeds, as for weeds in general, are not usually spread evenly across the whole field in the early stages of resistance development. Patches of resistant weeds around a site of origin of resistance can be expected and if resistance is detected early, the size of the infested areas can be restricted.

The biotypes were tested because of farmers concern of herbicide resistance within these biotypes. Some of the biotypes showed varied results and it must be kept in mind that temperature and other environmental factors may influence the results. Although the level of resistance within a certain biotype (for example Sonja) may vary between different methods used, the end result will be the same.

It is a possibility that farmers tend to try and save on their herbicide account and consequently apply dosage rates lower than the recommended dosage. When the expected level of control is not achieved, they suspect the weeds of being resistant to the herbicides and report that the herbicides is ineffective or that resistance is the cause of the problem. Samples are sent for resistance testing and it is often found that resistance does not occur at the recommended dose. What farmers do not realise is that by applying sub-lethal dosage rates of herbicides they are exacerbating the problem. Model simulations predict that lowering herbicide efficacy, by reducing the application rate, would slow the rate of increase of herbicide-resistant individuals in a weed population, but the resulting increase in density of susceptible plants would reduce crop yield and increase the weed seed bank (Beckie & Kirkland, 2003), thus leading to an increase in herbicide resistance. Reduced use rate theories and related predictive models are often of limited practical value to growers. Aside from inconsistent performance, weed control strategies based on reduced herbicide use rates are not a solution to prevent or even delay target site resistance. In fact, prolonged use of sub-lethal use rates may select for metabolic resistance and add future weed management challenges by replenishing the weed seed bank (Doyle & Stypa, 2004).

It must also be kept in mind that the growth stage of *C. bonariensis* may play a major role in the occurrence of resistance or in the level of resistance that is occurring. To achieve effective control of *C. bonariensis*, it is crucial to treat it when it is small at its early growth stages, actively growing and before stem elongation. Control efficacy declines as plants mature (Storrie, 2004). *Conyza* has two phases of resistance during vegetative (rosette) growth: (1) a low level of resistance with an I_{50} value 10 times that of the sensitive wild type during most of the vegetative growth phase; and (2) a high level of resistance at an age of 10 weeks, where it is 50-250 times more resistant to paraquat than the wild biotype. Both biotypes are exceedingly resistant to paraquat and other photooxidant stresses when *Conyza* bolts to flower, often in rain-free, subtropical summers (Gressel, 2002).

3.6 Conclusions

Prompt identification of the resistance status of surviving weeds before the seed bank becomes enriched with resistant seed is an important aspect of resistance management. If herbicide efficacy is declining and if the weed species and herbicides involved have been implicated in resistance at other sites, as in the case of the Breede Valley, then it is possible that resistance may be the cause. The quick and effective method of the petri dish assay can help a lot in the identification of herbicide resistance so that the necessary precautions can be taken. This is especially important with a weed such as *C. bonariensis* and the other species in this genus, which have a very effective seed dispersal mechanism. Prevention of weed seed set in the resistant patches by nonselective herbicides or by cutting is preferable to allowing the whole field to become infested with a large population of herbicide-resistant weed seeds. Under most field conditions the potential seed set of the weed population on maturing plants exceeds the actual resistant seed population in the soil seed bank many times over. It is thus important to identify herbicide resistance as quick as possible to try to stop the increase of herbicide resistance.

What is encouraging is that none of the biotypes that were tested were resistant to both herbicides. Therefore by switching herbicides from the one to the other (paraquat to glyphosate and visa versa) should in fact bring about a dramatic improvement in control. This in fact happened with the Klopper biotype, which is paraquat resistant, and the Goudini biotype, which is resistant to glyphosate. When the two producers

were advised to switch products the Klopper biotype was pratically eliminated when sprayed with glyphosate and the same happened to the Goudini biotype when paraquat was applied. (A.L.P. Cairns, August 2005, Department of Agronomy, University of Stellenbosch, pers. comm.). However, evidence of multiple resistance where populations are resistant to both herbicides is emerging (see Chapter 4).

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CHAPTER IV

THE EFFECT OF GROWTH STAGE ON THE CONTROL OF *CONYZA BONARIENSIS*

4.1 Abstract

Resistance of *Conyza bonariensis* to glyphosate and paraquat is causing problems on wine farms in the Breede Valley. In this study the effect of growth stage on the efficacy of various herbicides on the level of control of C. bonariensis was evaluated. Herbicides were sprayed at the 2, 8, 15, 22 and 25 leaf stage. It was found that growth stage does play an important role in the level of herbicide efficacy. Control by different herbicides decreased with increase in growth stage. Control was better at leaf stage 22 than at leaf stage 25. It was found that MCPA gave good control of most of the different biotypes tested at all of the different leaf stages. It was also observed that no biotype (with the possible exception of Groenrivier) showed resistance to both paraquat and glyphosate, which means that one or other of these two herbicides, can be used to successfully control all populations tested.

Keywords: glyphosate, herbicide resistance, MCPA, paraquat, Sorgomil Gold

4.2 Introduction

Populations of horseweed (*Conyza canadensis*) have developed resistance to herbicides in 11 countries worldwide (Heap, 2003). Glyphosate resistance was reported in *C. bonariensis* in January 2003 in the Breede Valley, South Africa, but recently resistance to paraquat was also reported (Heap, 2003). Resistance to herbicides with five different modes of action has been reported in *C. bonariensis*: Photosystem II inhibitors, bipyridiliums, acetolactate synthase (ALS) inhibitors, ureas and amides and most recently glycines (VanGessel, 2001). *C. canadensis* populations with multiple resistance to ALS and photosystem II inhibitors have been identified in Israel (Heap, 2003), to atrazine and paraquat in Poland (Pölos, Mikulas, Szigeti, Matkovics, Hai, Parducz & Lehoczki, 1988) and to atrazine, simazine and diuron in the United States (Heap, 2003). Populations that are resistant to paraquat are usually also resistant to diquat, another bipyridilium, but at reduced levels (Preston, 1994). *C. canadensis* populations resistant to a particular mode of action may exhibit negative

cross-resistance to herbicides with different modes of action (Gadamski, Ciarka, Gressel & Gawronski, 2000).

Sorgomil Gold (Terbuthylazine (triazine) at 497,2gL^{-1} and S-metalochlor (chloro-acetanilide) at 102,8gL^{-1} was registered in 2001 by the company Syngenta (www.syngenta.co.za). Sorgomil Gold 600 SC is a suspension concentrate herbicide for selective pre- and post-emergence control of most annual broadleaf weeds and some annual grasses. Sorgomil Gold may be applied post-emergence before the broadleaf weeds have developed beyond the 4-leaf stage. Sorgomil Gold is a group code C1 and K3 herbicide.

The auxin analog herbicides were the first selective organic herbicides to be developed primarily for the selective control of broadleaf weeds in cereal crops. English and American workers discovered MCPA and 2,4-D independently during the 1940s (Zimmerman & Hitchcock, 1942; Nutman, Thornton & Quastel, 1945; Kirby, 1980). MCPA was introduced in 1945 (www.inchem.org).

