IMPROVING WEED CONTROL OPTIONS FOR RYEGRASS/CLOVER PASTURES THAT CONTAIN PLANTAIN *(Plantago lanceolata)*

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Abstract

The inclusion of narrow-leaved plantain (*Plantago lanceolata*) in the traditional pasture system of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) is preferred by many New Zealand farmers nowadays to assist with nitrogen loss mitigation and to improve summer production. Control of weeds using selective herbicides becomes more difficult after adding extra species to a grass/clover sward. The work in this thesis investigated weed control options for ryegrass/clover pastures in New Zealand that contain plantain. This included determining the tolerance of plantain to different herbicides and identifying the herbicide options most effective for weeds.

There has been some breeding of phenoxy herbicide tolerance into the Agritonic cultivar of plantain in New Zealand. In this thesis, the level of tolerance in Agritonic plantain was compared with Tonic plantain to MCPB, MCPA, MCPB/MCPA mix, 2,4-D and 2,4-DB in two glasshouse experiments. The tolerance generally appeared to be 1.3 to 3.4-fold, so not large but potentially useful.

The thesis also investigated the tolerance of plantain, white clover and perennial ryegrass to a range of herbicides applied to a mixed sward of these species at an early stage of establishment in spring. The effectiveness of the herbicides was also assessed for controlling weeds that established within the swards using two field trials. Half of the recommended rate of MCPB was less damaging to both cultivars of plantain than the recommended rates of MCPB and MCPB/MCPA, but could not control most of the weeds present. The recommended rate of MCPB/MCPA had very little detrimental effect on young clover or Agritonic plantain and gave good weed control. Flumetsulam was fairly safe to use in the plantain-based pasture though it suppressed plantain initially, which recovered after 3-6 months in each trial. Bentazone was safe for the plantain, ryegrass and clover and suppressed most of the weeds, but only if there was no rainfall in the hours after herbicide application. Mowing after each harvest controlled redroot, black nightshade and fathen and also suppressed docks temporarily. The most effective weed control strategy that was also selective involved a combination of bentazone + half rates of MCPB/MCPA followed by mowing which gave useful control of weeds including docks for many months.

The mechanism of tolerance of Agritonic plantain to 2,4-D was investigated using radiolabeled herbicide (^{14}C-2,4-D) in two experiments. Absorption/translocation and metabolism of the herbicide was studied in both this cultivar and Tonic plantain for comparison. The tolerance to 2,4-D in Agritonic plantain appeared to involve reduced translocation of the herbicide, though reduced absorption may have also contributed.

Two glasshouse experiments were conducted to test the tolerance of mature plantain plants (both Agritonic and Tonic) to application of some herbicides suitable for use in weed wipers (glyphosate, clopyralid, aminopyralid, dicamba, picloram and triclopyr) to the seed-heads, simulating potential contact during wiper application to pasture weeds. Aminopyralid and a low rate of glyphosate were found to be the least damaging treatments and should be safe to use for weed wiping within swards containing plantain. A high rate of glyphosate and a glyphosate/metsulfuron mix caused the most damage to plantain, and this damage was greater following simulated rainfall after application.

Keywords: Plantain, Agritonic, Tonic, pasture, ryegrass, clover, herbicide, phenoxy herbicides, tolerance, MCPA, MCPB, 2,4-D, flumetsulam, bentazone, weeds, weed control.

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Dedication

This work is dedicated to my parents, Jyantri Lal Shrivastav and Saroj Devi Shrivastav and in-laws Ayodhya Prasad Shrivastava and Uma Devi Shrivastava for their love, encouragement, and support.

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Abbreviations

2,4-D	2,4-dichlorophenoxyacetic acid
2,4-DB	4-(2,4-dichlorophenoxy)butyric acid
A	Agritonic
ai	Active ingredient
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
dpm	Disintegrations per minute
drc	Dose-response curve
GR_{50}	Dose that caused growth reduction of 50%
kBq	Kilobecquerel
Kg ha^{-1}	Kilograms per hectare
LD_{50}	Lethal dose for 50% of population
LSD	Least Significant Difference
MBq	Megabecquerel
MCPA	2-methyl-4-chlorophenoxyacetic acid
MCPB	4-(4-chloro-2-methylphenoxy)butanoic acid
rpm	Revolution per minute
T	Tonic
t	Tonne

Chapter 1

Introduction, objectives and thesis structure

1.1 Introduction

Narrow-leaved plantain (*Plantago lanceolata*, hereafter referred to as plantain) was once regarded as a serious weed of lawns, grasslands, and pasture. However, the interest in plantain has increased in New Zealand following breeding to increase its productivity and nutritional values for pasture production. The traditional pastoral system of New Zealand was based on grass species with clover where the predominant pasture mix was perennial ryegrass (*Lolium perenne*) with white clover (*Trifolium repens*) (Kemp et al., 1999). The production of both ryegrass and white clover can be limited throughout summer and autumn months (Moorhead et al., 2002) and feed deficits can occur when dry. Lots of research has been done with pure plantain but less with mixed swards. The mixed swards comprise plantain, red clover, white clover and ryegrass which is preferred by farmers nowadays as it provides improved persistence, a longer grazing season and produces greater herbage quality and yield as compared to mono species herb swards, which directly improves animal performance (Nie et al., 2008; Golding et al., 2011; Hutton et al., 2011; Sinhadipathige et al., 2012).

Weeds can also be defined as any plant or vegetation that interferes with the objective of farming or forestry like growing crops, grazing animals or cultivating forest plantations (Popay, 2008b). Despite using correct management practices when establishing new forages, there is still rapid establishment of weeds in the field (Fraser et al., 2016). There are generally less herbicide options available to control weeds in plantain mixed swards as compared to other forages and legumes (Lockley and Wu, 2008) and also the effect of herbicide on plantain is less known. Therefore, more research needs to be done to control weeds in mixed swards of pasture.

1.2 Objectives

The overall objective of this PhD was to improve weed control options for ryegrass/clover pastures in New Zealand that contain plantain. This included determining the tolerance of pasture species to different herbicides and identifying the herbicide options most effective for weeds. The results from experiments discussed in each chapter in this thesis provided further understanding of some aspects of tolerance to phenoxy herbicides by plantain in New Zealand.

The specific objectives of this study were:

1. To determine how tolerant Agritonic is compared with Tonic to various phenoxy herbicides by comparing dose response curves for seedlings grown in a glasshouse.
2. To conduct field trials to assess the tolerance of various herbicides in newly establishing pastures in which plantain, white clover and ryegrass are grown together, and also to assess their effectiveness on weeds.
3. To look at the mechanism of tolerance to phenoxy herbicides in the Agritonic cultivar using radiolabeled herbicide.
4. To determine whether the herbicides that could be used in weed wipers to selectively control tall weeds damage plantain with seed-heads that get the herbicides wiped on them. assess the use of weed wipers in mixed swards to control tall weeds without damaging pasture, especially plantain with seed-heads.

1.3 Thesis Structure

This thesis consists of eight chapters. Chapter 1 is an introductory chapter with a problem statement and PhD project direction and outlook including objectives to address. Chapter 2 is a literature review that contains background information which has three parts. The first part reviews literature published on narrow-leaved plantain (*Plantago lanceolata*), its biology, uses, cultivars and agronomic practices used for growing it. In the second part, problems caused by weeds in pasture are discussed, while the last part explores the options for controlling weeds in plantain-based pasture which include non-chemical and chemical (herbicides) methods, including the use of weed wipers to control tall weeds. In Chapter 3, glasshouse experiments are described to investigate the tolerance of Agritonic plantain compared to Tonic plantain to several phenoxy herbicides. In Chapter 4, a field experiment was conducted to assess the tolerance of plantain cultivar (Agritonic and Tonic) to a variety of herbicides relative to clover and weeds. Chapter 5 describes a second field trial which repeated the one discussed in Chapter 4, to confirm the results obtained with a few changes. This field trial was set up at another location with more weeds and also increased seeding rate for ryegrass. In Chapter 6, the mechanism of tolerance in Agritonic plantain to 2,4-D was investigated using radiolabeled herbicide (^{14}C-2,4-D). In Chapter 7, glasshouse experiments were described which were to test the tolerance of plantain (Agritonic and Tonic) with seed heads to the application of some herbicides suitable for use in weed wipers (glyphosate, clopyralid, aminopyralid, dicamba, picloram and triclopyr) to the seed-heads simulating potential contact during weed wiping of pasture weeds. Chapter 8 summarizes the main findings of this PhD project including a brief discussion of implications of the results, and priorities for future research are suggested.

Chapter 2

Literature review

2.1 Narrow-leaved plantain (*Plantago lanceolata*)

2.1.1 Biology of plantain

Plantago lanceolata is a flowering plant from the family Plantaginaceae. It is commonly known as narrow-leaved plantain in New Zealand, but in other countries it is called ribwort plantain, English plantain, rib leaf and lamb's tongue (Stewart, 1996;). Within this thesis, it will generally be referred to simply as plantain. The plant is a perennial herb with broad distribution in native grasslands of the temperate world (Stewart, 1996). It is apparently native to Europe, North Africa and West and South Asia (USDA-Agricultural Research Service, 2019) but is now present and widespread in the Americas, Australia and New Zealand as an introduced species , and also throughout Japan (Morita, 2002) and many countries of Africa.

The plant is erect, leafy with a deep, dense, branching fibrous root system (Reed, 2009) spreading downwards from the crown. Leaves are smooth, with rosette growth from a central crown (Stewart and Charlton, 2006) which are up to 25 cm long, purplish at the base, and fine hairs cover the upper surface and also along the lower side ribs (Reed, 2009). The leaves are lance shaped, spreading or erect, and scarcely toothed with three to seven strong parallel veins narrowed to a short petiole (Laws and Genever, 2013). The flower stalks are erect, leafless, deeply furrowed, and about 10-50 cm long, ending in an ovoid inflorescence of many small flowers densely crowded together each with a pointed bract (Reed, 2009). It is self-sterile and entirely cross fertilised (Warwick and Briggs, 1979). Flowering occurs from the base of the head upwards (Rowarth, 1998). There is a single spike per stem which is up to 2.5 cm long, conical or cylindrical shaped which turns brown to black from grey green on ripening (Reed, 2009). Flowers are tiny and creamy white coloured with long stamens. Each flower can produce up to two hundred seeds with 36,000 seeds per plant (Donaldson and Bowers, 1998). Each seed is oblong, dark brown to black, strongly indented on one side and 1 mm long (Reed, 2009). The flowers are generally pollinated by wind and sometimes by insects collecting pollen (Bond et al., 2007; Sharma et al., 2008).

Important agronomic characteristics that have led plantain to become a widely used pasture species in many parts of Australia and New Zealand are discussed below (Moorhead et al., 2002).

2.1.2 Uses of plantain

Plantain was once regarded as a serious weed of lawns, dry grasslands and roadsides (Cavers et al., 1980). However, there has now been increased interest in plantain in New Zealand for pasture production following breeding to increase its productivity. It has become a popular pasture species sown either alone or in a mix with other pasture species (Muir, 2012). It can establish rapidly (Peri et al., 2000) and grow on a wide range of agricultural soils varying in soil acidity from pH 4.2 to 7.8, with 5.8 considered as the optimum (Laws and Genever, 2013). It also tolerates a range of soil fertility levels (Charlton and Stewart, 1999) and many common pests and diseases (Moorhead et al., 2002).

The root system of plantain consists of a mix of tap and fibrous roots which provides some degree of tolerance to drought. The growth pattern is similar to perennial ryegrass where there is low to medium growth in winter and the main growth is in spring and autumn (Moorhead and Piggot, 2009). The use of plantain on farms is mainly to act as a component of mixed pasture where it contributes by filling the gaps in the sward (mostly in the low fertility dryland pastures) and where growth of grass is less vigorous (Stewart, 1996). Similarly, a report from a sheep and beef farm shows that weed infestation was reduced when the forage herbs chicory (*Cichorium intybus*) or plantain, were added in grass legume mixtures (Tozer et al., 2011).

Plantain can produce a high annual dry matter yield, with high nutritive value for ruminants when quality declines for other grazed forages during summer (Cranston, 2014; Somasiri et al., 2016). Powell et al. (2007) mentioned that a pure sward of plantain is as productive as a perennial ryegrass and white clover pasture over a year. Moorhead and Piggot (2009) found that plantain mixed pasture which included plantain, red clover, white clover and ryegrass produced yields significantly more than ryegrass-based swards by 1.8 t dry matter (DM)/ha and 0.9 t DM/ha during summer and autumn, respectively. During summer and autumn when ryegrass growth and quality is limited by soil moisture deficit, plantain provides large amounts of high quality feed (Glassey et al., 2012).

Plantain is highly palatable to grazing animals, providing a mineral rich forage. Stewart (1996) mentioned that calcium retention is increased by animals when even a small proportion of plantain is present in the swards. It has lower neutral detergent fibre (NDF) and acid detergent fibre (ADF) content as compared to perennial ryegrass which indicates that it is more digestible than perennial ryegrass (Cranston, 2014). The concentration of copper and zinc was found to be higher in plantain compared to ryegrass or clover (Harrington et al., 2006).

In addition to minerals, plantain is also rich in biologically active compounds (including antimicrobial compounds) which can inhibit rumen fermentation and change the volatile fatty acid composition which reduces scouring and daggyness in lambs (Stewart, 1996). Trials conducted for animal liveweight gain with plantain found variable results depending on whether swards were pure or mixed plantain. The liveweight gain was equal to or less than endophyte-free perennial ryegrass for pure plantain swards (Derrick et al., 1993; Fraser and Rowarth, 1996). Mixed swards containing chicory, plantain, red clover and white clover offered to ewes in late pregnancy and during lactation increased ewe and lamb liveweight gain, milk production, body condition score and improved lamb survival in comparison to ewes grazing perennial ryegrass and white clover (Golding et al., 2011; Hutton et al., 2011; Kenyon et al., 2017). During summer and autumn, a plantain-clover mixture can increase the growth rates of lambs and young cattle when compared to grass-based swards (Somasiri et al., 2016). Plantain can also be used for medical purposes like treatment of respiratory and inflammatory skin diseases (Marchesan et al., 1998).

Plantain inclusion in pastures has been identified as a potential tool for nitrogen loss mitigation in the environment (Judson et al., 2019) from dairy farm systems (Bryant et al., 2019; Dodd et.al., 2019). Some studies suggest that plantain, when incorporated into mixed swards, can reduce the amount of N excreted in urine from livestock production system, while maintaining similar herbage production to perennial ryegrass and white clover mixes (Woodward et al., 2013; Box et al., 2016; Box and Judson, 2018).

2.1.3 Plantain cultivars

The ability to establish on a wide range of soils (even low fertility), higher responsiveness to N, and the good palatability for livestock of narrow-leaved plantain (Stewart, 1996; Reed, 2009; Cranston, 2014) has resulted in release of a number of cultivars of this species for growing alone or in pasture mixes. Several commercial plantain cultivars have been developed in New Zealand which have better suitability for livestock grazing and higher forage yield than the wild plants. The first plantain cultivar Grasslands Lancelot was released in New Zealand in 1993 (Fraser and Rowarth, 1996; Laws and Genever, 2013). It was recognised by Dr W. Rumball and developed for pasture use by AgResearch Grasslands, selected from ecotypes in North Island of New Zealand (Rumball et al., 1997). Along with this, other plantain cultivars are Boston, Endurance, Hercules, Captain, Tonic and Agritonic. Tonic and Agritonic cultivars are only discussed here.

The improved cultivar 'Ceres Tonic' is believed to be derived from selections made in 50 poly cross parental clones originating from northern Portugal and selected in New Zealand for very erect growth habit (Pyne Gould Guiness Ltd, 1996; Stewart, 1996; Muir, 2012). It remains erect under a wide range of conditions while Grasslands Lancelot has plasticity to become prostrate under close grazing (Stewart, 1996). It is a perennial plantain cultivar which is different from Grasslands Lancelot and common weed types found in pasture with larger leaves, six days earlier flowering under New Zealand conditions and better autumn winter production (Muir, 2012; Laws and Genever, 2013; DairyNZ, n.d). It has a higher crown and is more suited to rotational grazing (Muir, 2012; Department of Primary Industries/ NSW Government, n.d.). Pure swards of Ceres Tonic have produced 8.4 to 19.1 t DM/ha/year (Stewart, 1996; Powell et al., 2007; Minneé et al., 2013).

'Agritonic' is a forage plantain from the breeding program which created Tonic. It has an upright growth habit, higher shoot, increased leaf number than Tonic and some additional tolerance to grazing and other farm management stresses. It is claimed to be an ideal option for inclusion in dense grass/legume pasture mixes at 1-3 kg/ha where grazing pressure is intense and often unnoticed (Agricom, 2018).