MCPA (4-chloro-2-methylphenoxy) acetic acid is a phenoxyacetic herbicide (www.hclrss.demon.co.uk). MCPA is a systemic post-emergence phenoxy herbicide used to control annual and perennial broad-leaved weeds. This herbicide is very compatible and may be used in formulation with many other products. MCPA is an acid, but is often formulated as a salt (e.g. dimethylamine salt) or an ester (e.g. isooctyl ester). Soil micro-organisms rapidly degrade MCPA and its formulations and it has a low persistence, with a reported field half-life of 14 days to 1 month, depending on soil moisture and soil organic matter. MCPA is readily absorbed and translocated in most plants. It works by concentrating in the actively growing regions of a plant (meristematic tissue), where it interferes with protein synthesis, cell division and ultimately the growth of non-resistant plants. It is actively broken down in plants, the major metabolite being 2-methyl-4-chlorophenol (www.extoxnet.orst.edu).

Observations made by agents and farmers in the Breede Valley, Western Cape indicated that *C. bonariensis* seed germinates from the beginning of May till the end of January. The peak flush of germination occurs in June and July, in the rainy season of the Western Cape. Because *C. bonariensis* germinates continuously during the

rainy season, it is difficult to control the weed with one application of herbicide. For complete control of *C. bonariensis* plants (when plants are ±2 mature leaf stage), farmers will have to apply herbicides every 14 days. Not only is this not practical, but it is not economical (P.E. de Wet, August 2005, PO BOX 102, Worcester, 6849, pers. comm.). In Australia it was found that emergence appears to be year round. Flushes of emergence can occur after significant rain events, resulting in the simultaneous presence of *C. bonariensis* plants at various growth stages ranging from small seedlings to mature plants (Wu & Walker, 2004).

The aim of this study is a) to determine if growth stage plays an important role in the level of resistance and b) to identify alternative herbicides to control resistant *C. bonariensis* plants.

4.3 Material and Methods

4.3.1 Plant Material

Seed from biotypes of *C. bonariensis* suspected of being resistant to either glyphosate or paraquat were collected from several farms in the Breede Valley (approximately 100km north east of Cape Town) in the Western Cape, South Africa. The seed were collected from vineyards where farmers reported that glyphosate and/or paraquat failed to control *C. bonariensis*. Seed from one biotype was also obtained from Millers Point in Cape Town. The seed of one biotype from Wolseley in the Western Cape was also obtained from A.L.P. Cairns from the Department of Agronomy at the University of Stellenbosch. The seed were then taken to Welgevallen Experimental Farm at Stellenbosch, where the seeds were stored in brown paper bags at room temperature until it was used for the tests.

4.3.2 Whole-plant dose-response

Seeds from six different *C. bonariensis* biotypes were collected from the Breede Valley. These six biotypes were reported to be resistant to either glyphosate and/or paraquat. These biotypes were obtained from the following farms: Sonja, Wysersdrift, Groenrivier, Die Hoek, Kloppersbosch and Boskloof. One biotype, Millers Point, was obtained from the Cape Peninsula near Cape Town and used as the susceptible biotype. One other biotype, Heimat (near Wolseley in the Western Cape) was obtained from A.L.P. Cairns (Department of Agronomy, University of

Stellenbosch). Seeds from the different biotypes were planted in 16cm diameter plastic pots. This experiment was conducted in a greenhouse at a 12 hour 25/18°C day/night temperature using a sandy potting medium. Four of the emerged seedlings were planted out in 7.5x8x7.5cm rectangular pots. Pots were watered daily by an automatic sprinkler system delivering a balanced nutrient solution.

For these experiments four different herbicides were used. Paraquat dichloride (200 g L^{-1}), glyphosate-isopropylamine (360 gL^{-1}), MCPA (4-chloro-2-methylphenoxy) acetic acid and Sorgomil Gold 600 were used. Sorgomil Gold is terbuthylazine (triazine) at 497,2 gL^{-1} and S-metalochlor (chloro-acetanilide) at 102,8 gL^{-1} (www.syngenta.co.za). Paraquat was applied at 3 L ha^{-1}, glyphosate was applied at 3 L ha^{-1}, MCPA was applied at 3 L ha^{-1} and Sorgomil Gold 600 was applied at 4.2 L ha^{-1}. The wetting agent Agral 600 (0.5% v/v) was used with paraquat at 0.04 ml per 100ml. Herbicides were applied to the plants within a spray chamber with a flat-fan nozzle, delivering 100 L ha^{-1} at a pressure of 210 kPa. Plants sprayed with paraquat were left overnight in the spray room to maximize the effect of paraquat (Brian & Headford, 1968) and returned to the greenhouse the following day. The plants treated with glyphosate, MCPA and Sorgomil Gold 600 were returned to the greenhouse immediately. The plants were monitored weekly and the final count of survivor plants was made 21 days after spraying.

Plants were sprayed at 2 leaf, 8 leaf, 15 leaf, 22 and 25-leaf stage. For the 2 leaf stage there were two replicates for each herbicide treatment on each biotype. Biotypes tested were Sonja, Miller Point, Wysersdrift, Groenrivier, Die Hoek, Kloppersbosch and Heimat. The experimental design was a randomised complete block with a two-factor factorial arrangement of treatments (Biotype and herbicide). For the 8, 15, 22 and 25 leaf stage there were three replicates for each herbicide treatment on each biotype. Biotypes tested were Die Hoek, Sonja, Wysersdrift, Heimat, Groenrivier and Boskloof. The experimental design was a randomised complete block with a three-factor factorial arrangement of treatments (Biotype, herbicide and leaf stage).

Analysis of variance was performed on the data using the Statistica 7 package. Fisher's protected LSD at a 5% level of probability was used to compare treatments.

4.4 Results

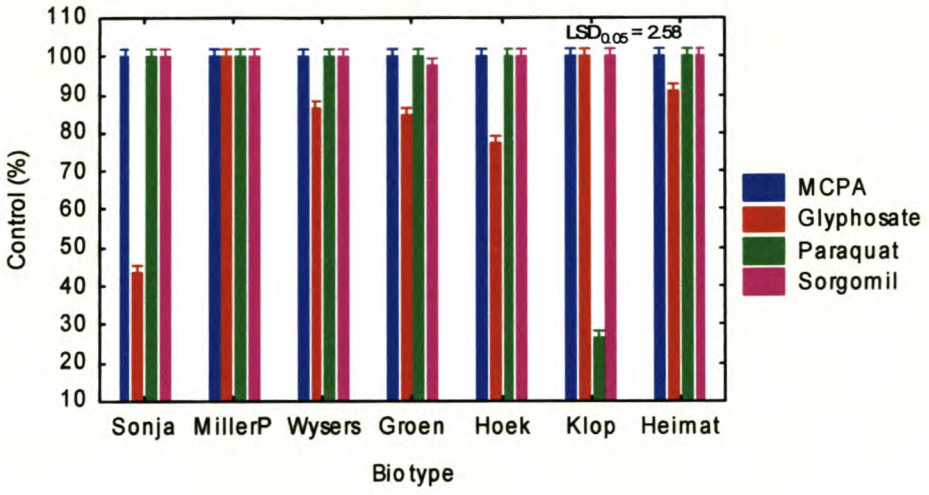

Figure 4.1 Control of *Conyza bonariensis* biotypes using 4 herbicides applied at the two leaf stage

Fig 4.1, Plate 4.1 and Plate 4.3 show that good control was achieved with MCPA and Sorgomil in all but one biotype at the two-leaf stage. Groenrivier was the only biotype that was not controlled 100% by Sorgomil. It is important to notice that there are survivors in Plate 4.1 from the MCPA application, but the two pots with the survivors were planted at a later date than the other pots on the plate and were thus sprayed later to determine control at the 2 leaf stage. From Fig 4.1 and Plate 4.4 it is clear that paraquat gave good control in most of the biotypes, but control was poor in the Kloppersbosch biotype. Kloppersbosch is known to be resistant to paraquat (see chapter 3). Glyphosate (Plate 4.2) did not achieve good control in the Sonja biotype, but Sonja is also known to be resistant to glyphosate (see chapter 3). The p value ($p = 0.00$) indicates that there is a significant difference in the percentage control achieved in different biotypes by using different herbicides. It is clear that there is no biotype at the two-leaf stage that showed resistance to both glyphosate and paraquat. The biotypes are completely controlled by one or the other herbicide. Where paraquat resistance occurs, glyphosate can be used to successfully control the resistant biotype and vice versa.

Plate 4.1 Survivors of MCPA at 2 leaf stage

Plate 4.2 Survivors of glyphosate at 2 leaf stage

Plate 4.3 Survivors of Sorgomil Gold at 2 leaf stage

Plate 4.4 Survivors of paraquat at 2 leaf stage

Although overall no significent interaction were observed between the three factors (biotype, herbicide and leaf stage), significant interaction occurred within biotypes for herbicides used and leaf stage.

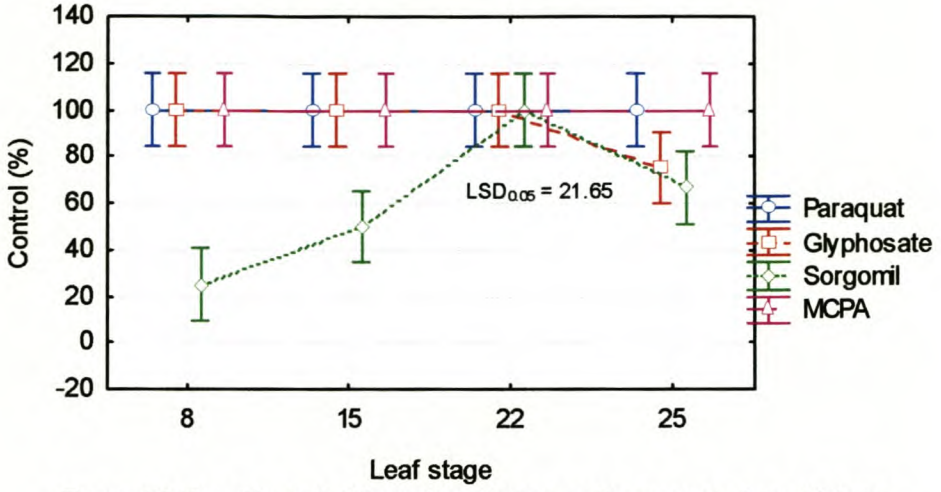

Figure 4.2 The effect of different herbicides on the control of one biotype (Die Hoek) of *Conyza bonariensis* at different leaf stages

According to Fig 4.2 it is clear that this specific biotype of *C. bonariensis* is not well controlled by Sorgomil Gold at the 8, 15 and 25 leaf stage. It is also clear that resistance to glyphosate and Sorgomil occurs at the 25-leaf stage. Sorgomil gave 100% control at the 2-leaf stage (Fig 4.1), but then the control declined at the 8-leaf stage. The percentage control achieved with Sorgomil varies greatly over different leaf stages and although the control was 100% at leaf stage 22, the percentage control decreased to reach 63.5% at the 25-leaf stage. The Sorgomil results are the main reason for the significant (p=0.000216) interaction between leaf stage and herbicide treatment.

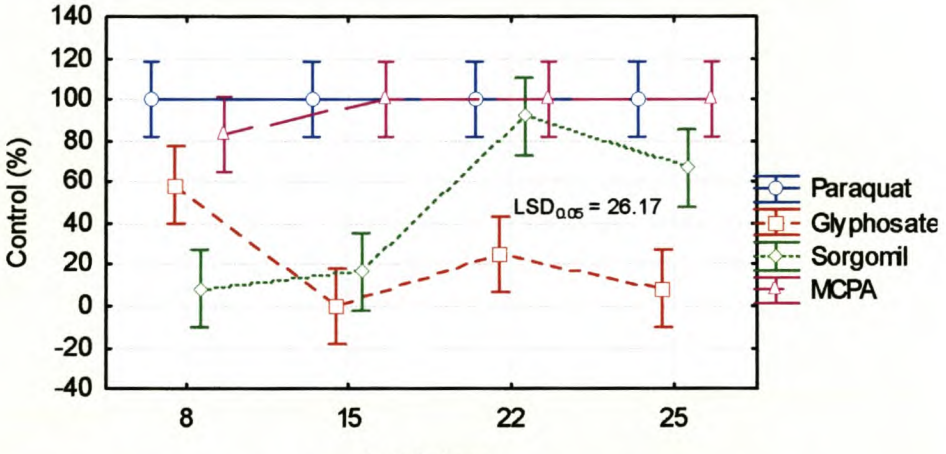

Figure 4.3 The effect of different herbicides on a biotype (Sonja) of *Conyza bonariensis* at different leaf stages

Fig 4.3 shows that this biotype (Sonja) is not controlled well by Sorgomil and glyphosate. This biotype however, is known to be resistant to glyphosate (see chapter 3). Paraquat gave 100% control at all leaf stages and can be used to control this biotype of *C. bonariensis*. The lowest control with all herbicides where achieved at leaf stage 15, with glyphosate (0%) and Sorgomil (16.67%). Better control was achieved at leaf stage 22, but control decreased at leaf stage 25. The mean percentage control at leaf stage 25 was 8.3% for glyphosate and 66.67% for Sorgomil. The p value of 0.00007 indicates that there is a significant difference in the percentage control observed at different leaf stages with different herbicides. Fig 4.3 clearly indicates that paraquat can be used successfully to control this biotype at all tested leaf stages.

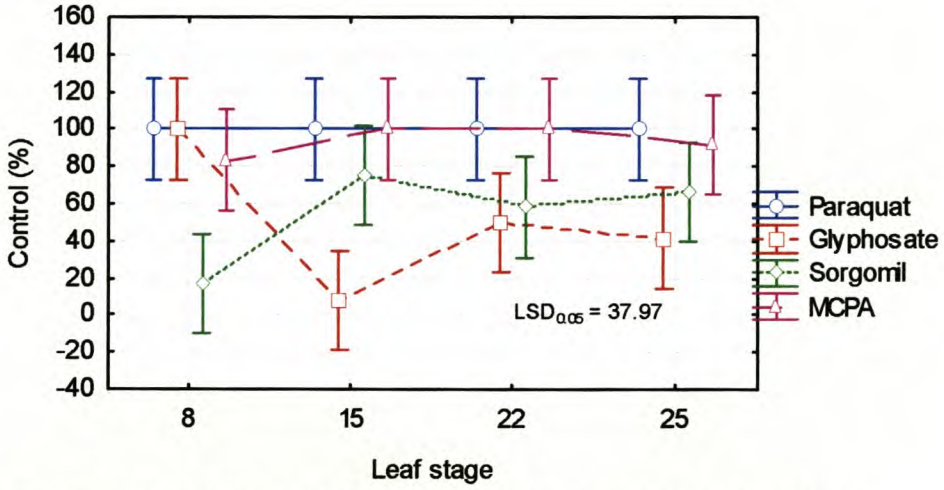

Figure 4.4 The effect of different herbicides on a biotype (Wysersdrift) of *Conyza bonariensis* at different leaf stages

According to Fig 4.4 it is clear that this biotype (Wysersdrift) was poorly controlled with Sorgomil over all leaf stages tested. Glyphosate gave 100% control at leaf stage 8, but control decreased sharply at leaf stage 15. MCPA gave 100% control at leaf stage 15 and 22, but only 83.33% control at leaf stage 8 and 91.67% control at leaf stage 25. Paraquat showed 100% control at all leaf stages and can be used successfully to control this biotype. The p value ($p = 0.001373$) indicates that the differences in the percentage control achieved with different herbicides at different leaf stages are significant.