2.1.4 Agronomic practices for growing plantain

Plantain requires careful management to obtain optimal animal production and persistence in the sward.

2.1.4.1 ***Soil requirements***

Plantain can grow well on a wide range of agricultural soils (even low fertility soils), being drought, frost and pest tolerant (Stewart and Charlton, 2006; Laws and Genever, 2013). However, it performs best and persists longer when grown on free draining soils that have high fertility and are not prone to water logging or treading damage in wet weather (DairyNZ, October 2013a). Although it can also grow in soils with a wide range of acidity (pH 4.2 to 7.8), 5.8 is considered optimum (Stewart, 1996) on clay soil.

2.1.4.2 Sowing and establishment

Plantain establishes best in soil temperatures of 10–12°C at planting (Laws and Genever, 2013). Due to its small sized seed, it can be broadcast or drilled at a maximum depth of 10 mm into new seed beds (DairyNZ, October 2013a). Broadcasting of seed is the cheapest and simplest technique, but it reduces plant establishment, increases weed invasion and reduces yield (DairyNZ, October 2013a). Direct drilling is considered to be the better method for seedling establishment and survival (Glassey et al., 2012) than broadcasting (Bryant et al., 2019).

Plantain can be sown in spring or autumn after weed control (Reed, 2009) but spring sowing is preferred as plants develop quickly. Late autumn sowing is not suggested as plantain development is slower in cooler weather (DairyNZ, October 2013a).

2.1.4.3 Seed rate

The recommended seed rates for Agritonic are 1-3 kg/ha and Tonic 2-4 kg/ha in mixed swards, and 8-14 kg/ha and 8-10 kg/ha as pure stands, respectively (Agricom, 2018; DairyNZ, October 2013a). However, recent research conducted in South America suggested best sowing rates were 11-12 kg/ha in pure swards and 6 kg/ha in mixed swards where average soil temperature at planting was around 11.7°C (Laws and Genever, 2013). According to Reed (2009), plantain can be sown at a rate of 5-10 kg/ha as a single species, 5-10 kg/ha with specialist clover pastures and 2-4 kg/ha with grass pastures or brassica fodder species.

2.1.4.4 Fertiliser requirements

Plantain is highly responsive to nitrogen fertiliser, i.e. 70 kg/ha of urea improves its establishment (DairyNZ, October 2013a). Rowarth (1998) suggested 120 kg N/ha, half of which is applied in September (at the time of rapid growth of plantain) and the remaining half in early October. Plantain sown with 10-15 kg/ha of phosphate gives better yields than no phosphate. At least two to four applications of 35 kg N/ha post grazing is recommended over the spring/summer period (DairyNZ, October 2013b).

2.1.4.5 Insect pest and disease control

Plantain is affected by a range of insects and is an alternate host of several aphid species (Bond et al., 2007). Also, red-legged earth mites (*Halotydeus destructor*), slugs and snails are potential threats to plantain at all stages of growth (Reed, 2009). It is a host to different Lepidopterans such as *Junonia coenia* (common buckeye), *Spilosoma congrua* (Agreeable tiger moth), and *Melitaea cinxia* (Glanville fritillary) which lay eggs to provide a food source for the larvae when they hatch (*Plantago lanceolata*, n.d.). With the increasing use of plantain within New Zealand, two species of Geometridae moths, *Scopula rubraria* and *Epyaxa rosearia*, have now become very common on infested plantain swards, particularly in the North Island (Gerard et al., 2018). The caterpillars of *Epirrhoe alternata* (common carpet moth), *Pieris rapae* (white butterfly), *Plutella xylostella* (diamondback/ cabbage moth) start feeding on plantain leaves in late February to mid-March, which can be controlled using an approved insecticide and grazing every 21-24 days to reduce caterpillar populations and damage from the populations during this interval (DairyNZ, October 2013b). The fungus *Phomopsis subordinaria* has been found establishing after the first harvest in plantain stands but the infestation was less than 5% of seed-heads by the end of autumn (Rowarth, 1998).

2.1.4.6 Weed management

Rowarth (1998) recommended that fields should be weed free before sowing as there are very few recommended post establishment herbicides for this species. Low sowing rates are not recommended as bare ground allows weed establishment and competition with plantain

(Rowarth, 1998). Plantain swards should be established by applying herbicide to existing pasture and direct-drilling seed for reduced weed establishment and optimal economic outcomes (Glassey et al., 2012).

2.1.4.7 Grazing management and persistence

Plantain is moderately tolerant to treading and soil compaction and hence is best suited to rotational grazing (Kemp et al., 1999). Plantain requires a longer time after sowing before it can be grazed for the first time compared to perennial ryegrass (Stewart, 1996). Plantain should not be grazed until it is at the six leaf stage, i.e. the plants have six fully grown leaves, in order to minimise plant losses and ensure well developed root systems to improve survival (Powell et al., 2007; DairyNZ, October 2013a). This is normally 7-8 weeks after spring sowing. Plantain can tolerate continuous and close grazing. A 4-5 week grazing interval is recommended to gain maximum production (Sanderson et al., 2003), though Reed (2009) recommends a 3-4 week rotation in late spring to maintain seed-head palatability (Reed, 2009). Leafy plantain has higher quality compared to plants with seed-heads, so grazing management should aim to minimize seed-head frequency (Charlton and Stewart, 1999).

2.2 Problems caused by weeds in pasture

Weeds can be defined as plants growing out of place or where they are not desired (Monaco et al., 2002). Weeds can also be defined as any plant or vegetation that interferes with the objective of farming or forestry like growing crops, grazing animals or cultivating forest plantations (Popay, 2008b). There are estimated to be about 245 species of weeds from 40 different families in New Zealand pastures (Popay et al., 2010; Champion et al., 2012). From published papers, the top 10 most important weeds are *Ulex europaeus* (gorse), *Critesion* spp. (barley grass), *Jacobaea vulgaris* (ragwort), *Carduus nutans* (nodding thistle), *Cirsium arvense* (Californian thistle), *Ranunculus acris* (giant buttercup), *Rubus fruticosus* (blackberry), *Cytisus scoparius* (broom), *Achillea millefolium* (yarrow) and *Juncus sarophorus* (rushes) (Ghanizadeh and Harrington, 2019). Weeds present in pasture not only reduce production of pastures and increase cost of production but also cause problems in many ways which are discussed below.

2.2.1 Competition by weeds

Competition between weeds and pastures generally inhibits growth of pasture by weeds. It is a major issue in establishing pastures, where new pasture can get killed by weed competition and result in pasture with very low density of useful plant species (Matthews et al., 1999). Weeds compete for moisture, light and nutrients thus reducing the yield (Bradley et al., 2004). They can compete directly with pastures replacing desirable pasture species, filling in gaps, reducing their nutritional value, quality and yield (Bourdot and Saville, 2002). The weeds present in pasture can affect the canopy of desirable plants, influencing their productive and reproductive capacity (Bowcher, 2002). Competitive effects of weeds can be seen in nodding thistles which caused root competition with pasture, effects on soil moisture, nitrogen immobilisation and light availability (Wardle et al., 1994). Haggar et al. (1986) found 15% yield loss in pasture through the competition created by Californian thistle for nutrients, moisture and light.

2.2.2 Effect on livestock health

There are some weeds of pasture that affect the health of livestock. Among them, *Silybum marianum* (variegated thistle) can cause nitrate poisoning in sheep; *Senecio jacobaca* (ragwort), *Galega officinalis* (goat's rue), *Ranunculus* spp. and *Hypericum perforatum* (St John's wort) are listed as poisonous to both sheep and cattle and can cause acute poisoning if consumed by livestock (Matthew, 1982; Popay, 2008a). Thistles and barley grass seed (*Critesion* spp.) damage skin and get in eyes causing blindness while Californian thistle can cause infections such as scabby mouth in sheep (Popay, 2008a). Similarly, *Nassella trichotoma* (nassella tussock grass) causes physical injury to livestock leading to secondary viral infections through punctures in the mouth (Dowling et al., 2000), *Pteridium esculentum* (bracken) can cause haemorrhaging in cattle, *Alternanthera pungens* (khaki weed) and *Tribulis terrestris* (caltrop) can cause external and internal injuries to livestock and *Chondrilla juncea* (skeleton weed) may cause choking in cattle.

2.2.3 Effects on product quality

The quality and value of livestock products can be reduced by weeds. Weeds such as *Coronopus didymus* (twin cress) and *Anthemis cotula* (stinking mayweed) can taint milk when consumed by livestock (Dowling et al., 2000). The seed-heads from barley grass, thorns of thistles and burs of *Xanthium spinosum* (Bathurst burr) can adhere to wool and pelts, reducing the value by contaminating them (Popay, 2008a). Hay that is contaminated by unwanted weeds such as nodding thistle has reduced quality and becomes difficult to sell.

2.2.4 Avoidance and reduced pasture utilisation

Weeds in pasture prevent livestock from eating the pasture, thereby reducing utilisation of pasture. Thistles cover the pasture with prickly leaves and poisonous weeds such as ragwort and goat's rue are recognised by livestock as being bad to eat and are avoided. Some weed species like *Rumex* spp.(docks) and rushes are of low nutritive value and unpalatable (Matthew, 1982) and the space utilised by these can be used to grow more nutritious species. Some scrub weeds like gorse and blackberry reduce pasture available to livestock. Giant buttercup foliage contain ranunculin (an acrid tasting glycoside) which is avoided by grazing dairy cattle (Bourdôt and Lamoureaux, 2002).

2.2.5 Higher cost of pasture production

Weeds are common in many pastures which causes loss of pasture production by competition and reduced utilisation. The conservative estimated cost of loss in production of pasture per year by weeds is about $1.3 billion which considered only the topmost weed species of New Zealand. Californian thistle causes the most production losses in New Zealand pastures followed by *Setaria pumila* (yellow bristle grass) (Saunders et al., 2017).

2.3 Methods currently used to control weeds in plantain-based pasture

Several methods are used to control the weeds of pasture. This section has been divided into two categories i.e. use of non-chemical methods and chemicals (herbicides) including use of weed wipers to control tall weeds of pasture.

2.3.1 Non-chemical methods

Non-chemical methods mostly use agronomic tactics to reduce the presence of weeds in pasture. It includes prevention, cultural, mechanical and biological methods.

2.3.1.1 Cultural methods

This technique aims to control weeds by providing a healthy sward to compete with the weeds. Cultural methods includes monitoring of soil pH and fertility and applying fertiliser and lime to correct problems; selecting the best pasture varieties to ensure rapid establishment; seeds sown uniformly at the correct sowing rate and depth; inspection of fields regularly for insects and diseases and controlling them when needed; planting dense pasture populations at proper timing; water management to avoid damage by pugging; using crop rotations and grazing by animals (Lingenfelter and Hartwig, 2007; Sellers, 2019).

Eerens et al. (2002) suggested maximising competition by pastoral species with weeds for pastoral weed management. If pasture is dense throughout the year, it provides less chances for establishment of annual or biennial weeds such as barley grass, thistles and ragwort. When diverse forage species are included in grass legume mixtures, it reduces weed ingress due to greater herbage mass inhibiting weed invasion (Sanderson et al., 2005). Similarly, weed infestations can be reduced when the forage herbs chicory or plantain, are added in grass legume mixtures (Tozer et al., 2011).

Grazing management improves weed management in pastures by keeping pasture dense throughout the year, improving plant competition which reduces weed populations and establishment rates (Bourdôt et al., 2007). Popay and Field (1996) found that success of grazing management in pasture for weed control depends on grazing time, grazing pressure and intensity, animal stocking rate and class of stock (sheep, goats or cattle). Once the pasture seedlings are established (for plantain, it should be at six leaf stage), they need light grazing to remove the upright weed species like *Chenopodium album* (fathen) and *Solanum nigrum* (black nightshade) which can cause shading in the first few months (Harrington, 2018; DairyNZ, October 2013a). However, grazing does not eradicate mature weed infestations. Grazing too heavily in summer can weaken the regrowth of perennial grasses which results in increased weed infestation (Harker et al., 2000). Winter grazed fodder crops can reduce the number of weed species, number of weed seedlings emerged and weed seed bank size (Schuster et al., 2016). The intensity of damage to weeds varies between types of livestock because of different diet selection behaviour. For example, goats give effective control of upright, prickly and spiny weeds like thistles, gorse, blackberry and *Rosa rubiginosa* (sweet-briar) (Bourdôt et al., 2007). Ragwort, buttercup and docks are the weeds which cattle don't like to eat which sheep don't mind. Likewise, cattle eat thistles and rushes more readily than sheep will (Matthews et al., 1999).

2.3.1.2 Mechanical method

Mechanical methods can destroy weeds using strategies such as hand pulling, hoeing, mowing, ploughing, disking, and other forms of cultivation.

Mowing is one of the most common methods used to control weeds in pastures. It helps to improve the appearance of a pasture; reduces the establishment, spreading and competitive ability of weeds; depletes carbohydrate reserves in the roots of some weed species and prevents them from producing seeds if done on time (Curran and Lingenfelter, 2001; Sellers, 2019). It can kill or suppress annual, biennial and perennial weeds and help restrict their spread. It is more effective on broadleaf weeds as compared to grass weeds but misses prostrate weeds such as many thistle species and dandelions. Californian thistle can be reduced if mowing is done three to four times per year (Curran and Lingenfelter, 2001).

Hand weeding is the easiest and most economical method to control weeds of pasture on small land holdings where weed numbers are relatively small. This method is appropriate for annual and biennial weeds but not for perennial weeds because it is difficult to remove all vegetative structures effectively (Curran and Lingenfelter, 2001).

2.3.1.3 Biological control

Biological control involves use of natural enemies of a weed such as plants, herbivores, insects, nematodes and phytopathogens (Sellers, 2019). In New Zealand pastures, biological weed control is categorised into two methods: classical by using insects and bioherbicides using pathogenic agents (Bourdôt et al., 2007). Classical biological control has been found effective in reducing ragwort, and also St John's wort populations by the beetle *Chrysolina hypericin* (Fowler et al., 2016) which are poisonous to farm stock (Popay, 2008a). The spread of nodding thistle was controlled by release of nodding thistle crown weevil (*Trichosirocalus* sp.), nodding thistle gall fly (*Urophora solstitialis*), and nodding thistle receptacle weevil (*Rhinocyllus conicus*) (Popay, 2008a). Bioherbicides are typically indigenous facultative parasites (fungi or bacteria), able to be mass produced in vitro and can be applied as herbicides (Bourdôt et al., 2007). The pathogens that have been developed to control the weeds in pastures are *Sclerotinia sclerotiorum* as a selective mycoherbicide for controlling Californian thistle and giant buttercup (Verkaaik et al., 2004), and *Fusarium tumidum* and *Chondrostereum purpureum* for woody weeds (Ramsfield, 2006). There are very few bioherbicides commercialised successfully worldwide because of formulation issues which can take several years to develop and then are often still unsuccessful (Ash, 2010).

2.3.2 Herbicides

Herbicides are chemicals used to control weeds, and are only useful if they give convenient, economical and effective control. They generally kill weeds by inhibiting plant processes necessary for growth. Herbicides have been commonly used to control weeds in both agricultural as well as non-agricultural situations for over 70 years (Monaco et al., 2002).

Many herbicides are available to control broadleaf weeds in grass pasture but very few herbicides are available for mixed pastures. In New Zealand, most of the pastures are grown as mixtures of grasses with legumes and sometimes with herbs like plantain and chicory (Kemp et al., 1999). Although a few selective herbicides can be used, many weed species require less selective herbicides to be applied through wiper applicators or spot spraying (Matthews et al., 1999; Harrington and Ghanizadeh, 2017; Harrington et al., 2017; Ghanizadeh and Harrington, 2019). Some of the commonly used herbicides in New Zealand pastures are discussed below.

2.3.2.1 Phenoxy herbicides

The phenoxys have a common structure that includes a phenyl (benzene) ring attached to an oxygen atom which is in turn attached to an acid, and various substituents on the ring. These herbicides are weak acids which are only slightly soluble in water and oils and are normally formulated as water soluble amines or oil soluble esters for easier handling and application (Monaco, 2002).

MCPA and 2,4-D

In New Zealand pastures, these herbicides are commonly preferred by farmers for boom spraying because they are more effective against older weeds and cheaper than some alternative herbicides (Matthews et al., 1999). These herbicides selectively control many broadleaf plants without harming grass (Thompson and Saunders, 1984). Despite having a similar chemical structure, their effectiveness differs against certain species. An experiment conducted by Thompson and Saunders (1984) found 2,4-D gives better control of ragwort (compared to MCPA, though MCPA is more effective against thistles and giant buttercup (Bourdôt et al., 2007; Chalak-Haghighi et al., 2008). 2,4-D and MCPA have short soil residual lives of up to 4 and 6 weeks respectively which is an important consideration for clover recovery (CAST, 1975).