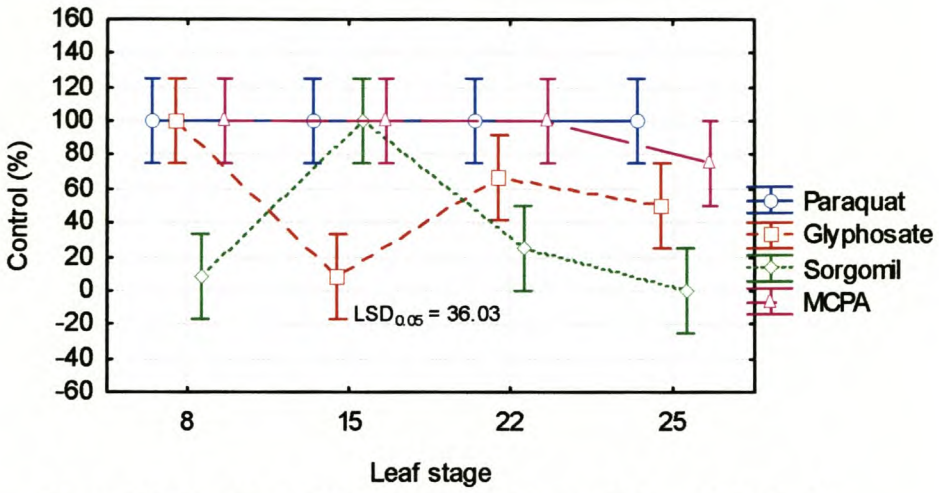

Figure 4.5 The effect of different herbicides on a biotype (Heimat) of *Conyza bonariensis* at different leaf stages

In Fig 4.5 it is clear that paraquat, with a 100% control over all leaf stages, can be used to control this biotype of *C. bonariensis*. Glyphosate gave 100% control at leaf stage 8, but the percentage control decreased after the 8-leaf stage. Glyphosate gave a 91% control at leaf stage 2 (see Fig 4.1). Sorgomil gave varying results, with only 100% control at leaf stage 15. Control achieved with Sorgomil decreased sharply to reach 0% at leaf stage 25. MCPA gave 100% control at leaf stages 8, 15 and 22, but the percentage control at leaf stage 25 was only 75%. The p value ($p = 0.000015$) indicates that there is a significant difference in the percentage control achieved by using different herbicides at different leaf stages.

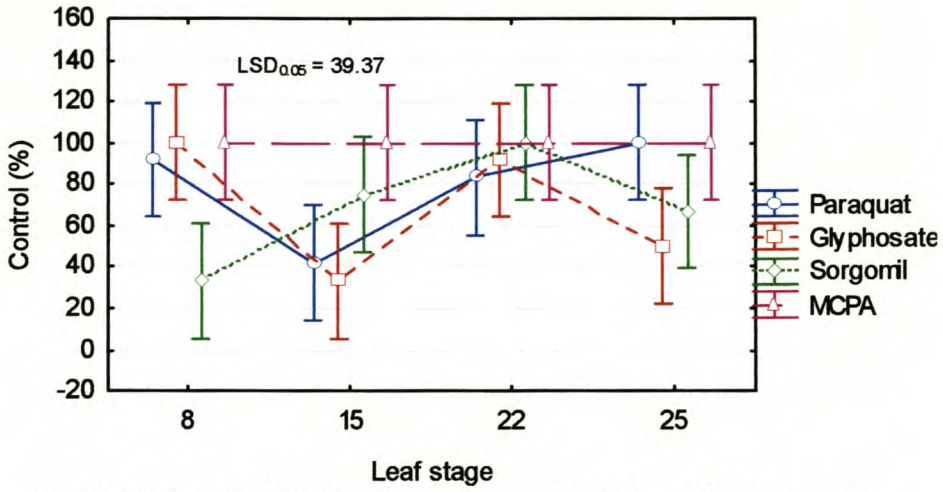

Figure 4.6 The effect of different herbicides on a biotype (Groenrivier) of *Conyza bonariensis* at different leaf stages

According to Fig 4.6, the only herbicide capable of controlling this specific biotype of *C. bonariensis* (Groenrivier) is MCPA. MCPA gave 100% control at all leaf stages tested. Paraquat gave varying results, but gave 100% control at leaf stage 25. Glyphosate gave 100% control at leaf stage 8, but then the percentage control decreased at leaf stage 15 to 33.33%. Glyphosate only control 50% of the plants when applied at leaf stage 25. Sorgomil gave the lowest percentage control at leaf stage 8 (33.33%), where after the percentage control increased to reach a 100% at leaf stage 22. At leaf stage 25 however, the percentage control decreased to only 66.67% when using Sorgomil. According to Fig 4.6, it is clear that the best results with the different herbicides were achieved at leaf stage 22, after which the percentage control declined again. The p value ($p = 0.006816$) indicates that there is a significant difference between the different herbicides used at different leaf stages.

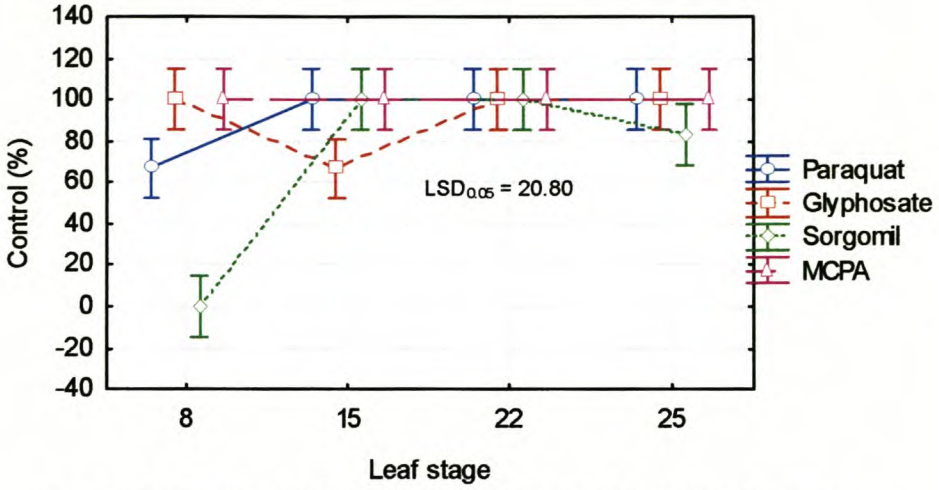

Figure 4.7 The effect of different herbicides on a biotype (Boskloof) of *Conyza bonariensis* at different leaf stages

According to Fig 4.7 it is clear that the only herbicide that gave 100% control over all leaf stages was MCPA. Paraquat gave a 66.67% control at leaf stage 8, but 100% control at all the other leaf stages. Sorgomil gave 0% control at leaf stage 8, but 100% control at leaf stages 15 and 22. The percentage control of Sorgomil decreased after leaf stage 22, to reach 83.33% control at leaf stage 25. Glyphosate gave 100% control at all leaf stages, except at leaf stage 15, where the mean percentage control was 66.67%. The p value of 0.00 indicates that there is a significant difference in the percentage control achieved by using different herbicides at different leaf stages.

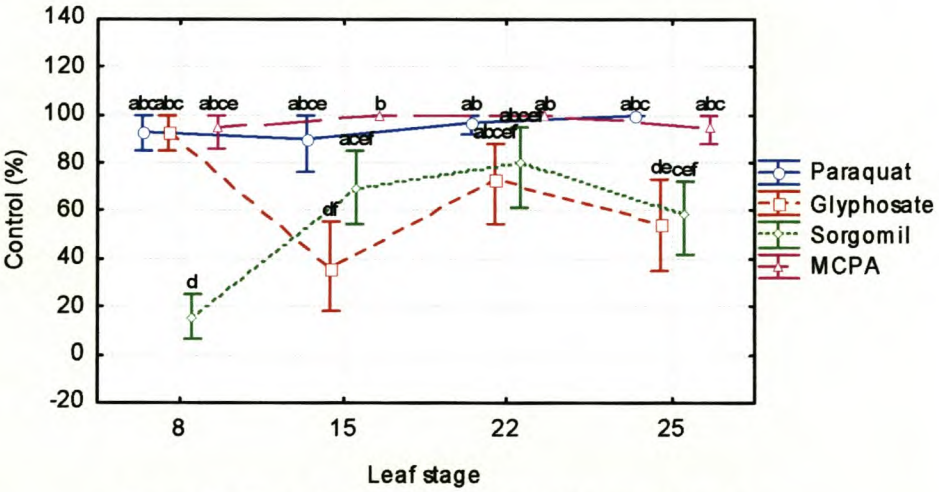

Figure 4.8 The effect of different herbicide treatments at different leaf stages on *Conyza bonariensis*

Fig 4.8 shows the combined results of the weed control by the herbicides over all biotypes and leaf stages. It is clear that paraquat gave good control over the different leaf stages. MCPA was the only herbicide whose efficacy was not influenced by stage of application. What is worrying is the overall poor control achieved with glyphosate. Although glyphosate gave a 100% over all biotypes tested at leaf stage 8, the percentage control decreased, to reach a low 36.11% percent control at leaf stage 15. The percentage control achieved with glyphosate increased at leaf stage 22 (72.22%), but decreased again to reach a percentage control of only 54.17% at leaf stage 25. Sorgomil gave low mean percentage control over all leaf stages. The p value ($p = 0.00$) indicates that there is significant difference in control by the herbicides used at different leaf stages.

4.5 Discussion

Overall, control by all the herbicides over all application stages fluctuated widely. It is clear however, that the optimum application stages for all herbicides are at the two leaf stage. Poor control with paraquat was observed in the Kloppersbosch biotype at the 2 leaf stage, but Kloppersbosch is a known paraquat resistant biotype (see chapter 3). Glyphosate gave poor control in the Sonja biotype at the 2 leaf stage, but again Sonja is known to be resistant to glyphosate (see chapter 3). What is reassuring, is the fact that using glyphosate in the case of Kloppersbosch and paraquat in the case of Sonja can control these resistant biotypes. MCPA can also be used successfully to control all the tested biotypes. What is worrying, is that Groenrivier is not well controlled by paraquat or glyphosate up to the 22-leaf stage. This may be an indication of cross-resistance to these two herbicides.

According to the label specifications of Sorgomil Gold 600, this herbicide must be applied post-emergence before the 4 leaf stage of any broadleaf weed. This can explain the reason why low percentages of control were achieved at the 8, 15, 22 and 25 leaf stages. According to Fig 4.1 it is clear that high percentages of control were achieved at the 2 leaf stage. If biotypes of *C. bonariensis* are sprayed early enough, before 4 leaf stage, good control will be achieved by using Sorgomil Gold 600 (www.syngenta.co.za).

The experiments indicated that there was a significant difference in the percentage control achieved at different leaf stages. In most of the biotypes tested, good control was achieved at the 2 leaf stage (Fig 4.1). It is noticeable that low percentages of control were achieved at the 15 leaf stage for most of the herbicides used. As explained later, temperature might have an influence on the results achieved at leaf stage 15. The weaker control in leaf stage 25 as opposed to leaf stage 22 must clearly be due to the increase in leaf stage.

As previously mentioned, it is possible that temperature may have an influence on the results achieved at different leaf stages. According to Fig 4.1 it is clear that good control were achieved at leaf stage 2. Leaf stage 2 was sprayed in the autumn when the temperatures were higher than in the winter when the other leaf stages where sprayed. Leaf stage 8 was sprayed in sunny and warm weather, which could have lead to higher control percentages. Leaf stage 15 was sprayed at the beginning of winter when it was cloudy and the temperature was lower. When looking at Fig 4.8, it is clear that lower control percentages were achieved at leaf stage 15. When leaf stage 22 was sprayed, it was a sunny winters day, which can be one of the reasons for the better control achieved at leaf stage 22 (Fig 4.8). Leaf stage 25 was also sprayed in warmer winter weather, so climate should not play a major role in the difference between leaf stage 22 and leaf stage 25. Growth stage must have an influence in the weaker control percentages achieved at leaf stage 25.

The resistance factor (RF) is generally higher in the flowering stage, as mentioned in the case of *C. bonariensis* (Amsellem, Jansen, Driesenaar & Gressel, 1993). They found that the concentration required to achieve 50% inhibition of the resistant biotype was about 30 times that of the susceptible one just after germination. This increased to >300 times that of the susceptible biotype after 10 weeks of growth and then decreased to 20-fold, remaining constant except for a brief increase while bolting. This rise in the RF value could be due to an increase in the degree of sensitivity of sensitive plants when they start to flower. The differences in sensitivity as a function of developmental stage may be due to the fact that treated resistant plants at the rosette stage contain four times as much paraquat as plants with stems, which are about to flower (Szigeti & Lehoczki, 2003). The I_{50} of resistant biotypes is typically 15- to 30-fold for 2 week old vegetative *C. bonariensis* plants and 50- to

100-fold for 10 week old vegetative *C. bonariensis* plants (Amsellem, *et al.*, 1993; Ye & Gressel, 1994). Ye, Müller, Zhang and Gressel (1997) reported that the I_{50} of 2 week old paraquat resistant *C. bonariensis* seedlings was 15 times higher than that of the sensitive plants. These results are consistent with the data of Szigeti, Rácz, Darkó, Lásztity and Lehoczki (1996), who showed that paraquat resistance in was correlated with a 2.5-fold higher level of putrescine in a paraquat-resistant biotype of *C. canadensis*. Ye *et al.* (1997) reported that the I_{50} for paraquat in the resistant plants of 10 weeks old, is >50 times that of the sensitive plants at this time.

Studies to determine the effect of growth stage and resistance were done on glyphosate and paraquat resistant *C. canadensis* and *C. bonariensis*. Results also indicate that there is an increase in resistance with an increase in growth stage. The glyphosate rates required to reduce biomass of glyphosate-resistant *C. canadensis* by 50%, increased from 0.14 to 2.2 kg/ha as plant size increased from the 5-leaf to 25- to 30-leaf growth stage. The GR_{50} rate for the susceptible biotype increased from 0.02 to 0.2 kg/ha glyphosate. These results demonstrate that the difficult-to-control biotypes were resistant to glyphosate, that resistant biotypes could survive glyphosate rates of up to 6.72 kg/ha and that plant size affected both resistant and susceptible biotypes in a similar manner (Koger, Poston, Hayes & Montgomery, 2004). The resistance factor (RF) value for the rosette stage in *C. canadensis* plants resistant only to paraquat was 180. In plants resistant to both paraquat and atrazine, RF had values between 210 and 650 irrespective of the antecedents of the plant. In the flowering stage, RF values as high as 1000 were reported. It was found that, in the rosette stage of development, RF depended greatly on the treatment conditions, on possible previous treatments and on the site of origin of the plants. The effect recorded was also substantially influenced by the light intensity to which the plants were exposed during the bipyridyl treatment.