The major problems with using MCPA and 2,4-D are that they suppress and damage clover and plantain for several months, and also are not as effective as other herbicides like picloram, dicamba and clopyralid in controlling perennial and mature weeds (Matthews et al., 1999; Young, 2019).

MCPB and 2,4-DB

MCPB and 2,4-DB are not actually herbicides themselves, but are inactive precursors of MCPA and 2,4-D respectively (CAST, 1975). They do not cause any damage to pasture grasses or legumes (Bourdôt et al., 2007; Chalak-Haghighi et al., 2008). This is because grasses and legumes have less ability to convert MCPB and 2,4-DB from their non-toxic forms to the toxic molecules of MCPA and 2,4-D, respectively (Chalak-Haghighi et al., 2008). These herbicides are preferred herbicides for boom spraying to control broadleaf weeds including docks and Californian thistles in young pasture or within a few months of major weed germination in established pasture (Matthews et al., 1999). Lockley and Wu (2008) mentioned that plantain seedlings had some tolerance of 2,4-DB and diuron. MCPB and 2,4-DB are two herbicides which are very suitable to control weeds in young pasture with respect to cost, range of weeds killed and selectivity to clover and ryegrass seedlings. MCPB tends to be better on thistles and buttercups while 2,4- DB is preferred on docks and ragwort (Matthews et al., 1999). Farmers do not use MCPB and 2,4-DB as widely as MCPA and 2,4-D due to relatively higher cost and lower effectiveness against mature weeds than these phenoxyacetic herbicides (Bourdôt et al., 2007; Chalak-Haghighi et al., 2008).

2.3.2.2 Acetolactate Synthase-Inhibitor (ALS-inhibitor) herbicide

ALS-inhibitor herbicides move in the xylem and phloem in plants preventing biosynthesis of branched-chain amino acids (valine, leucine and isoleucine) and so inhibit plant cell division (Monaco et al., 2002). As a group, these herbicides are effective against a broad range of weed species in pastures and arable crops (Whitcomb, 1999; Monaco et al., 2002).

Flumetsulam has been available since 1993 (Lusk et al., 2015) and sold under many trade names in New Zealand such as Preside, Aim and Valdo 800WG (Young, 2019). In New Zealand, flumetsulam is mainly used for selective control of weeds in young pasture, especially weeds not controlled by MCPB such as chickweed, spurrey, mallow and *Anthemis cotula* (stinking mayweed) (Matthews et al., 1999; Bourdôt et al., 2007). When flumetsulam was applied to a sward of plantain and clover seedlings in a trial, plantain was severely suppressed initially but did recover well (Gawn et al., 2012).

2.3.2.3 Bentazone

Bentazone was first reported in 1968 in a sodium salt formulation sold as Basagran (Rao, 2000). It is a selective contact herbicide used in cereals, clover and grass seed crops, lucerne, beans, new pasture, plantain, onion, peas, potatoes, soyabean and turf (Young, 2019). In New Zealand it is sold under a number of trade names as Troy 480SL, Agpro Bentazone, Pasture Guard

Bentazone and in a mixture with MCPB as Pulsar, Pasture Guard Elite and Quasar (Young, 2019). Weeds such as Onehunga weed (*Soliva sessilis*) in lawn, stinking mayweed, *Galium aparine* (cleavers) and *Erodium cicutarium* (storksbill) can be controlled by bentazone which are poorly controlled by phenoxy herbicides (Matthews et al., 1999). Bentazone when tank mixed with MCPB in new pasture, provides good control of young docks (Young, 2019). Bentazone has little or no residual activity with 20 days of field half-life (Rao, 2000), thus it is used for control of young broadleaf weeds in both new and established pastures (Bourdôt et al., 2007) along with *Conium maculatum* (hemlock) (Gawn et al., 2012). A report from Farmers Weekly (21st April, 2016) mentioned that for both establishing and established plantain and clover pasture, broadleaf weeds and thistles are big issues and bentazone has been widely used to control young thistles.

2.3.2.4 Paraquat/diquat

Diquat and paraquat are the heterocyclic organic compounds that belong to the bipyridilium class of pesticides. The herbicidal activity of diquat was introduced in 1955 in England while paraquat was discovered a few years later (Monaco et al., 2002). In New Zealand, these herbicides are mainly sold under trade names of Gramoxone, Agpro Paraquat 200 and Flash for paraquat by itself; Reglone for diquat by itself; and Preeglone, Speedy and PDQ for mixtures of paraquat and diquat (Young, 2019). They kill plants by producing super-oxide ions and hydrogen peroxide as a result of interacting with chemical reactions that occur during photosynthesis (Rao, 2000).

These are contact herbicides which are broadly used in crop and non-crop areas (Rao, 2000). Paraquat controls grasses and many broadleaf weeds while diquat is not as effective on grass species, thus paraquat is often sold with some mixture of diquat (Zimdahl, 2007). These are generally non-selective herbicides that almost affect or kill all plant tissue to which they are applied, and paraquat is effective on young weeds and used as a site preparation herbicide prior to planting, as a directed spray to avoid contact with crop or foliage, or as a harvest aid (Monaco et al., 2002). However, its inability to translocate in plants makes it suitable for selective weed control in deep-rooted perennial crops such as lucerne, and its tolerance by white clover makes it useful in white clover seed crops (Young, 2019).

Rowarth (1998) found that plantain seedlings are tolerant of diuron, paraquat, diquat, bentazone, haloxyfop and dicamba. Results from a trial conducted in Palmerston North by Gawn et al. (2012) showed several useful chemicals especially the paraquat/diquat mixture, bentazone and diuron for farmers wanting to grow plantain/clover mixtures. The application of low rates of paraquat allows pasture to become clover dominant at certain times of the year (as grasses are suppressed), allowing good health of livestock on those pasture (white clover has high nutritive value) (Rao, 2000). A major disadvantage of these herbicides is their high toxicity level which make them the most toxic herbicides available in New Zealand. The acute oral LD_{50} for rats is 150 mg/kg for paraquat and 231 mg/kg for diquat, and there is no antidote for these chemicals (Monaco et al., 2002).

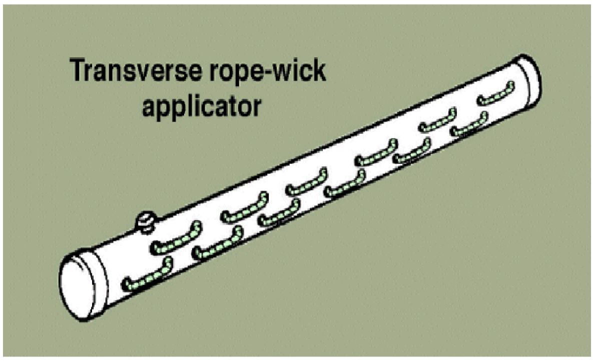
2.3.3 Weed wipers

A weed wiper is a device that applies herbicides to control upright weeds with minimal damage to non-target species, by wiping herbicide directly onto the foliage or stems of the weeds (Martin et al., 1990). The concept of a weed wiper applicator is similar to painting with a pad or roller where absorbent material is saturated with herbicide solution and used to wipe the herbicide onto plants (Johnson, July 2011). Weed wipers have been available for several decades but there is a limited understanding of factors affecting their use, and only limited information is available on the use of herbicides applied with wipers to control weeds of pastures (Harrington and Ghanizadeh, 2017). The history and uses of herbicide wipers, and the herbicides commonly used in weed wipers, are discussed further in the following sections.

2.3.3.1 History of wiper applicators

In the early 1900s, the concept of wiper application originated in USA with a horse drawn device to apply insecticides on to plant foliage by means of wicks and absorbent material (McWhorter and Derting, 1985). Many developments were made through the 1920s including use of rotating drums mounted on horse drawn ploughs. Herbicide application through wipers was first adopted for control of broadleaf weeds using 2,4-D after its development in the late 1940s. The most common method used was conventional spray booms wrapped with absorbent material (Derting, 1987). Due to lack of selectivity of 2,4-D on dicotyledons, use of this technology was limited (McWhorter and Derting, 1985).

In the 1970s, after the introduction of glyphosate, use of wicks, wiper applicators or weed wipers were greatly increased, and rope-wick applicators and roller applicators were the first commercial wiper applicators developed to apply glyphosate (Derting, 1987). Rope wick wipers were comprised of transverse or longitudinal wicks through which herbicide flowed by capillary action from a PVC tube (Fig 2.1). The wicks were abrasion-resistant material or a combination of materials such as nylon fabrics braided into ropes which wiped the herbicide solution from the tube onto taller plants (McWhorter and Derting, 1985).



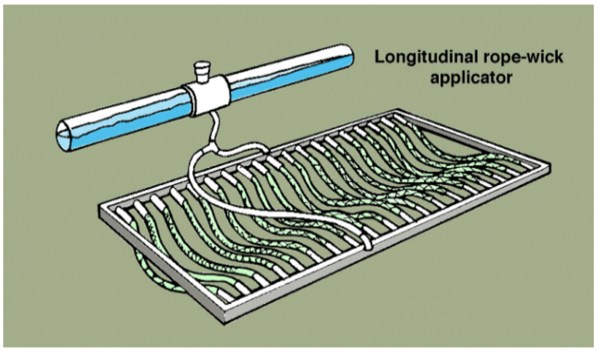


Figure 2.1 Rope wick applicator showing transverse rope-wick applicator and longitudinal rope-wick applicator.

Source: James H. Miller, USDA Forest Service, Bugwood.org

There have been several different types of rope wick applicators developed around the world, from passive to pressurized types and simple to multiple wick applicators. The transverse rope-wick applicator shown in Fig 1, the multiple rope-wick applicator and the pressurized rope-wick applicator are the three basic designs of tractor mounted rope-wick applicator (McWhorter and Derting, 1985; Rao, 2000), of which only the transverse rope-wick applicator was ever sold in New Zealand.

Roller applicators work on the same basic principle as rope-wick applicators but instead of moving by capillary flow through a series of short ropes, herbicide solution is dripped onto a carpet-covered roller which is rotated as the applicator is driven across the field (Derting, 1987; Ozkan, 1995) (Figure 2.2). The roller rotates in a direction counter to the rotation of the wheels to minimise dripping and maximise wiping to weeds impacting the roller. This has a much larger wiping surface than the rope wicks and herbicide can be delivered more rapidly than is possible by capillary flow. The main problem of this wiper is that most depend on a farmer operated pump to dispense herbicides onto the carpet while driving across a field which causes variability in amount of herbicide solution applied (Van Toor, 1994). However, some models were developed overseas to electronically maintain the predetermined wetness of the wiper using moisture sensors and automatically regulated the flow of the herbicides (Van Toor and Brewster, 1995).

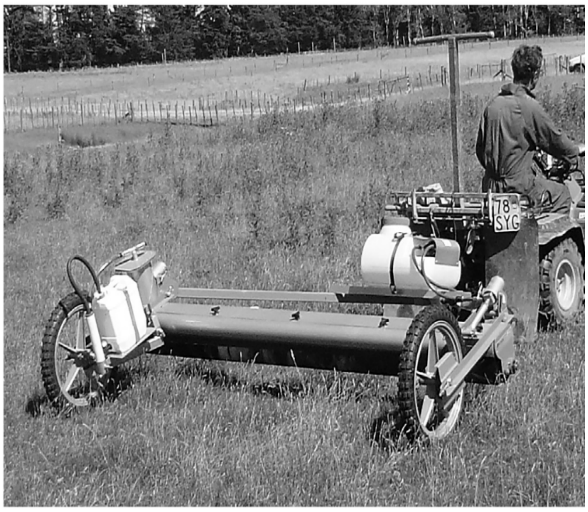


Figure 2.2 Roller applicator

Source: sciencedirect.com

Wiper applicators were widely adopted by soybean and cotton growing farmers in USA to selectively control weeds which grew taller than the crops such as *Sorghum bicolor, Amaranthus* spp., *Sorghum halepense* and *Ambrosia* spp. by applying glyphosate in the 1980s (McWhorter and Derting, 1985). In New Zealand pasture, farmers started to use wiping devices in the 1970s for the control of thistles, ragwort and rushes among other weeds (Makepeace and Thompson, 1982). Farmers became disillusioned with rope-wick applicators due to the slow rate of herbicide delivery and make very little use of this design now. They looked for more effective wiper applicators and are now using the rotary weed wiper and some newer design applicators like WeedSwiper and C-Dax Eliminator.

2.3.3.2 Uses of weed wipers

Weed wipers are specialised devices designed to supplement standard weed control practices. They provide another method for controlling weeds taller than the crops or pastures when there are not many satisfactory alternative methods available. Wiper applicators may provide good control of upright weeds such as Californian thistle (Grekul et al., 2005) and rushes selectively in pastures by applying concentrated solutions of translocated herbicides such as glyphosate on to their foliage and stems above the height of surrounding vegetation (Moyo et al., 2006b). The herbicides available to selectively control troublesome perennials such as Californian thistles and rushes in grasses and legumes are limited (Gabruck et al., 2013). Wiper applicators enabled highly effective but non-selective herbicides like glyphosate to be applied selectively to control these weeds with minimal damage to pasture (Martin et al., 1990; Grekul et al., 2005; Moyo et al., 2006a). Hand held wiping devices are also used by home gardeners to apply glyphosate selectively around their properties, commercial vegetable crops (Harrison, 1983), and for controlling emergent aquatic weeds (Kay, 1995). Roller devices that run along the ground surface have occasionally also been used to wipe selective herbicides directly on turf (Harrington et al., 2000) and onto weeds underneath fruit trees and vines (Derting, 1987).

For wiper applicators that wipe herbicide to the weeds above the crops or pasture, the volumes of herbicides used are much less as only weeds receive herbicide, so herbicide savings can be up to 80% compared with broadcast spraying (Schepers and Burnside, 1979; Rao, 2000). This diminished chemical use can reduce soil contamination and cost per hectare. Other advantages are that herbicide application can occur even when it is windy as no spray is released, eliminating the risk of herbicide drift, environmental risks are reduced, and less skill is required by the personnel who use them (Derting, 1987).

2.3.3.3 Herbicides used for wipers

Wipers were initially developed to apply phenoxy herbicides but their popularity and use was increased only after the introduction of glyphosate (McWhorter and Derting, 1985). The various formulations of glyphosate and dicamba were labeled as the primary chemicals for wiper application (Johnson, July 2011). Glyphosate is considered to be the best herbicide for the wiper application as it controls a broad-spectrum of weeds and is highly systemic in plants (Duke and Powles, 2009). However, other herbicides are also used with rotary weed wipers, including metsulfuron, clopyralid, triclopyr and picloram (Harrington et al., 2000; Grekul et al., 2005; Moyo, 2008; Harrington and Ghanizadeh, 2017). Moyo et al. (2006a) found that glyphosate and clopyralid were highly effective in reducing Californian thistle density by over

90% control with a double pass and by 80% for a single pass when assessed 8 months after treatment.

There is only a limited number of herbicides recommended for use with weed wipers, though much more use of translocated herbicides such as metsulfuron and aminopyralid could be possible if enough research was done. Research is also needed to determine the best formulations and adjuvants that can be used for the various herbicides that can be applied by weed wipers to control various problematic weed species and minimise damage to pasture species if some transfer from the weeds down to the pasture should occur. Some of the main herbicides used in wipers are described below.

Glyphosate

Glyphosate is a non-selective, systemic and broad-spectrum herbicide which was first introduced as a herbicide by Monsanto Agricultural Products company in 1974 under the trade name of Roundup (Franz et al., 1997). Generally, it is formulated as a salt, is stable under normal temperature conditions (-20 to 40°C), is essentially photostable and non-volatile (Monaco et al., 2002). In New Zealand, it is sold under different trade names differing in salts used, range of concentration and different surfactants (Young, 2019).

Glyphosate is a foliage active herbicide which translocate rapidly throughout aerial and underground parts of plants following foliar absorption (Monaco et al., 2002). In New Zealand, glyphosate is recommended for use in agriculture and horticulture to prepare seed beds for almost every crop and pasture because it is immediately deactivated on contact with soil (Young, 2019). It is also used for general weed control in many different situations including under perennial fruit crops and in urban and waste places (Monaco et al., 2002). It is also used for pasture and turf renovation (Young, 2019). It is used to control a broad spectrum of annual and perennial weeds including troublesome weeds with below ground organs such as rhizomes and tap-roots (Bourdôt et al., 2007; Cobb and Reade, 2010; Ghanizadeh et al., 2015). It has been widely used for wiper application to tall weeds due to its ability to translocate and reach underground parts of perennial weeds such as Californian thistles (McWhorter and Derting, 1985; K.C. Harrington and Ghanizadeh, 2017). It is also extensively used for spot treatment as an alternative to hoeing, in non-crop areas to control undesirable species and in forestry (Monaco et al., 2002).

Clopyralid

Clopyralid is an off-white crystalline solid and growth regulator herbicide with a pyridine ring in the chemical structure and high mobility in the soil which means it is very leachable (Zimdahl, 2007). Versatill was the first product available in New Zealand. Clopyralid has stand-alone products as well as mixtures on the market containing amine, mono-ethalolamine and potassium salts (Young, 2019).

Clopyralid controls a broad spectrum of broadleaf weeds in the Asteraceae, Fabaceae and Solanaceae families through foliar and soil application (Bukun et al., 2010), but does not control grasses (Monaco et al., 2002). Clopyralid is a selective herbicide used for control of Californian and other thistles, yarrow and many difficult flat weeds in crops, plantations and pre-cultivation and troublesome weeds of turf like Onehunga weed in New Zealand (Young, 2019). However it is toxic to clover and has persistent residual activity which will preclude replanting of clover for months (Bourdôt et al., 2007). DiTomaso et al. (2006) found that prescribed burning followed by application of clopyralid is much more effective to maintain grassland in natural conditions and for prevention of weedy species like yellow star thistle in California.

The use of selective herbicides for wiping may produce greater forage production than wiping with glyphosate. The systemic and non-selective nature of glyphosate could damage taller grasses and desirable pasture grown with weeds. Thus, wiping with selective broadleaf herbicide may cause less damage to shorter desirable species. However, Moyo (2008) found that glyphosate caused less damage to pasture than clopyralid, as the clopyralid totally killed off all clover when it rained, whereas glyphosate caused less trouble as both ryegrass and clover can tolerate low amounts of glyphosate. Similar findings were reported by Grekul et al. (2005) in controlling Californian thistle.

Chapter 3

Glasshouse experiments comparing tolerance of two plantain cultivars to phenoxy herbicides

3.1 Introduction

Several commercial plantain cultivars have been developed in New Zealand, which have better suitability for livestock grazing and higher forage yield than wild plants. The difference between Agritonic and Tonic was reviewed in Section 2.1.3. A detailed description about the phenoxy herbicides used for this experiment was provided in Section 2.3.2.1. The Agritonic cultivar was bred to tolerate phenoxy herbicides better than other cultivars such as Tonic, but the level of tolerance achieved by this breeding needs to be quantified. Thus, the main objective of this study was to test the hypothesis that Agritonic plants are more tolerant than Tonic plants to several phenoxy herbicides when compared using dose-response experiments for plants grown in a glasshouse, and also to determine which of the herbicides are tolerated better by Agritonic than Tonic.

3.2 Materials and methods

3.2.1 First dose-response experiment

Plantain plants were grown in 400 PB ¾ planter bags (200 each for Agritonic and Tonic), each filled with 0.4 L of potting mix and slow-release fertiliser (short term Osmocote i.e. 400 g, 50 g Osmoform, and 150 g dolomite per 100 L of potting mix). Two seeds were sown per bag about 1 cm apart in the middle of the bag at 0.5 cm deep, and these were placed in a heated glasshouse (with average daily maximum and minimum temperatures of 25.2ºC and 12.8ºC, respectively) with automated sub-irrigation below the pots via felt mats on 21 May 2019 at Plant Growth Unit, Massey University. Almost 95% of the seeds germinated, and they were thinned to one plant per pot after 3 weeks when they were at the 2 to 3 leaf stage. Scoring (from 0 to 100 where 0 indicated no germination and 100 indicated fully developed plants) was done on 24 June 2019 to select good seedlings in pots based on germination, physical appearance, growth pattern and low incidence of disease and pests; the 50 worst pots (25 each of Agritonic and Tonic) were removed. A range of herbicides (Table 3.1) at different rates (Table 3.2) were applied to Agritonic and Tonic cultivars of plantain at the 4 to 5 leaf stage on 1 July 2019 using a laboratory track sprayer calibrated to deliver 251 L ha^{-1} of spray solution at 200 kPa.

Table 3.1 Trade name and recommended rate of herbicides used for the experiment.

Herbicides	Trade name	Recommended rate	g ai ha^{-1}
2,4-DB	Dow AgroSciences 2,4-DB	6.0 L ha^{-1}	2400
2,4-D amine	Synergy 2,4-D Amine 720	1.0 kg ha^{-1}	720
MCPB	Dow AgroSciences MCPB	3.0 L ha^{-1}	1155
MCPA	Nufarm Agritone 750	1.5 L ha^{-1}	1125
MCPB/ MCPA	Thistrol Plus	3.0 L ha^{-1}	1125 + 75

Table 3.2 Treatments applied to Agritonic and Tonic cultivars of plantain for each herbicide in a glasshouse, expressed as g ai ha^{-1}.

Treatment description	2,4-DB	2,4-D amine	MCPB	MCPA	MCPB+MCPA
Untreated control	0	0	0	0	0
Eighth recommended rate	300	90	144	141	141 + 9
Quarter recommended rate	600	180	289	281	281 + 19
Half recommended rate	1200	360	577	562	562 + 37
Recommended rate	2400	720	1155	1125	1125 + 75
Two times recommended rate	4800	1440	2310	2250	2250 + 150
Four times recommended rate	9600	2880	4620	4500	4500 + 300

The experiment was conducted using a randomised complete block design with five replicates/blocks of two cultivars of plantain and seven different rates of five herbicides. The daily maximum and minimum temperatures during the 2 weeks after spraying averaged 26.5 ºC and 16.5 ºC, respectively. Scoring of plants to quantify the effect of herbicides on plants was done every week after herbicide application starting from 8 July 2019 (1 week after application of herbicides). Scores were allocated based on a 0-10 scale where 0 signified no effect and 10 signified total death. Two months after treatment, all plants were cut to a height of 7 cm, and the cut material was oven dried at 70ºC for 48 hours and weighed. Three months after herbicide treatment, another dry weight assessment was done using the same method. On 22 November (about 4 months after herbicide treatment), all above-ground parts of plants were cut to ground level, and dry weight was measured. The final scoring of the plants were done immediately before this final harvest for dry weight. Note that scores were used to help interpret dry weight results, though only dry weight results are reported as these were sufficient to show treatment effects.

3.2.2 Second dose-response experiment

The experiment was repeated to confirm the results from the first experiment, using the same procedure as described above but with six replicates and a slightly higher fertiliser rate (long-term Osmocote i.e. 500 g per 100 L, 50 g Osmoform, and 150 g dolomite). Fresh seeds of Agritonic and Tonic received from Agricom (NZ) Ltd were planted on 8 June 2020 in a heated glasshouse with daily maximum and minimum temperatures averaging 24.2 ºC and 16.2 ºC, respectively. Thinning was done to remove the weaker plants on 29 June 2020 (3 weeks after sowing). Treatments were applied on 16 July 2020 when plants were at the 4 to 5 leaf stage using a laboratory track sprayer calibrated to deliver 244 L ha^{-1} of spray solution at 200 kPa. The daily maximum and minimum temperatures during the 2 weeks after spraying were 25ºC and 16.5ºC, respectively.

The scoring of the plants started 1 week after herbicide spraying and continued weekly until 8 weeks after spraying (11 September). After the eighth scoring, the first harvest was done 7 cm above the ground for each plant (2 months after spraying). One month after the first cut, a second harvest was done, and the final harvest occurred 4 months after spraying when all above-ground material was removed. The harvested samples were oven dried at 70ºC for 48 hours and weighed.

3.2.3 Statistical analysis

A three-parametric logistic model was fitted to the data using the following equation:

$$Y = \frac{\mathrm{d}}{1 + \exp(\mathrm{b} * (\log \mathrm{x}) - \log(\mathrm{GR50}))}$$

where Y is plantain dry weight as a percentage of the untreated control, d is the upper limit, x is herbicide rate, GR_{50} is the rate of herbicide corresponding to 50% reduction in total plant dry weight and b is the slope around GR_{50}. The total dry weight data were fitted to this model using the statistical software RStudio 4.1.3 with its dose-response curve (drc) package to estimate the GR_{50} (Ritz et al., 2015; Ritz et al., 2019) values for each herbicide. The data were checked for normality and homogeneity of variance, and the Box-Cox transformation (Christian and Jens, 2005) was used to improve data homogeneity where appropriate. The dose-response curves based on reduction in total dry weight as a percentage of untreated plants (Figure 3.2) were fitted for each herbicide and cultivar. For MCPB/MCPA, the sum of grams of MCPB and MCPA was used to give a single number to create dose response curves. The relative tolerance was determined by dividing the GR_{50} value of Agritonic by the GR50 value of Tonic plantain. A two-way ANOVA was also conducted to compare the effectiveness of the herbicides for the two cultivars at the recommended rates in SPSS 27. The means were separated using Fisher`s protected test at a 5% level of probability.

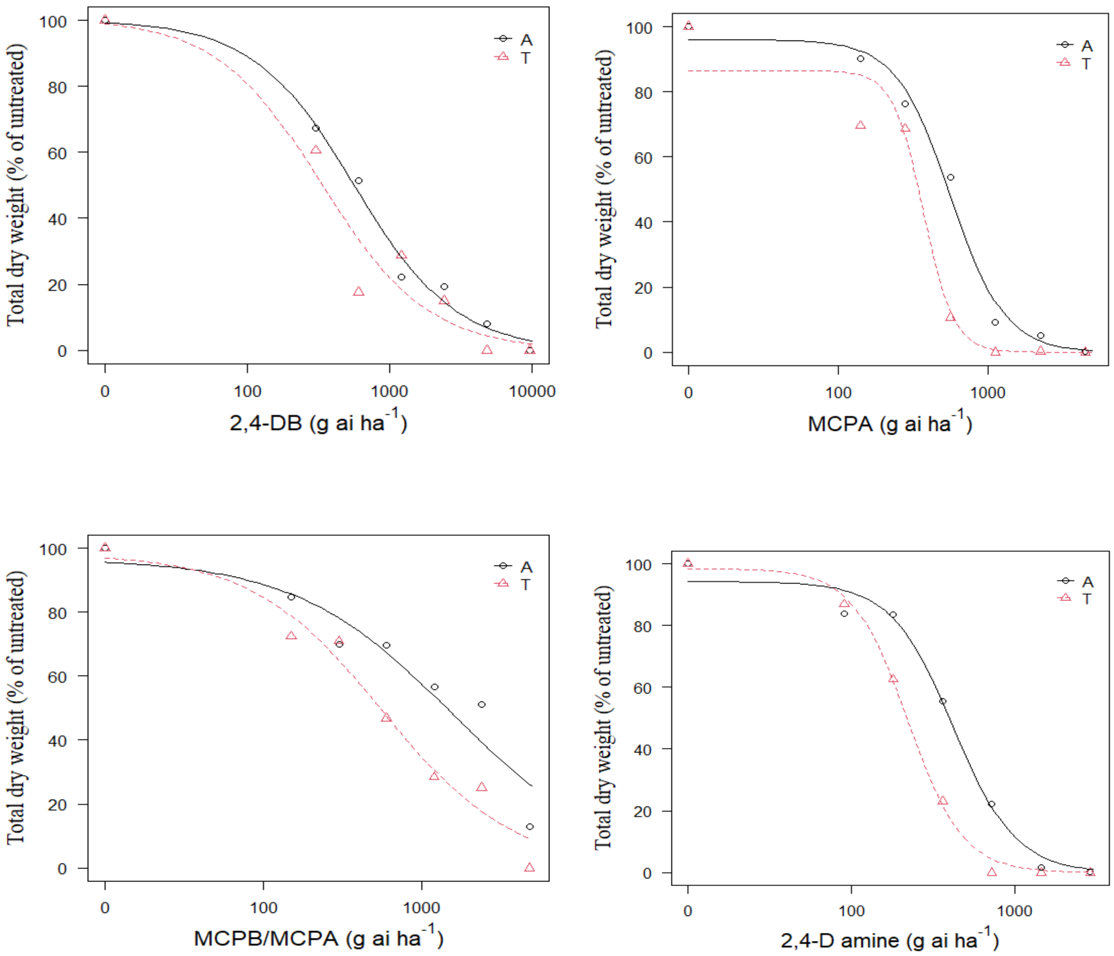
3.3 Results

3.3.1 First dose-response experiment

The experiment showed that all the herbicides at the recommended rate for use in pastures had an adverse effect on plantain seedlings, with some herbicides being particularly damaging. Agritonic plants appeared less affected by each of the herbicides than the Tonic plants, though the differences were not large (Figure 3.1).

Figure 3.1 Effects of different rates of various phenoxy herbicides on Agritonic and Tonic cultivars of plantain 5 weeks after treatment in the first dose-response experiment. The asterisk with a yellow background is the plant sprayed with recommended rates of these herbicides.

As expected, total dry weight decreased with increasing herbicide rates for both Agritonic and Tonic cultivars for all herbicides. The estimated GR_{50} value for Agritonic was 569.6, 201.1, 1301.2, 388.0 and 1164.2 g ai ha^{-1} and Tonic was 164.9, 74.1, 609.2, 188 and 876.9 g ai ha^{-1} for 2,4-DB, MCPA, MCPA/MCPB, 2,4-D amine and MCPB, respectively (Figure 3.2 and Table 3.3).



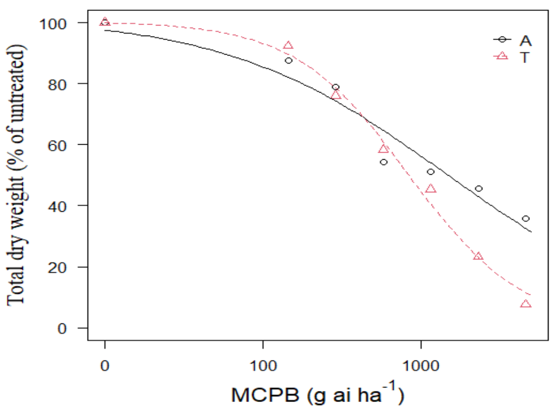


Figure 3.2 Fitted dose-response curves (on a logarithmic scale) from the first dose-response experiment based on percentage reduction in total dry weight of plants compared to untreated ones for two cultivars of plantain (A is Agritonic and T is Tonic).

Based on GR_{50} values derived from the reduction in total dry weight (percentage of untreated control) from the first dose-response experiment, the ratio of Agritonic GR_{50} compared with Tonic GR_{50} (A/T GR_{50}) showed that level of tolerance of Agritonic compared to Tonic is 3.45, 2.71, 2.14, 2.06 and 1.33 times greater to 2,4-DB, MCPA, MCPB/MCPA, 2,4-D amine and MCPB, respectively. However, the statistical analysis suggested this difference between the two cultivars was only significant for 2,4-D amine ($p<0.05$).

Table 3.3 Parameters estimated for the three-parameter logistic model analysis of five different phenoxy herbicides for two plantain cultivars in the first dose-response experiment.

Herbicides	Cultivars	d±SE	b±SE	GR_{50}±SE	A/T GR_{50} ratio	P value
2,4-DB	A	87.9±95.3	3.9±0.9	569.6±322.2	3.45	0.27
	T	115.3±163.2	3.2±0.5	164.9±104.1		
MCPA	A	111.2±126.3	3.6±0.7	201.1±110.9	2.71	0.32
	T	121.0±168.5	3.3±0.5	74.1±43.6		
MCPB/MCPA	A	76.0±23.9	2.5±1.6	1301.2±837.6	2.14	0.45
	T	81.9±25.1	3.0±1.0	609.2±242.2		
2,4-D amine	A	88.9±17.4	4.6±1.1	388.0±73.6	2.06	0.04
	T	89.2±23.1	5.3±1.3	188.0±39.5		
MCPB	A	101.3±17.7	0.6±0.2	1164.2±853.5	1.33	0.76
	T	89.6±14.1	2.0±0.7	876.9±316.3		

A = Agritonic, T = Tonic, d = upper limit, b = the slope around GR_{50}, GR_{50} = rate of herbicide (g ai ha^{-1}) corresponding to 50% reduction in total plant dry weight.

Although the results have been presented as a percent reduction in yield, there was mortality in some cases. The data have not been presented as percent mortality as not all herbicides killed plants, and it was the suppression of yield that was of more interest. All the Tonic plants died at the recommended rate for 2,4-D amine of 720 g ai ha^{-1}, whereas 60% of plants survived this rate for the Agritonic cultivar. Most of the Agritonic plants treated with higher rates (1440 and 2880 g ai ha^{-1}) of 2,4-D amine survived till 2 months after herbicide application though they were severely damaged and then died.

Similar results were found in Tonic and Agritonic plants treated with 2,4-DB and MCPA where Tonic plants did not survive even the recommended rates, but Agritonic did. Both Agritonic and Tonic plants sprayed with MCPB and MCPB/MCPA were little affected. The double rate of MCPB/MCPA showed some severe effects on plants within 1 month but most of these plants recovered later; only 20% of Agritonic plants died in contrast to 40% recorded for Tonic plants. The Agritonic plants treated with MCPB did not suffer from severe effects even at rates up to four times its recommended rate, whereas 60% of Tonic plants died after 1 month when treated with twice the recommended rate.