Although these experiments only tested MCPA and Sorgomil Gold 600 as additional herbicides for the control of resistant *C. bonariensis*, it is important to mention that other herbicides can be successfully used for the control of resistant *C. bonariensis* plants. Herbicides used for control of *C. canadensis* in crop fields include atrazine, alachlor, cyanazine and metolachlor (Buhler, 1992), triazine (de Prado, Dominguez & Tena, 1989) and paraquat (Vaughn & Vaughan, 1989). Negative cross-resistance can be a most useful pre-emptive, cost-effective tool for delaying the evolution of

resistance, as well as for resistance management, after resistant populations evolve. Gadamski *et al.* (2000) reported that eleven of 18 herbicides tested exerted significant negative cross-resistance to atrazine-resistant weeds, ranging from 0.03 to 0.67 of the concentration required to affect the triazine-sensitive type. No synergism was found between bentazon and fluroxypyr in a mixture on *Conyza*, even though both separately exerted negative cross-resistance. Using a mixture with half the amount of each component lowers the environmental effect of each component while controlling a broader spectrum of other weeds.

In a study done by Weaver, Downs and Neufeld (2004) it was found that susceptible and resistant *C. canadensis* populations showed a two- to three-fold difference in sensitivity to paraquat. The discriminating dose, at which all susceptible plants but none of the resistant plants were killed, was 1.12 kg ha^{-1}. *C. canadensis* is generally not controlled by linuron at the low rates used in many field crops (0.5 to 2.25 kg ha^{-1}). However, the higher use rates in orchards (4.5 kg ha^{-1}) and the addition of a surfactant had provided control of small rosettes in early spring.

Addition of other chemicals to glyphosate can improve the control of *C. bonariensis*. These chemicals include 2,4-D, Grazon ® DS, Tordon ® 75D, metsulfuron, atrazine and Dual ® Gold. Research has shown that Amitrole ® T could be strategically used as a late treatment to target mature survivors. Its excellent damaging effects on elongated shoots and flowering heads greatly reduced the addition of new seeds into the soil, although it did not completely kill the plant (Storrie, 2004).

4.6 Conclusions

Growth stage plays an important role in the manifestation of herbicide resistance in *C. bonariensis* in the Breede Valley. To prevent herbicide resistance in this weed, it is important to spray it as early as possible, because resistance increases with increase in leaf stage. Although farmers of the Breede Valley do not normally spray in the winter, it may become necessary to spray at that time if resistance is to be overcome. It has been shown that only one of the biotypes (Groenrivier) tested showed slight cross-resistance to both glyphosate and paraquat at the 15 and 22 leaf stages, which means

that these herbicides may become ineffective as control measures in the future due to the increasing developing cross-resistance.

This study has emphasised that producers have the means at their disposal to eliminate herbicide-resistant populations. A combination of early spraying and rotation of modes of action of herbicides can be used to eliminate recalcitrant populations. Another important aspect of successful herbicide usage on weed populations is the application rate of herbicides and the use of herbicide mixtures. Lower herbicide doses allow the accumulation of resistance alleles in the surviving population. At low herbicide rates there can be lower weed mortality, meaning that individuals expressing relatively weak resistance mechanisms will survive and contribute to the resistance gene pool. At low herbicide rates, both heterozygous and homozygous resistant individuals are likely to survive. When herbicides are applied at higher rates, heterozygous resistant individuals and individuals possessing only weak resistance mechanisms may be killed. Hence, higher herbicide doses result in higher weed mortality and less diversity of resistant genes in the surviving population. However, surviving individuals in such a population will be highly resistant and the continued use of high doses of the same herbicides will result in the rapid spread of highly resistant individuals – especially in a weed such as *C. bonariensis, which* has such an efficient seed dispersal system. The natural tendency of producers to spray at higher dosage rates when control at registered dosage rates is inadequate must be strongly discouraged.

Successful field management of herbicide-resistant weed populations depends upon the integration of alternative weed control methods. Available control strategies include the scope for effective herbicide use, exploitation of unique aspects of the biology of the weed species and manipulating the cropping system to maximize both chemical and nonchemical weed control.

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CHAPTER V

SUMMARY

Biological processes and characteristics are important factors in the introduction, spread and establishment of invasive weeds. These biological traits include reproduction, dispersal, phenology, physiology, protection from herbivores, tolerance to environmental extremes and interspecific interactions. Measures and methods to prevent dispersal, establishment and persistence of non-native weeds include knowledge of the most vulnerable growth stages, management strategies, maintenance of weed-free areas and sanitation of vehicles, transportation equipment, animals and other modes of dispersal (Bryson & Carter, 2004).

Conyza bonariensis is widespread in the world and it is very common in the Western Cape, South Africa. Since the weed was first reported in 1895, it has spread throughout South Africa to become one of the most abundant and troublesome problem plants. Reports of herbicide resistance in *C. bonariensis* to both paraquat and glyphosate were made in 2003 by Heap. This was the first report of resistance to glyphosate in this weed. The resistant populations appeared to be restricted to the Western Cape. Subsequently, resistance to glyphosate in *C. bonariensis* has been reported in Spain (Heap, 2004).

Ten years ago spraying of herbicides specifically for the control of *C. bonariensis* in the Western Cape would have been rare, now it is quite common. The reasons why a relatively insignificant weed exploded into aggressive herbicide resistant populations during a relatively short space of time are not obvious. Possible reasons might include:

1. The use of inferior sub-standard herbicides or the application of sub-lethal dosage rates.
2. A succession of dry years where drought stressed plants were not effectively controlled.
3. Lack of rotation of herbicides with different modes of action leading to strong selection pressure resulting in resistant biotypes.
4. Absence of effective cover crops to provide competition for *C bonariensis.*

The success of *C. bonariensis* as a weed can, to a large extent, be attributed to the plant's prolific seeding, its ability to grow rapidly and flower in a range of photoperiods and its relatively pubescent cuticula which makes for poor contact between sprayed herbicide droplets and the leaf. The fecundity of this weed has determined that the best long-term management strategy for *C. bonariensis* is to treat weeds at early growth stages, to manage survivors and to prevent the replenishment of new seeds into the soil seed bank. The rapid development of herbicide resistance in *Conyza* spp. overseas suggests that an integrated management program would need to be implemented in order to prevent or retard its resistance to herbicides (Wu & Walker, 2004).