The overall percentage reduction in total dry weight at recommended rates of herbicides was not significantly different between the two cultivars ($p = 0.06$), whereas the herbicides were found to be statistically significant from each other in their effect on plantain ($p < 0.01$). There was no significant interaction of cultivars with recommended rates of herbicides (Appendix 3.1). The recommended rates of MCPB and MCPB/MCPA had significantly less adverse effects on plant dry weight than other treatments. All plants died when treated with the recommended rate of MCPA and 2,4-D amine, which resulted in the large reduction shown in Figure 3.3.

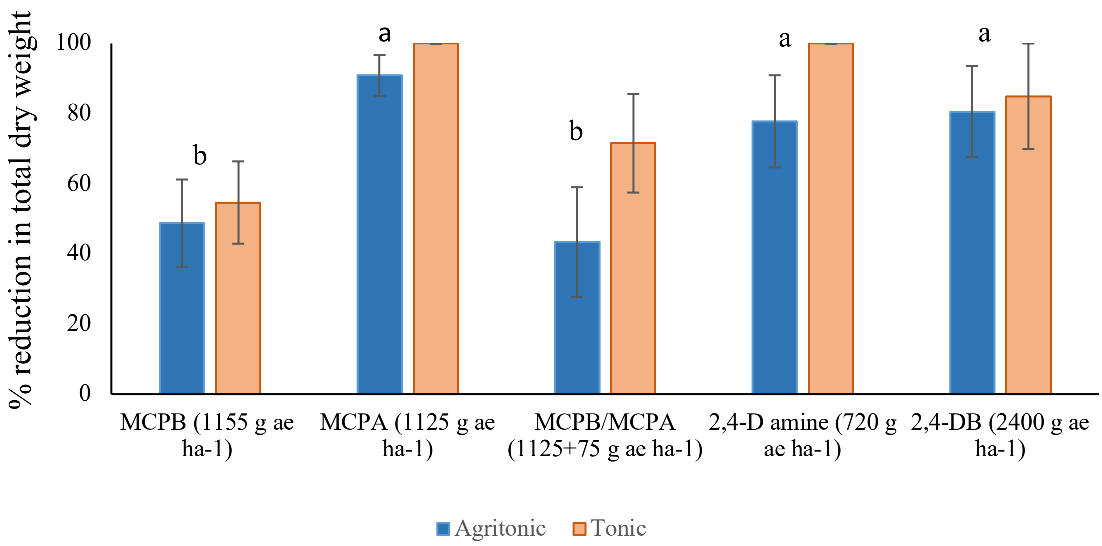


Figure 3.3 Percentage reduction in total dry weight at recommended rates of five different herbicides in Agritonic and Tonic cultivars of plantain, measured over the 4 months following treatment in the first dose-reponse experiment. Vertical bars are ± the standard errors of the mean (n=5). Herbicides with different lowercase letters are significantly different according to Fisher`s protected test (p<0.05).

3.3.2 Second dose-response experiment

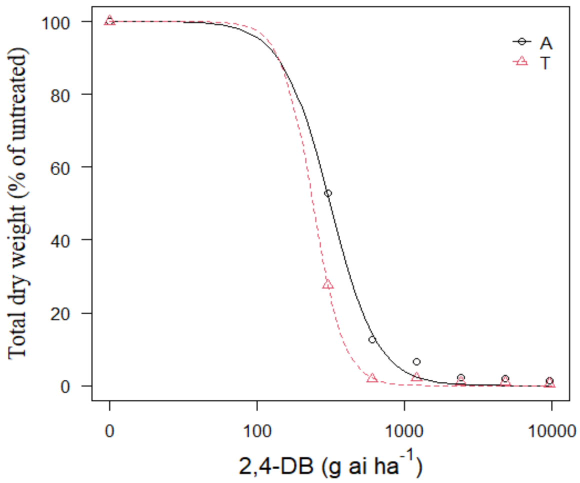
The graphed results (Figure 3.2) were similar for both first and second dose-response experiments in that Tonic was consistently more affected than Agritonic, and the gap between the curves was smaller for some herbicides than others. Results were not exactly the same between both experiments, with graphs showing some cross-over of curves which was how the model determined the relationship (Figure 3.5). The estimated GR_{50} values for Agritonic were 62.9, 283.8, 105.2, 167.2 and 160.3 g ai ha^{-1} and for Tonic were 25.1, 118.6, 70.1, 121.4 and 121.2 g ai ha^{-1} for 2,4-DB, MCPA, MCPA/MCPB, 2,4-D amine and MCPB, respectively. The estimated GR_{50} values in the first run were greater than those in the second run for all the herbicides. The level of tolerance to 2,4-DB, MCPB/MCPA, 2,4-D amine, MCPB and MCPA were 2.50, 2.39, 1.50, 1.38 and 1.32, respectively, higher in Agritonic than Tonic (Table 3.4). However, the statistical analysis showed this difference between the two cultivars was only significant for the MCPB/MCPA mix ($p<0.05$). As the GR_{50} values of all the herbicides were different between experiments, the results have been presented separately.

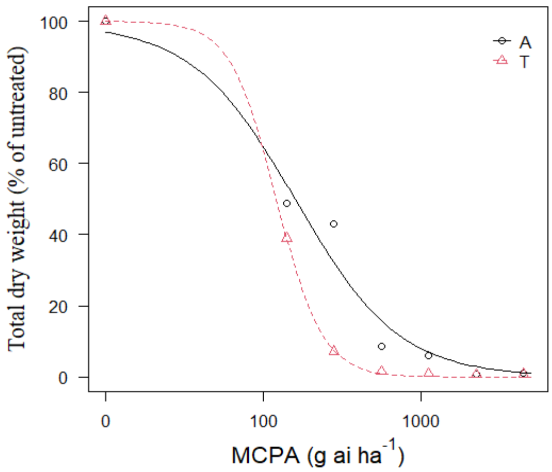
Table 3.4 Parameters estimated for the non-linear regression analysis of five different phenoxy herbicides for two plantain cultivars in the second dose-response experiment.

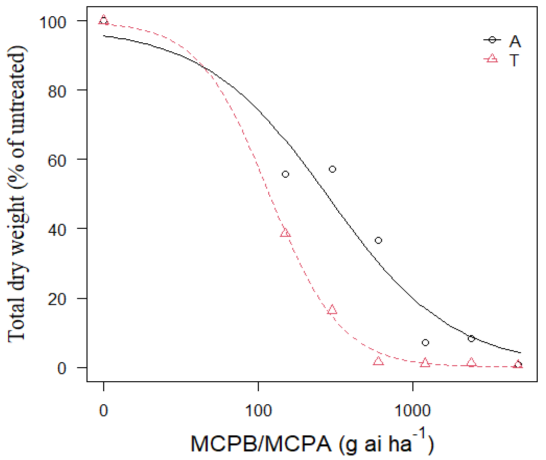
Herbicides	Cultivars	d±SE	b±SE	GR50±SE	A/T GR_{50} ratio	P value
2,4-DB	A	108.8±37.4	1.0±0.1	62.9±39.4	2.50	0.40
	T	102.7±36.2	1.0±0.1	25.1±16.8		
MCPA	A	99.4±4.2	1.3±0.2	160.3±20.2	1.32	0.12
	T	100.0±4.2	3.0±0.9	121.2±10.4		
MCPB/MCPA	A	98.2±4.8	1.1±0.1	283.8±44.5	2.39	0.01
	T	100.0±4.7	1.9±0.5	118.6±17.1		
2,4-D amine	A	103.0±16.2	1.7±0.2	105.2±23.6	1.50	0.23
	T	106.9±16.7	1.8±0.2	70.1±14.2		
MCPB	A	106.8±24.4	1.2±0.2	167.2±72.0	1.38	0.60
	T	104.1±24.7	1.5±0.2	121.4±44.3		

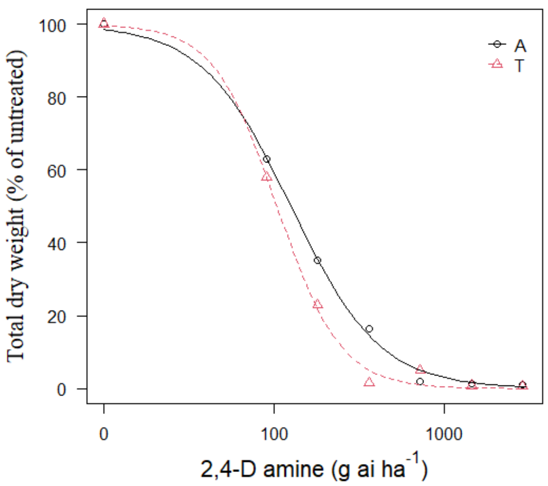
A = Agritonic, T = Tonic, d = upper limit, b = the slope around GR_{50}, GR_{50} = rate of herbicide (g ai ha^{-1}) corresponding to 50% reduction in total plant dry weight.

Figure 3.4 Effects of different rates of various phenoxy herbicides on Agritonic and Tonic cultivars of plantain 7 weeks after treatment in the second dose-response experiment. The asterisk with a yellow background is the plant sprayed with recommended rates of these herbicides.









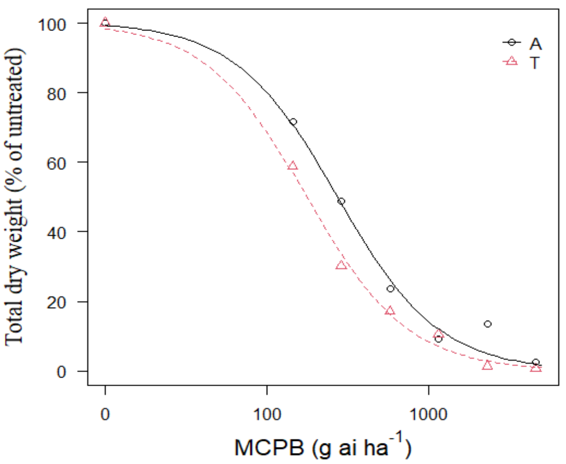


Figure 3.5 Fitted dose-response curves (on a logarithmic scale) from the dose-response experiment based on percentage reduction in total dry weight of plants compared to untreated ones for two cultivars of plantain (A is Agritonic and T is Tonic).

The percentage reduction in total dry weight compared to untreated control at recommended rates of herbicides was not significant between cultivars and herbicides in the second dose-response experiment (Appendix 3.2). However, MCPB and MCPB/MCPA had fewer effects on plant dry weight compared to other herbicides (Figure 3.6), which were similar to the first dose-response experiment. The plants treated with half and full recommended rates of 2,4-DB got knocked down initially and did not recover, whereas those treated with MCPB and MCPB/MCPA had good regrowth in the third dry weight. Some plants even survived the higher rates of these two herbicides.

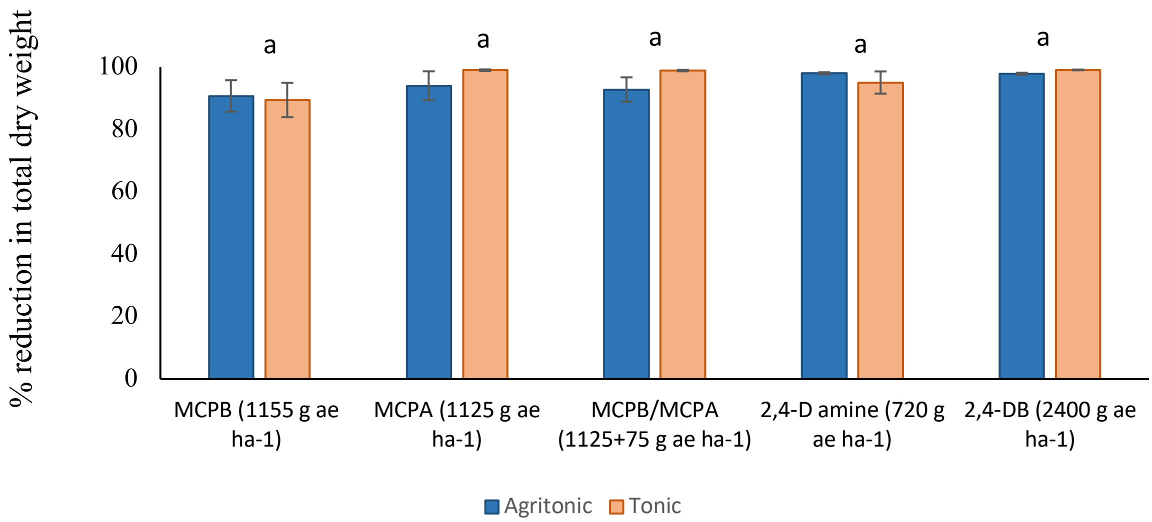


Figure 3.6 Percentage reduction in total dry weight at recommended rates of five different herbicides in Agritonic and Tonic cultivars of plantain, measured over the 4 months following treatment in the second dose-response experiment. Vertical bars are + the standard errors of the mean (n=6). Herbicides with the same lowercase letters over their bars are not significantly different according to Fisher`s protected test (p<0.05).

An observation to note from the experiments was the large variability between replicates/blocks of Agritonic plants for some treatments, which suggested some plants were tolerant and others susceptible, as shown in Figure 3.7. MCPB was applied to six replicates, and the photos show the variability in response 7 weeks after spraying with the same treatment. Similar results were found for plants treated with other herbicides. If all the plants had been like the tolerant ones, then there might have been completely different results and perhaps closer to a 5-fold tolerance.

Figure 3.7 Agritonic plants showing variability in response to the recommended rate of MCPB sprayed 7 weeks after spraying.

3.4 Discussion

Weed control is needed in pastures that contain plantain. Some weeds, such as thistles are only controlled well by phenoxy herbicides when clover is present, as dicamba or aminopyralid cannot be used because although tolerated by plantain they are not by clover. The results from dose-response experiments showed that all of these herbicides are damaging to plantain, though perhaps there is a possibility of using some of the less damaging ones, especially if growing Agritonic. Agritonic appears to be more tolerant of phenoxy herbicides like MCPB, MCPB/MCPA, 2,4-D amine, 2,4-DB and MCPA than Tonic. MCPB and MCPB/MCPA looked to be the most promising overall as they caused the least damage. Farmers would be particularly interested in knowing the recommended rates of application as to which herbicides damaged the plantain least, and whether the difference between the cultivars is large enough to make it worth using Agritonic rather than Tonic (Figure 3.3).

Herbicide-resistant weeds had been reported in 98 crops in 72 countries, where 41 species are resistant to synthetic auxin herbicides (Heap, 2022). In New Zealand, herbicide resistance cases have been reported in 12 arable, 5 pasture, 6 horticultural and 2 turf weeds with resistance to nine herbicidal sites of action (Ghanizadeh and Harrington, 2019). A study in Hawkes Bay found that the biotypes of *Carduus nutans* (nodding thistle) developed a 6-fold level of resistance to MCPA, MCPB and 2,4-D, and a five-fold increase in MCPA application for *Ranunculus acris* (giant buttercup) in Takaka, Golden bay (Harrington and Woolley, 2006; Bourdôt et al., 2007; Bourdot and Hurrel, 1988). It has been reported that resistance to one phenoxy herbicide gives resistance to other phenoxy herbicides in weeds. For instance, dicamba-resistant *Bassia scoparia* (kochia) was cross-resistant to dichlorprop, 2,4-D, mecoprop, MCPA and picloram; *Sinapis arvensis* (wild mustard) to 2,4-D, dicamba, mecoprop and picloram and dicamba-resistant fathen to clopyralid and aminopyralid (Cranston et al., 2001; Heap and Morrison, 2002; Ghanizadeh and Harrington, 2017b). Globally, resistance to 2,4-D in plantain was first reported from the USA in the cemetery turf where this herbicide had been used continuously for 30 years (Patton et al., 2018). The authors noted that the resistant biotype was 6.2 times more resistant to 2,4-D compared with the susceptible one, while it was still susceptible to triclopyr. In another 2,4-D-resistant biotype of plantain, Russell et al. (2021) recorded 4-fold resistance, which was slightly lower than that reported by Patton et al. (2018).

In this research, however, a lower level of tolerance to phenoxy herbicides compared to the studies mentioned above. In both dose-response experiments, the plantain treated with different herbicides were affected differently by the end of the experiment, and the level of tolerance varied between 1.33 to 3.45-fold, depending on the phenoxy herbicide. All plants treated with these phenoxy herbicides were hit hard at the start, whereas those treated with the recommended rate of MCPB and MCPB/MCPA recovered very well by the end of the experiment. Some plants even survived the higher rates of these two herbicides. Also, there was variability between the Agritonic plants in their tolerance. Among the five Agritonic plants treated with the same herbicide, some were quite tolerant. The tolerance was very variable, partly due to how seeds were produced from bred plants (i.e. pollinated by susceptible plants), so there is potential to breed for higher levels of tolerance. But perhaps plantain will always be variable because of the pollination system. Plantain is self-sterile, cross fertilised and pollinated by wind (Warwick and Briggs, 1979; Bond et al., 2007), which increases the chances of being pollinated by a susceptible plant. Thus, breeders intend to breed in higher levels of tolerance and do more work on the variability of the plantain.

The data from Table 3.3 and Table 3.4 have now been summarised in Table 3.5 to show how different they are from each other side by side. The GR_{50} values for Agritonic and Tonic in the second dose-response experiment were considerably lower than those of the first dose-response experiment. However, the pattern of relative difference was the same and Agritonic had higher GR_{50} values compared to Tonic in both experiments (Table 3.5).

Table 3.5 Summary of GR_{50} (g ai ha^{-1}) and relative difference between two plantain cultivars sprayed with different phenoxy herbicides in the first and second dose-response experiments.

Herbicide	GR_{50} (g ai ha^{-1})				A/T GR_{50} ratio	
	Agritonic		Tonic		First experiment	Second experiment
	First experiment	Second experiment	First experiment	Second experiment		
2,4-DB	569.6	62.9	164.9	25.1	3.45	2.50
MCPA	201.1	160.3	74.1	121.2	2.71	1.32
MCPB/ MCPA	1301.2	283.8	609.2	118.6	2.14	2.39
2,4-D amine	388.0	105.2	188.0	70.1	2.06	1.50
MCPB	1164.2	167.2	876.9	121.4	1.33	1.38

Cultivars	First pot experiment	Second pot experiment
Agritonic		
Tonic		

Figure 3.8 Photos showing the difference in growth of untreated plants (Agritonic and Tonic) at 10 weeks of first and second dose-response experiments.

The reason for this difference between experiment was the untreated plants were much larger than the treated plants, making the GR_{50} values relatively smaller in the second dose-response experiment. There was some error in mixing the nutrients in the potting mix with 8-9 months longevity fertiliser instead of 3-4 months longevity as in the first experiment, which made plants grow much larger even at the same growth stage, in the second experiment than those of the first experiment (Figure 3.8).

3.5 Conclusion

The results from these experiments showed that plantain plants survived the recommended rates of MCPB and the MCPB/MCPA mixture. Some of the Agritonic plantain also survived the recommended rates of MCPA, 2,4-DB and 2,4-D, however Tonic plantain did not survive the recommended rates of the above mentioned herbicides. The half rate of MCPA, 2,4-DB and 2,4-D amine were enough to kill the Tonic plants. Therefore, these herbicides would need to be used at a very low rate (i.e. below half recommended rate) to control weeds if used in a field that contains plantain. Also, the low rates of MCPB/MCPA mix may be enough to kill weeds, which will be studied in the coming field trials.

Although the untreated plants were 5 times bigger in the second experiment, and thus there was a higher growth rate, and this had little effect on relative differences between Tonic and Agritonic. Growing conditions and experimental techniques were kept as similar as possible for both experiments, but it is still not clear why the large differences occurred.

Chapter 4

First field experiment studying weed control in plantain-based pastures

4.1 Introduction

The traditional pastoral system of New Zealand was based on grass species with clover where the predominant pasture mix was perennial ryegrass (*Lolium perenne*) with white clover (*Trifolium repens*) (Kemp et al., 1999). The production of both ryegrass and white clover can be limited throughout summer and autumn months (Moorhead et al., 2002). There has been much research on pure plantain but less when it is in a mixed sward. A mixed sward comprised of plantain, perennial ryegrass, and red or white clover, or both is preferred by farmers nowadays as it provides improved persistence, a longer grazing season and produces greater herbage quality and yield as compared to mono species herb swards, which directly improves animal performance (Nie et al., 2008; Golding et al., 2011; Hutton et al., 2011; Sinhadipathige et al., 2012). Including plantain in such mixes is also now considered important to assist with reducing nitrate leaching from grazed pastures (Carlton et al., 2019).

Despite using correct management practices when establishing new forages, there is still rapid establishment of weeds in the field (Fraser et al., 2016). There are generally less herbicide options available to control weeds in plantain mixed swards as compared to other forages and legumes (Lockley and Wu, 2008) and also the effect of many selective herbicides on plantain is less known. Therefore, more research needs to be done to control weeds in mixed swards of pasture incorporating plantain. In Chapter 3, it was found that Agritonic plantain can tolerate some selective herbicides under glasshouse conditions when grown alone. A field experiment was then conducted to test how tolerant this plantain was to a variety of herbicides relative to clover and weeds. Information about the herbicides used was provided in Section 2.3.2. Thus, the purpose of this experiment was to determine the tolerance of plantain, white clover and perennial ryegrass to a range of herbicides applied to a mixed sward of these species at an early stage of establishment in spring. The effectiveness of the herbicides was also assessed for controlling weeds that establish within the swards.

4.2 Materials and methods

A field trial was set up at the Pasture and Crop Research Unit located at Poultry Farm Road, Palmerston North, on soil classified as a Manawatu silt loam over sand. The paddock was prepared by spraying with Glyphosate 540 @ 4 L ha $^{-1}$ on 4th October 2019 as a boom application, followed by mouldboard ploughing and secondary cultivation. Tonic and Agritonic cultivars of plantain (7 kg ha^{-1}) were each sown in half of the plots, both being mixed with One50 diploid perennial ryegrass (10 kg ha^{-1}) and Apex white clover (3 kg ha^{-1}). These were sown on 31 October 2019 with a seed drill (Figure 4.3 A) where the maximum and minimum temperature was 16.3 ºC and 7.5 ºC, respectively. Soil samples were collected from the top 15 cm from different places in the paddock and sent to Hill Laboratories to get tested for the soil characteristics (Hill Laboratories, 2022) (Table 4.1). Soil samples were also analysed by the Environmental Chemistry Laboratory of Landcare Research for particle size distribution, which showed the soil at the site had 21% clay, 40% silt, 30% fine sand (0.06-0.2 mm) and 9% medium sand (0.2-0.6 mm) (Landcare Research, 2022).

Table 4.1 Soil characteristics at the research site (Pasture and Crop Research Unit).

Soil Characteristics	Value
pH	5.4
Organic matter %	4.13
Olsen P mg/L	58
Potassium (K) me/100g	0.29
Calcium (Ca) me/100g	5.5
Magnesium (Mg) me/100g	0.90
Sodium (Na) me/100g	0.07
Cation exchange capacity (CEC) me/100g	12
% Base Saturation	55
Volume weight g/mL	1.12
Total carbon %	1.9

The field trial was conducted using a randomised complete block design with four replicates/blocksand a split-plot arrangement of 12 herbicide treatments where cultivars

(Agritonic and Tonic) were allocated as the main plots and herbicide treatments as subplots (Figure 4.1). Thus, there was 96 plots in total, and each plot was 3 m × 4 m. Pegging was done on 27 November 2019 to separate the individual plots. The treatments (Table 4.2) were applied in the early afternoon on 2 December 2019 (2 months after sowing) with a precision plot sprayer pressurized by carbon dioxide at 125 kPa and Teejet 730231 flat fan nozzles when the plantain had 3-6 leaves, white clover had 3-4 trifoliate leaves and weeds were about 4-7 leaf stage (10 cm high) (Figure 4.3 B and C) and had started to compete with pasture for presumably light, nutrients and water. The plots were irrigated when required through an overhead sprinkler irrigation system. Weather conditions during the experiment are shown in Figure 4.2.

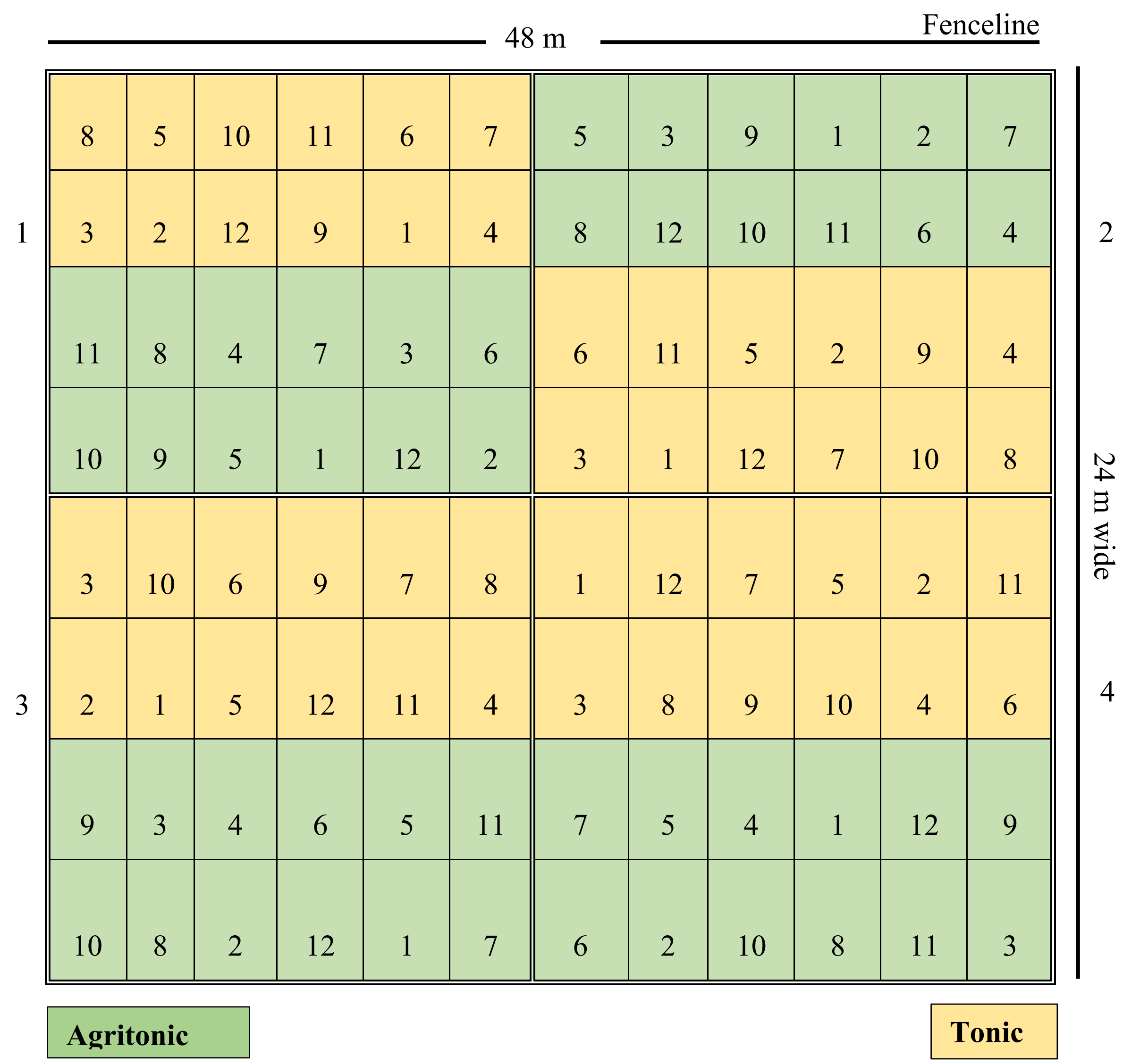


Figure 4.1 Layout of first field trial, 2019-2020. The number in each plot is the treatment number which is shown in Table 4.2, and the numbers to the sides are block numbers.

Table 4.2 Treatments applied to young plantain/clover/ryegrass sward.

Treatment		Trade name	Rates	g ai ha^{-1}
1	Untreated control			
2	Half recommended rate of MCPB	Dow AgroSciences MCPB	1.5 L ha^{-1}	577
3	Recommended rate of MCPB	Dow AgroSciences MCPB	3.0 L ha^{-1}	1155
4	Bentazone	Basagran	3.0 L ha^{-1}	1440
5	Flumetsulam	Preside	50 g ha $^{-1}$	40
6	MCPB/ MCPA	Thistrol Plus	3.0 L ha^{-1}	1125 + 75
7	Half MCPB + bentazone	Dow AgroSciences MCPB + Basagran	1.5 L ha^{-1} + 3.0 L ha^{-1}	577 + 1440
8	Flumetsulam + bentazone	Preside + Basagran	50 g ha $^{-1}$+ 3.0 L ha^{-1}	40 + 1440
9	Half MCPB/MCPA + bentazone	Thistrol Plus + Basagran	1.5 L ha^{-1} + 3.0 L ha^{-1}	562 + 37 + 1440
10	Flumetsulam + bentazone + half MCPB	Preside + Basagran + Dow AgroSciences MCPB	50 g ha $^{-1}$+ 3.0 L ha^{-1} + 1.5 L ha^{-1}	40 + 1440 + 577
11	Paraquat/diquat mix	Preeglone	2 L ha^{-1}	270 + 230
12	Diuron	Karmex DF	0.5 kg ha^{-1}	400

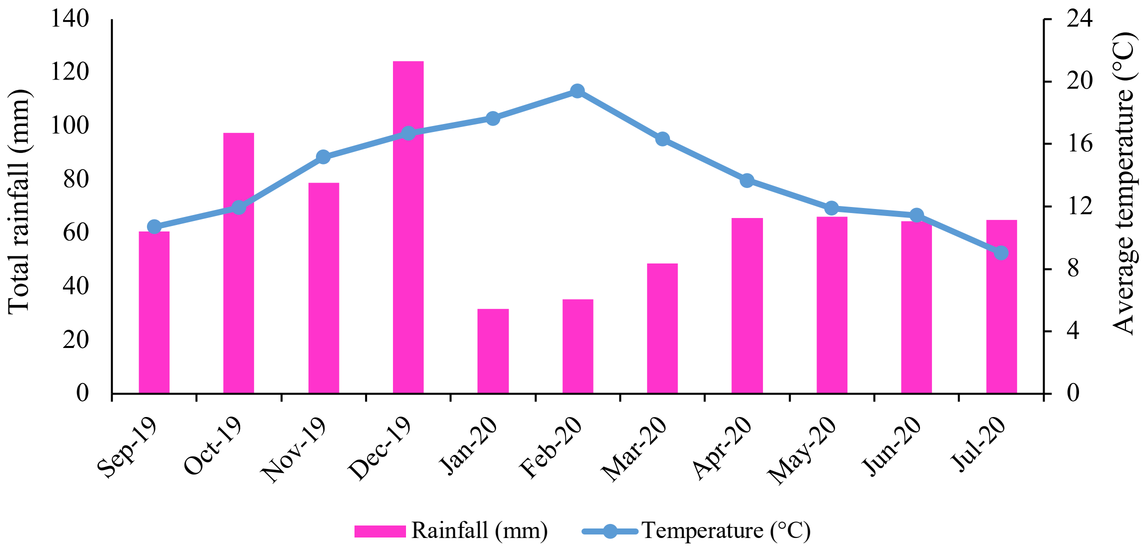


Figure 4.2 The average of maximum and minimum temperatures (°C) and total monthly rainfall (mm) for the duration of the trial, recorded at the AgResearch weather station approximately 1 km from the site.

The effects of herbicides were assessed through a scoring method for each pasture species as well as total weeds in each plot at various times after spraying where first scoring was 1 week after herbicide was sprayed and was repeated every 2 weeks until 16 January 2020. Scores were allocated based on a 0-10 scale where 0 signified no effect and 10 signified total death. Note that scores were used to interpret dry weight results, though only dry weight results are reported below as these were sufficient to show treatment effects. The first mowing was 3 weeks after herbicide treatment (Figure 4.3 D) as weeds were smothering the pasture in some plots which would have made it difficult to assess effects of herbicides on the pasture components if they weren't removed. After the first harvest, 35 kg ha^{-1} urea was applied prior to irrigation and after mowing plots, to overcome a nitrogen deficiency in the plantain. The main effects of the herbicides were assessed by taking cut quadrats (from just above the ground) from two randomly selected 0.1 m^2 (50 cm x 20 cm) quadrats per plot (Figure 4.3 E). Then the cut samples were separated into the following components: plantain, ryegrass, clover, weeds and dead matter was allocated to the respective species (Figure 4.3 F). These were then all oven dried for 48 hours and weighed. Harvests were conducted on four occasions, at 2, 3, 5 and 7 months after spraying, followed each time by mowing to a height of 5 cm to simulate grazing and to avoid excessive growth of pasture and weeds.

Figure 4.3 Various operations done in the field A. Seed sowing, B. pasture and weed establishment C. spraying of herbicides, D. mowing of plots, E. harvesting the plants in quadrats and F. separating plantain, ryegrass, clover and weeds from sub-sample.