In summarizing the results obtained from the study, it is obvious that herbicide resistance is no longer just a threat to farmers in Southern Africa, but a devastating reality. Heap (2005) has reported only six weeds species resistant to glyphosate worldwide. What is worrying is that four of these six weed species occur in South Africa and to be more specific, the Western Cape. This area has thus the highest incidence of glyphosate resistant species in the world (Chapter I). Paraquat resistance occurs in three of these four species. Currently, the most troublesome weeds with regards to resistance, are *Lolium multiflorum*, *L. rigidum* and *C. bonariensis*. South Africa was the first and only country to date to report glyphosate resistance in *Plantago lanceolata.*

There are different factors that influence the germination process of *C. bonariensis* seed. The optimum temperature range for different weeds is an important factor in the germination of weed seeds and is currently the best researched of all factors influencing germination. Other important factors include soil depth and the influence of light on the germination process. During this study it was found that the optimum temperature range for *Conyza bonariensis* germination is 15-30°C (Chapter II). By knowing the optimum temperature range, it is easier to determine when germination flushes will occur. However, due to the lack of dormancy germination of *C. bonariensis* can occur throughout the year. Soil depth also plays an important role in the germination process. When the optimum soil depth of germination is known, it is

possible to determine if there are any cultivation methods that can inhibit the germination of *C. bonariensis*. The results of this study indicate that the optimum depth for seedling emergence is 0-1cm. This factor is linked to the type of light required for germination. It is known that *C. bonariensis* seeds require light to germinate, so it is possible to cultivate soils and bury seeds at a depth of more than 2 cm so that the germination process is inhibited. The fact that many farmers with vineyards and plantations in the Western Cape do not make use of tillage, may explain the reason why so many cases of resistance are being reported.

Since herbicide resistance in *C. bonariensis* has been brought to the attention of producers in the Western Cape, many more farmers have recently complained about the occurrence of herbicide resistant populations of the weed. One of the aims of this study was to determine if paraquat and glyphosate resistance is really such a big problem in this region of South Africa. During this study it was found that many farmers have a reason to be concerned about resistance in the *C. bonariensis* biotypes occurring on their farms (Chapter III). However, it was found that some reported cases of resistance turned out to be false. These cases involve, for instance the Sandrivier population, that was reported as being resistant to glyphosate and to a lesser extent to paraquat, but after the greenhouse trials (Fig 3.5 and 3.6) it was found that this biotype (Sandrivier) was almost completely controlled by glyphosate applied at 2 L ha^{-1}. This same biotype was completely controlled by paraquat applied at 2 L ha^{-1}. It was therefor considered to be a susceptible biotype to paraquat and glyphosate. Another case of reported resistance that turned out to be negative was in the case of Alartskraal which was reported as being extremely resistant to glyphosate, but the greenhouse trial (Fig 3.6) showed that Alartskraal was completely controlled by glyphosate applied at 2 L ha^{-1}. The reasons why resistance may occur in the field but not in the greenhouse may be ascribed to many factors unlinked to resistance but it is beyond the scope of this study to comment on them here. However, later stages of application may responsible for poor control of many *C. bonariensis* biotypes under field conditions. Another cause of concern to farmers is the development of cross-resistance to paraquat and glyphosate in *C. bonariensis*. This study indicated that only one of the biotypes (Groenrivier) tested showed slight cross-resistance to these two herbicides. All of the other biotypes that were tested were completely controlled by one or the other herbicide.

It is relatively easy to determine if resistance occurs, but to find alternative ways of controlling resistance is much more difficult. In this study two herbicides with different mode of action were tested (Chapter IV). It was found that Sorgomil Gold 600 gave poor control after 2-leaf stage, but it is important to remember that Sorgomil must be applied before the 4-leaf stage for optimum effectiveness. MCPA gave good control in all biotypes tested and can be used effectively in controlling resistant *C. bonariensis*. Growth stage plays an important role in the appearance of resistance of many weeds, if not all weeds. One aim of this study was to determine if growth stage had an important influence on the appearance of herbicide resistance in *C. bonariensis*. The results of this study clearly indicate that growth stage does have an important influence on the percentage control of seedlings and plants. While the mean percentage control for all herbicides at 2-leaf stage were approximately 100%, the mean percentage control decreased, as the plants grew older. It was found that percentage control decreased at the 8-leaf stage, but increased again up to the 22-leaf stage. In most of the biotypes tested, the mean percentage control over most of the herbicides tested, decreased at the 25-leaf stage. This indicates that herbicide resistance increases with an increase in growth stage. Growth stage may play an important role in the control of *C. bonariensis*. If seedlings are sprayed at 2-leaf stage most of the seedlings will be controlled and the occurrence of herbicide resistance will be slowed down. If plants are allowed to grow to big before herbicide application, herbicide resistance will become a still bigger problem in the future.

Some factors that were not included in this study, but may be important in the germination process, are the influence of soil pH and viability of *C. bonariensis* seeds in the soil. Further studies could be conducted to find the pH range in which optimum germination occurs and to determine the period of viability of *C. bonariensis* seed in the soil. The results of these above mentioned studies could be used as a useful guide to determine to what extent tillage could have an influence on the germination of *C. bonariensis* and thus reduction in the soil seed bank. Further studies can also be done to determine the effect of other herbicides on the control of *C. bonariensis* and to determine the effect of paraquat and glyphosate with mixtures on the control of flowering and/or bolting *C. bonariensis* plants. The extent, to which herbicide

resistance and cross-resistance in *C. bonariensis* in the Western Cape occur, could be a study on its own.

To control herbicide resistant *C. bonariensis*, the focus should be on managing weed populations through time rather than minimizing the yield effect of weeds in a single season or year. Rather than viewing weeds as an annual production problem, the weed seed bank can be considered a renewable resource stock and the management goal is to deplete this resource stock through time.

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CHAPTER VI

ANEXURE A

ANOVA'S FOR EXPERIMENTS DONE DURING STUDY

A1: Chapter 2: The effect of light quality, temperature and depth of burial on the germination of *Conyza bonariensis*

A1.1: The percentage germination of *Conyza bonariensis* seed in different light qualities

Analysis of variance

Source of variation	s.s.	d.f.	m.s.	F	p
Intercept	24704.17	1	24704.17	653.2613	0.000000
Treatment	23599.50	3	7866.50	208.0167	0.000000
Error	756.33	20	37.82		

A1.2: The influence of soil depth on the percentage germination of *Conyza bonariensis* seeds at different days

Analysis of variance

Source of variation	s.s.	d.f.	m.s.	F	p
Intercept	11667.86	1	11667.86	309.2448	0.000000
Depth	46019.14	6	7669.86	203.2819	0.000000
Error	792.33	21	37.73		
DAY	320.86	2	160.43	26.5624	0.000000
DAY*Depth	400.14	12	33.35	5.5210	0.000015
Error	253.67	42	6.04		

A1.3: The different temperature optimums for the germination of different biotypes of *Conyza bonariensis*

Analysis of variance

Source of variation	s.s.	d.f.	m.s.	F	p
Intercept	25576.33	1	25576.33	3991.682	0.00000
Temperature	24478.17	8	3059.77	477.536	0.00000
Biotype	1457.06	2	728.53	113.701	0.00000
Temp*Biotype	2183.44	16	136.47	21.298	0.00000
Error	519.00	81	6.41		

A2: Chapter 3: Confirmation of paraquat and glyphosate resistance in *Conyza bonariensis*

A2.1: The effect of different glyphosate concentrations on the control of different *Conyza bonariensis* biotypes in a petri dish assay (Figure 3.1)

Analysis of variance

Source of variation	s.s.	d.f.	m.s.	F	p
Intercept	94453.78	1	94453.78	2648.237	0.000000
Biotype	5790.26	2	2895.13	81.172	0.000000
DOSAGE	55925.97	5	11185.19	313.604	0.000000
Biotype*Dosage	8590.49	10	859.05	24.085	0.000000
Error	642.00	18	35.67		