4.2.1 Statistical analysis

An analysis of variance was performed on all data using SPSS and least significant differences were calculated to test if treatment means differed significantly at 5% level of probability. A repeated-measures mixed model was used which had three fixed factors, i.e. time, cultivars and herbicides. The random factor was block, including those nested within interactions with fixed factors and the covariance structure selected was diagonal. The repeated measures enabled comparison across the harvests.

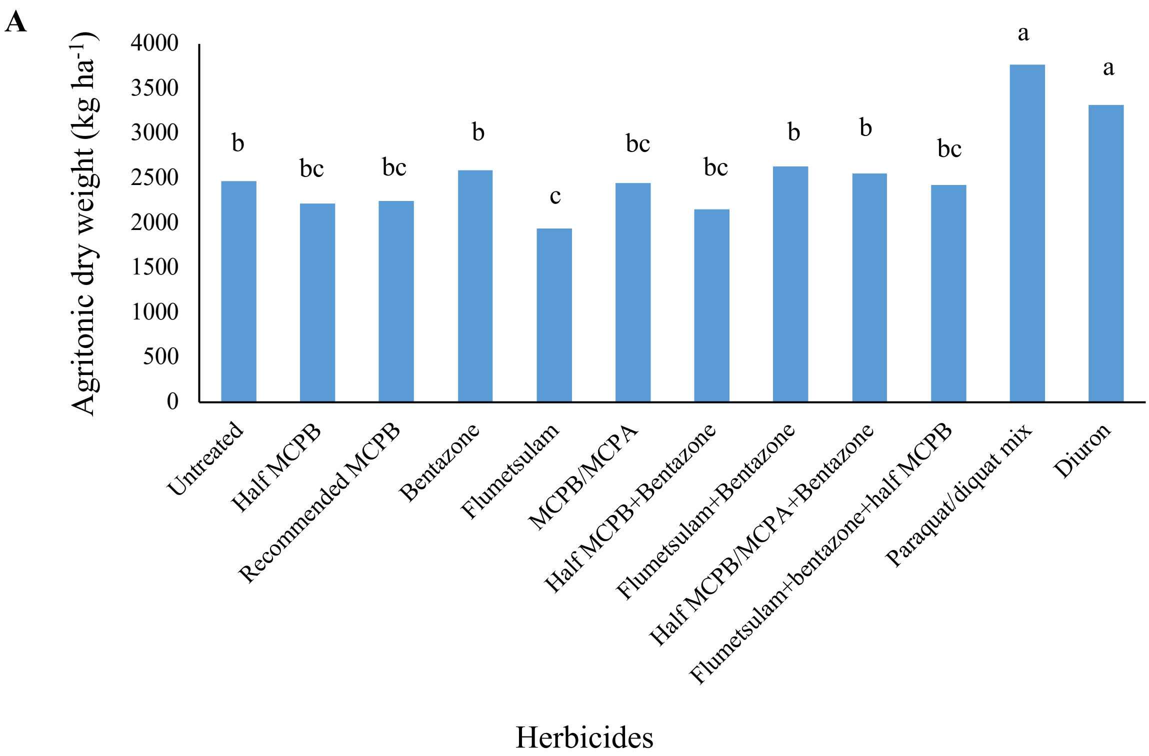
4.3 Results

4.3.1 Plantain

There was no significant difference between the two plantain cultivars in their overall dry weight (Table 4.3), suggesting that the cultivars responded similarly to herbicide treatments. However, the effect of herbicide treatments on dry weight of both cultivars was significant along with the interaction between cultivar and herbicides (p=0.023) as shown in Table 4.3. The dry weight of Agritonic was significantly less affected when treated with a paraquat/diquat mix and diuron compared to other treatments whereas the highest reduction in dry weight was recorded for flumetsulam (Figure 4.4A). The total dry weight of Agritonic treated with recommended rates of MPCB and MCPB/MCPA was not reduced significantly compared to the untreated control. Bentazone treated plants also reacted similarly to untreated plants. In contrast, the dry weight of Tonic treated with diuron was not significantly less than the untreated control and a significant reduction was found when treated with the recommended rates of MCPB and MCPB/MCPA (Figure 4.4B). A clear difference in the tolerance of recommended rates of MCPB and MCPB/MCPA was seen between cultivars, where Agritonic was found to be more tolerant than Tonic (Figure 4.5).

Table 4.3 ANOVA table for the effect of cultivar, herbicides, and time of harvest on plantain dry weight.

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	164.2	4425.4	<0.001
Time	3	177.6	216.4	<0.001
Plantain cultivar	1	164.2	0.3	0.586
Herbicide	11	164.2	10.1	<0.001
Time * Cultivar	3	177.6	4.1	0.008
Time * Herbicide	33	177.6	4.7	<0.001
Cultivar * Herbicide	11	164.2	2.1	0.023
Time * Cultivar * Herbicide	33	177.6	1.1	0.378



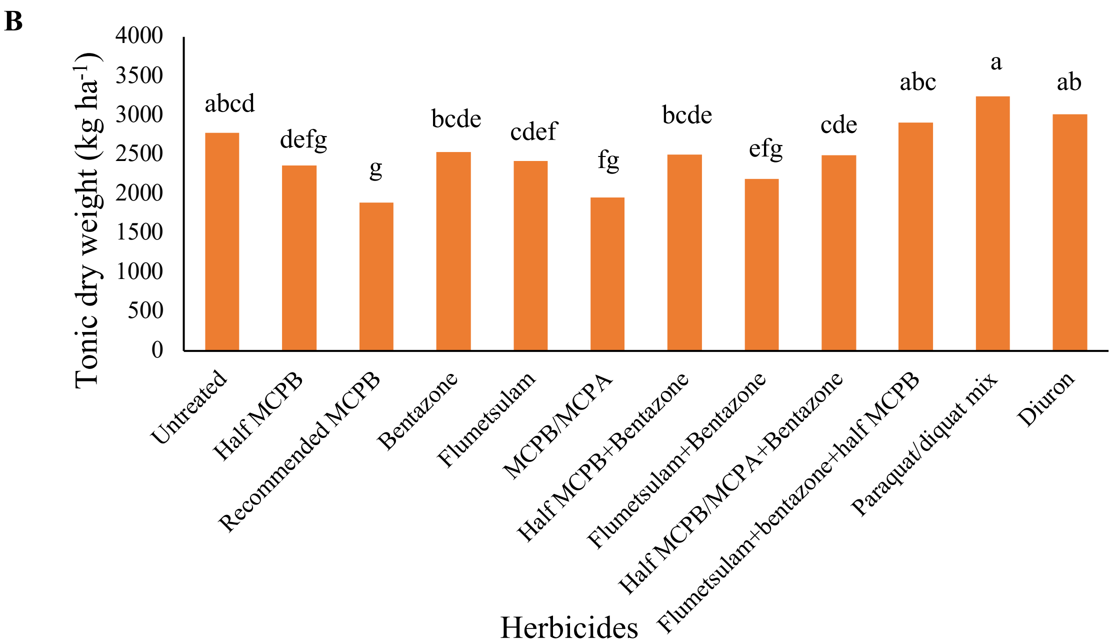


Figure 4.4 The effect of cultivar and herbicide treatments on A. Agritonic and B. Tonic dry weight averaged across all four harvests. Bars with different lowercase letters are significantly different according to LSD ($p < 0.05$).

Most of the herbicides affected the plantain severely a few days after spraying. The visual observation from the field showed that after spraying the lower rate of paraquat/diquat, it desiccated all the plants within just one day, but plantain recovered quickly, though clover and ryegrass didn't recover well. The results from 2 months after spraying showed that a paraquat/diquat mix had the least significant effect on the dry weight of plantain in comparison to other herbicides even with untreated control. The initial damage to plantain caused by treatments such as diuron, the mixture of flumetsulam, bentazone and half MCPB became less apparent over time and the dry weight measured at 3 months after spraying for these treatments was similar to the untreated control. There was still a lot of damage to plantain at the recommended rates of MCPB and MCPB/MCPA in the treated plots at 3 months. Flumetsulam also caused some adverse effects to plantain initially but plants recovered later. However, by the final harvest (7 months after spraying), there was no significant difference in dry weight of plantain between any of the treatments and there was good growth of plantain when the experiment ended in early July 2020.

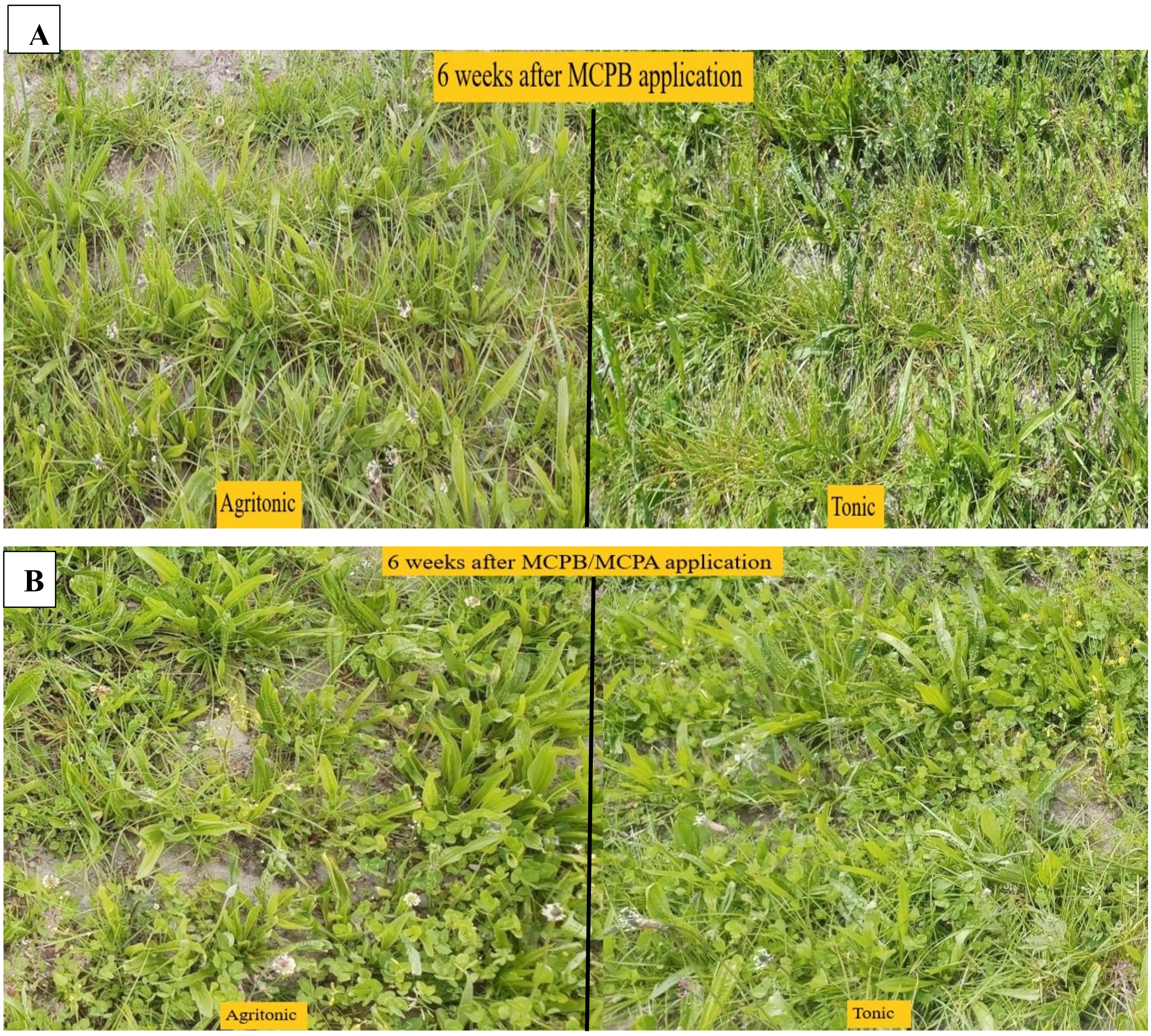


Figure 4.5 Difference in tolerance to A. MCPB and B. MCPB/MCPA of Agritonic and Tonic plantain plants, 6 weeks after application of herbicides.

The overall dry weight of Agritonic and Tonic plantain plants was significantly different only at the third harvest which was 5 months after spraying, where Tonic had greater overall dry weight than Agritonic plants (Figure 4.6). At the last harvest which was 7 months after spraying, Tonic appeared to have better growth and higher dry weight than Agritonic, but they were not significantly different.

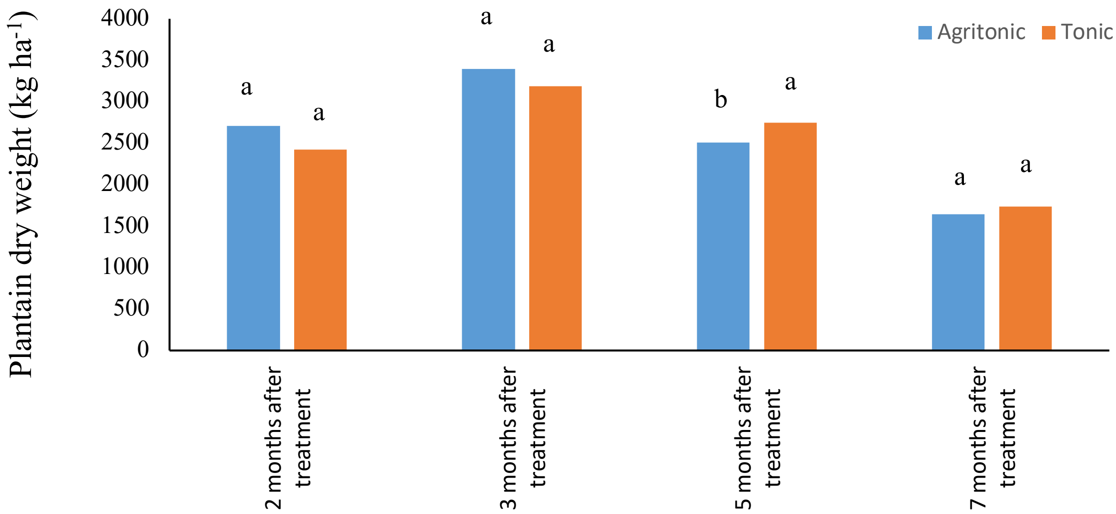


Figure 4.6 The interaction effect of plantain cultivar when harvested at different times after treatment. Bars with different lowercase letters are significantly different according to LSD ($p<0.05$).

4.3.2 Ryegrass

The plantain cultivar grown did not have a significant effect on the dry weight of perennial ryegrass (Table 4.4), suggesting that both cultivars responded similarly to herbicide treatments and so competed with ryegrass in a similar way. However, the effect of herbicide treatments on the dry weight of ryegrass was significantly different. As shown in Figure 4.7, the paraquat/diquat treatment had the greatest effect on dry weight of ryegrass. The ryegrass grew best in flumetsulam, recommended rate of MCPB, MCPB/MCPA, flumetsulam+bentazone and flumetsulam+bentazone+half MCPB treatments. MCPB/MCPA treated plots had significantly higher dry weight of ryegrass compared to untreated control whereas the effect of MCPB was found to be similar to untreated control. The dry weight of ryegrass was less in the plots sprayed with bentazone and was similar to untreated control.

Table 4.4 ANOVA table for the effect of cultivar, herbicides and time of harvest on ryegrass dry weight.