A2.2: The effect of different paraquat concentrations on the control of different *Conyza bonariensis* biotypes in a petri dish assay (Figure 3.2)

Analysis of variance

Source of variation	s.s.	d.f.	m.s.	F	p
Intercept	69876.04	1	69876.04	208.3261	0.000000
Biotype	3151.04	1	3151.04	9.3944	0.009807
TREATMENT	36330.21	5	7266.04	21.6627	0.000013

Biotype*TREATMENT	3742.71	5	748.54	2.2317	0.118200
Error	4025.00	12	335.42		

A2.3: The effect of different glyphosate concentrations on the control of two *Conyza bonariensis* biotypes in a petri dish assay (Figure 3.3)

Analysis of variance

Source of variation	s.s.	d.f.	m.s.	F	P
Intercept	135450.4	1	135450.4	9105.908	0.000000
Biotype	2583.4	1	2583.4	173.672	0.000000
TREATMENT	16876.9	5	3375.4	226.916	0.000000
Biotype*TREATMENT	3753.9	5	750.8	50.472	0.000000
Error	178.5	12	14.9		

A2.4: The effect of different paraquat concentrations on the control of two *Conyza bonariensis* biotypes in a petri dish assay (Figure 3.4)

Analysis of variance

Source of variation	s.s.	d.f.	m.s.	F	P
Intercept	159740.2	1	159740.2	23094.96	0.000000
Biotype	130.7	1	130.7	18.89	0.000951
TREATMENT	19382.8	5	3876.6	560.47	0.000000
Biotype*TREATMENT	167.3	5	33.5	4.84	0.011811
Error	83.0	12	6.9		

A2.5: The effect of different paraquat doses applied to six different *Conyza bonariensis* biotypes (Figure 3.5)

Analysis of variance

Source of variation	s.s.	d.f.	m.s.	F	P
Intercept	790273.7	1	790273.7	9199.192	0.000000
Biotype	37229.0	5	7445.8	86.673	0.000000
TREATMENT	158529.6	5	31705.9	369.073	0.000000

Biotype*TREATMENT	9930.3	25	397.2	4.624	0.000000
Error	8762.5	102	85.9		

A2.6: The effect of different glyphosate doses applied to six different *Conyza bonariensis* biotypes (Figure 3.6)

Analysis of variance

Source of variation	s.s.	d.f.	m.s.	F	P
Intercept	822808.8	1	822808.8	6012.645	0.000000
Biotype	2088.6	5	417.7	3.052	0.013104
TREATMENT	182130.8	5	36426.2	266.183	0.000000
Biotype*TREATMENT	9116.1	25	364.6	2.665	0.000294
Error	13958.3	102	136.8		

A2.7: The effect of different paraquat doses applied to seven different *Conyza bonariensis* biotypes (Figure 3.7)

Analysis of variance

Source of variation	s.s.	d.f.	m.s.	F	P
Intercept	371488.1	1	371488.1	1497.840	0.00
Biotype	187209.8	6	31201.6	125.805	0.00
TREATMENT	81770.8	5	16354.2	65.940	0.00
Biotype*TREATMENT	59531.3	30	1984.4	8.001	0.00
Error	31250.0	126	248.0		

A3: Chapter 4: The effect of growth stage on resistance in *Conyza bonariensis*

A3.1: The percentage control of different herbicides on *Conyza bonariensis* at two leaf stage (Figure 4.1)

Analysis of variance

Source of variation	s.s.	d.f.	m.s.	F	P
Intercept	486019.4	1	486019.4	299088.9	0.00
Biotype	2178.9	6	363.2	223.5	0.00
Treatment	2769.8	3	923.3	568.2	0.00
Biotype*Treatment	11505.4	18	639.2	393.3	0.00
Error	45.5	28	1.6		

A3.2: The effect of different herbicides on the control of one biotype (Die Hoek) of *Conyza bonariensis* at different leaf stages (Figure 4.2)

Analysis of variance

Source of variation	s.s.	d.f.	m.s.	F	p
Intercept	376302.1	1	376302.1	2223.077	0.000000
Treatment	12968.7	3	4322.9	25.538	0.000000
Leaf stage	2343.7	3	781.2	4.615	0.008572
Treatment*Leaf stage	7968.7	9	885.4	5.231	0.000216
Error	5416.7	32	169.3		

A3.3: The effect of different herbicides on a biotype (Sonja) of *Conyza bonariensis* at different leaf stages (Figure 4.3)

Analysis of variance

Source of variation	s.s.	d.f.	m.s.	F	p
Intercept	210013.0	1	210013.0	848.8947	0.000000
Treatment	51705.7	3	17235.2	69.6667	0.000000
Leaf stage	3997.4	3	1332.5	5.3860	0.004079
Treatment*Leaf stage	16992.2	9	1888.0	7.6316	0.000007

Error	7916.7	32	247.4

A3.4: The effect of different herbicides on a biotype (Wysersdrift) of *Conyza bonariensis* at different leaf stages (Figure 4.4)

Analysis of variance

Source of variation	s.s.	d.f.	m.s.	F	P
Intercept	266263.0	1	266263.0	511.2250	0.000000
Treatment	24414.1	3	8138.0	15.6250	0.000002
Leaf stage	247.4	3	82.5	0.1583	0.923530
Treatment*Leaf stage	19283.9	9	2142.7	4.1139	0.001373
Error	16666.7	32	520.8		

A3.5: The effect of different herbicides on a biotype (Heimat) of *Conyza bonariensis* at different leaf stages (Figure 4.5)

Analysis of variance

Source of variation	s.s.	d.f.	m.s.	F	p
Intercept	240833.3	1	240833.3	513.7778	0.000000
Treatment	35937.5	3	11979.2	25.5556	0.000000
Leaf stage	3541.7	3	1180.6	2.5185	0.075628
Treatment*Leaf stage	29687.5	9	3298.6	7.0370	0.000015
Error	15000.0	32	468.8		

A3.6: The effect of different herbicides on a biotype (Groenrivier) of *Conyza bonariensis* at different leaf stages (Figure 4.6)

Analysis of variance

Source of variation	s.s.	d.f.	m.s.	F	P
Intercept	300833.3	1	300833.3	537.3023	0.000000
Treatment	7812.5	3	2604.2	4.6512	0.008276
Leaf stage	5937.5	3	1979.2	3.5349	0.025590
Treatment*Leaf stage	16250.0	9	1805.6	3.2248	0.006816

Error	17916.7	32	559.9

A3.7: The effect of different herbicides on a biotype (Boskloof) of *Conyza bonariensis* at different leaf stages (Figure 4.7)

Analysis of variance

Source of variation	s.s.	d.f.	m.s.	F	P
Intercept	376302.1	1	376302.1	2408.333	0.000000
Treatment	5572.9	3	1857.6	11.889	0.000022
Leaf stage	8072.9	3	2691.0	17.222	0.000001
Treatment*Leaf stage	17552.1	9	1950.2	12.481	0.000000
Error	5000.0	32	156.3		

A3.8: The effect of different herbicide treatments at different leaf stages on *Conyza bonariensis* (Figure 4.8)

Analysis of variance

Source of variation	s.s.	d.f.	m.s.	F	p
Intercept	1750009	1	1750009	2589.510	0.000000
Treatment	98359	3	32786	48.515	0.000000
Leaf stage	8498	3	2833	4.192	0.006377
Treatment*Leaf stage	68064	9	7563	11.191	0.000000
Error	183819	272	676		