Source	**Numerator df**	**Denominator df**	**F**	**Sig.**
Intercept	1	100.0	1148.8	<0.001
Time	3	139.2	90.3	<0.001
Plantain cultivar	1	100.0	1.5	0.217
Herbicide	11	100.0	9.3	<0.001
Time * Cultivar	3	139.2	2.4	0.068
Time * Herbicide	33	139.2	1.6	0.025
Cultivar * Herbicide	11	100.0	1.7	0.081
Time * Cultivar * Herbicide	33	139.2	0.9	0.611

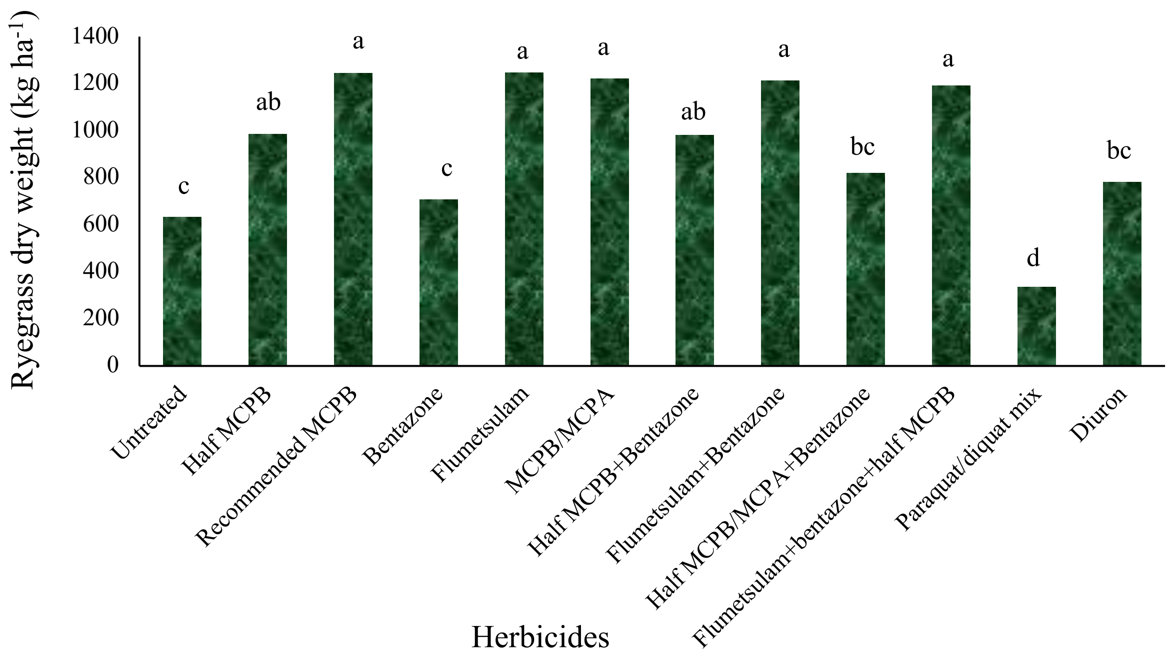


Figure 4.7 The effect of different herbicide treatments on ryegrass total dry weight averaged across all four harvests . Bars with different lowercase letters are significantly different according to LSD ($p < 0.05$).

4.3.3 Clover

There was a significant difference between the plantain cultivars, different herbicide treatments and interaction between time of harvest and herbicide effects on the dry weight of white clover as shown in Table 4.5. Thus, the dry weight of clover was significantly different between Agritonic and Tonic plots with a higher dry weight of clover in Agritonic plots than Tonic plots. As shown in Figure 4.8, diuron and paraquat/diquat significantly reduced the growth of white clover. The dry weight of clover was highest in flumetsulam, recommended rate of MCPB/MCPA, half recommended rate of MCPB+bentazone treatments, more than in the untreated control. The clover grew better in these plots due to adverse effect on plantain and weeds by these herbicides, reducing competition with clover.

Table 4.5 ANOVA table for the effect of cultivar, herbicides, and time of harvest on clover dry weight.

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	114.5	1161.2	<0.001
Time	3	127.7	23.2	<0.001
Plantain cultivar	1	114.5	7.7	0.007
Herbicide	11	114.5	11.7	<0.001
Time * Cultivar	3	127.7	0.9	0.435
Time * Herbicide	33	127.7	2.1	0.002
Cultivar * Herbicide	11	114.5	0.5	0.903
Time * Cultivar * Herbicide	33	127.7	0.6	0.951

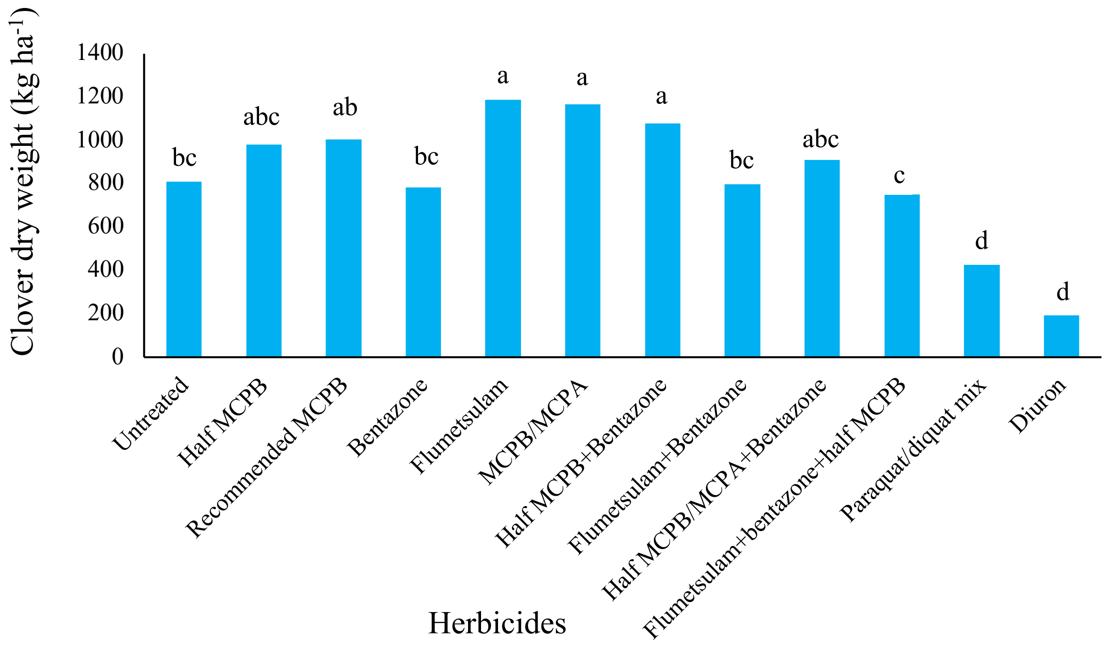


Figure 4.8 The effect of different herbicide treatments on clover total dry weight averaged across all four harvests. Bars with different lowercase letters are significantly different according to LSD ($p < 0.05$).

Diuron severely affected clover after a month compared to other herbicides, and this effect continued until the final harvest i.e. 7 months after herbicide application. Paraquat/diquat, had some effect on clover initially after spraying but it recovered later and was similar to the untreated control at 2 months after spraying (Figure 4.9A). Flumetsulam, bentazone, recommended rate of MCPB and MCPB/MCPA seemed to be safe on clover from first harvest to last harvest compared to all other herbicides (Figure 4.9A and 4.9B), but uncontrolled competition from weeds and/or plantain did have an effect on bentazone treated plots. Clover appeared to have produced good growth and ground was fully covered in most herbicide treated plots except diuron and mixture of paraquat/diquat at final harvest.

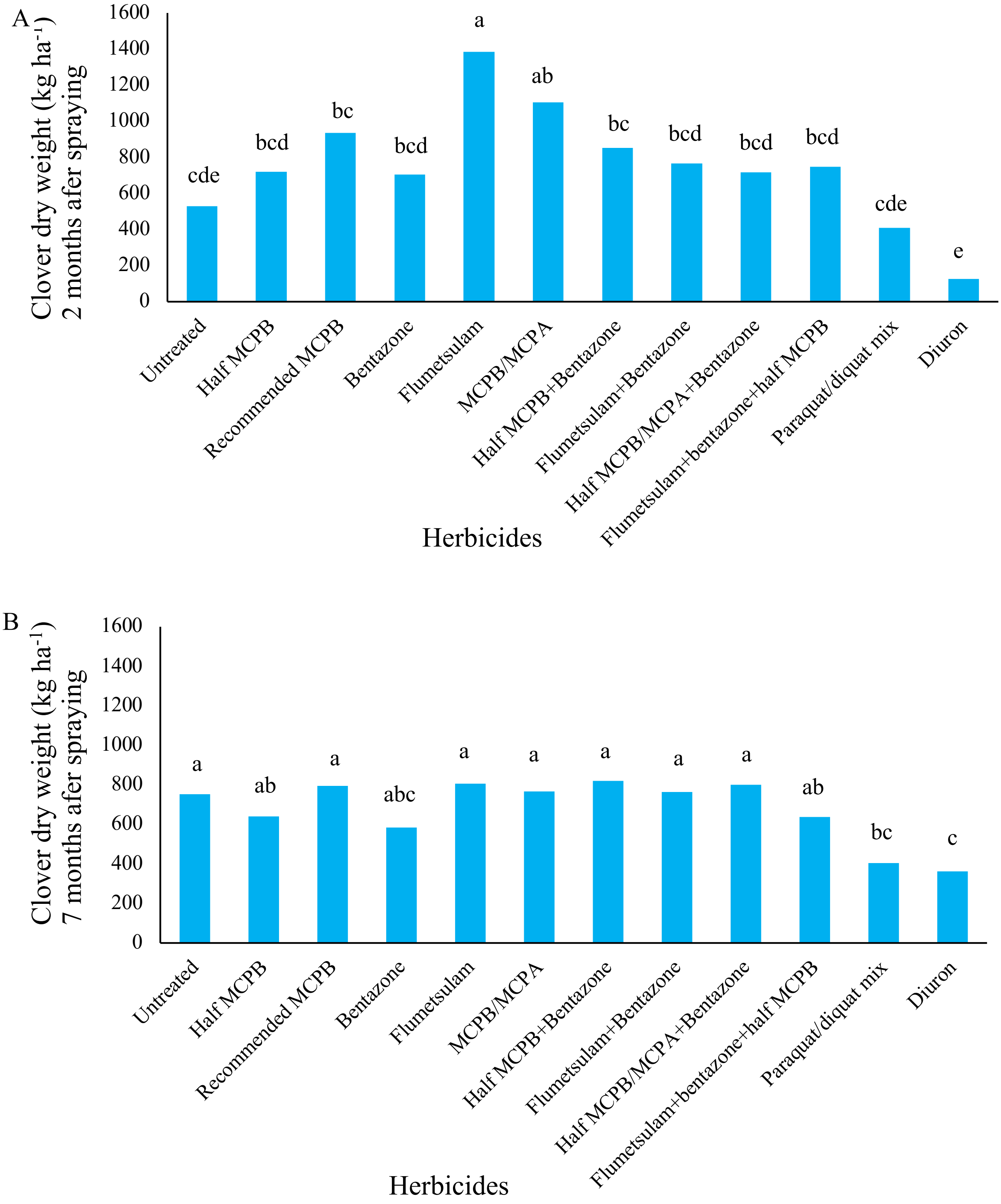


Figure 4.9 The effect of herbicides in clover dry weight averaged across all four harvests A. 2 months and B. 7 months after spraying. Bars with different lowercase letters are significantly different according to LSD ($p < 0.05$).

4.3.4 Weeds

The major weeds that were found in the field were redroot (*Amaranthus powellii*), black nightshade (*Solanum nigrum*), fathen (*Chenopodium album*), twin cress (*Coronopus didymus*) and groundsel (*Senecio vulgaris*); also uneven distribution of summer grass (*Digitaria sanguinalis*), wireweed (*Polygonum aviculare*), prickly sow thistle (*Sonchus asper*), galinsoga (*Galinsoga parviflora*), creeping mallow (*Modiola caroliniana*), and shepherd's purse (*Capsella bursa-pastoris*); and occasional broad-leaved dock (*Rumex obtusifolius*) across the plots. At the time of spraying the herbicides, the weeds ranged from small to flowering stage with redroot varying from small to 9 cm in height, black nightshade small to 7 cm, fathen small to flowering (13 cm), twin cress small to seed formation at the centre of the plant, and groundsel also small to 8 cm high (Figure 4.10).

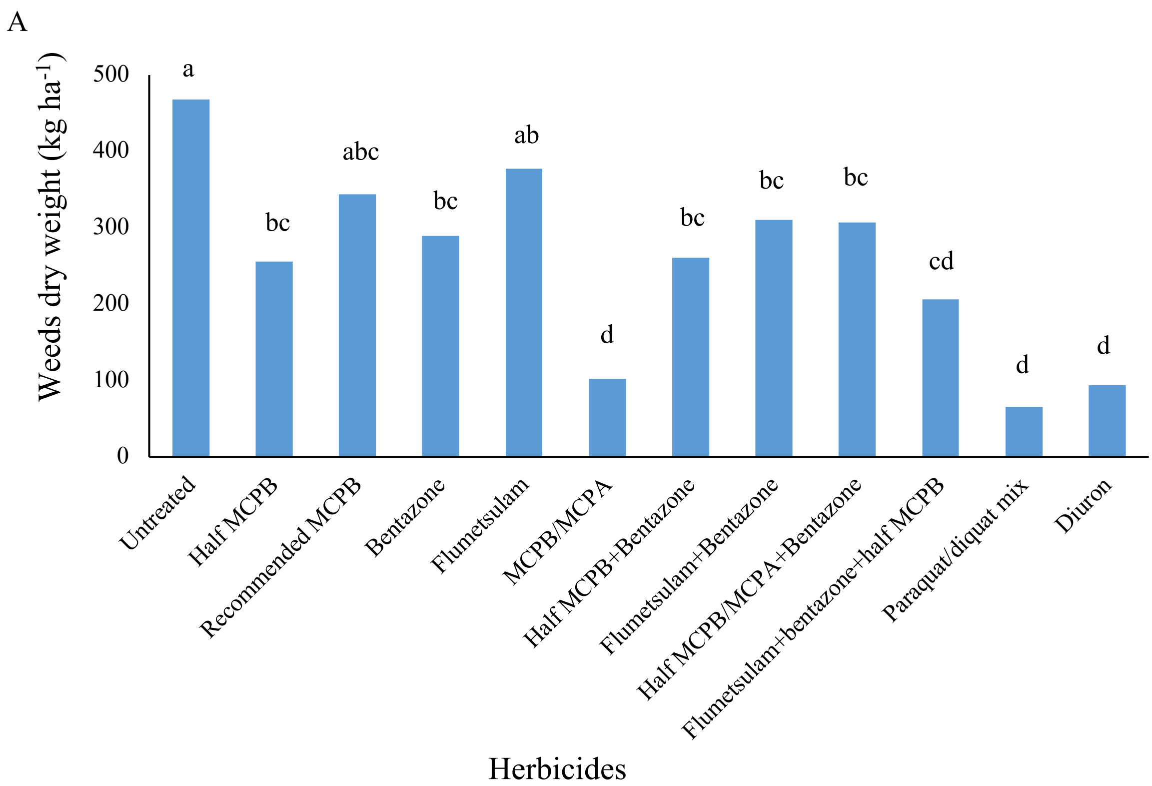
Figure 4.10 Different stages of pasture and weed plants at the time of spraying of herbicides.

The most dominant weed in the plots was redroot until the third harvest (5 months after spraying) then very few weeds were found during the third and fourth harvest as compared to earlier harvests, presumably because the upright weeds were removed by mowing after each harvest. Redroot, black nightshade and fathen were not found at the end of this experiment (July) because these are summer annual species which die naturally in autumn (Gawn et al., 2012). Thus, there were significantly fewer weeds in the final harvest than in earlier harvests. Flumetsulam gave good suppression of redroot, fathen, twin cress and black nightshade initially, but did not kill them completely and so they later recovered and spread over the plots by 6 weeks after spraying. Flumetsulam had little effect on docks and grasses. The recommended rate of MCPB controlled fathen and gave some suppression of the twin cress and groundsel. However, MCPB/MCPA worked better to control the twin cress, even the larger ones, and also had more effect on redroot. However, both MCPB and MCPB/MCPA mix had no effect on black nightshade and summer grass. Paraquat/diquat killed most of the weeds apart from twin cress which recovered along with the plantain. Bentazone caused some necrotic leaves on redroot, twin cress and galinsoga but had no effect on black nightshade and docks. A few weeks after spraying, the weeds in the bentazone-sprayed plots had recovered and were found to be tall and spreading over the plots like in the untreated control plots.

The plantain cultivar grown, and the herbicide treatments used both had significant effects on the dry weight of weeds (Table 4.6). Tonic plantain plots had a significantly lower dry weight of weeds compared to Agritonic plots. The Agritonic untreated plots were weedier compared to the Tonic plots (Figure 4.11A and 4.11B). There was a significant interaction effect of cultivar and herbicide on the reduction of weeds (Table 4.6). In Agritonic plots, paraquat/diquat mix, diuron and the recommended rate of MCPB/MCPA gave the best reduction of weeds compared to all other treatments. The dry weight of weeds which had been treated with MCPB and flumetsulam was similar to the untreated ones showing not much effect on weeds (Figure 4.11A). In Tonic plots, because of the less weedy untreated plots, most of the herbicides had little effect on the total dry weight of weeds compared with untreated plots except diuron which had significantly less weed dry weights compared to the plots with the most weeds, which were those treated with half MCPB/MCPA+bentazone, bentazone and half MCPB+bentazone (Figure 4.11B).

Table 4.6 ANOVA table for the effect of cultivar, herbicides, and time of harvest on weeds dry weight.

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	169.7	419.8	<0.001
Time	3	95.5	103.4	<0.001
Plantain cultivar	1	169.7	6.7	0.010
Herbicide	11	169.7	4.4	<0.001
Time * Cultivar	3	95.5	2.2	0.091
Time * Herbicide	33	95.5	2.0	0.006
Cultivar * Herbicide	11	169.7	2.2	0.018
Time * Cultivar * Herbicide	33	95.5	1.3	0.153



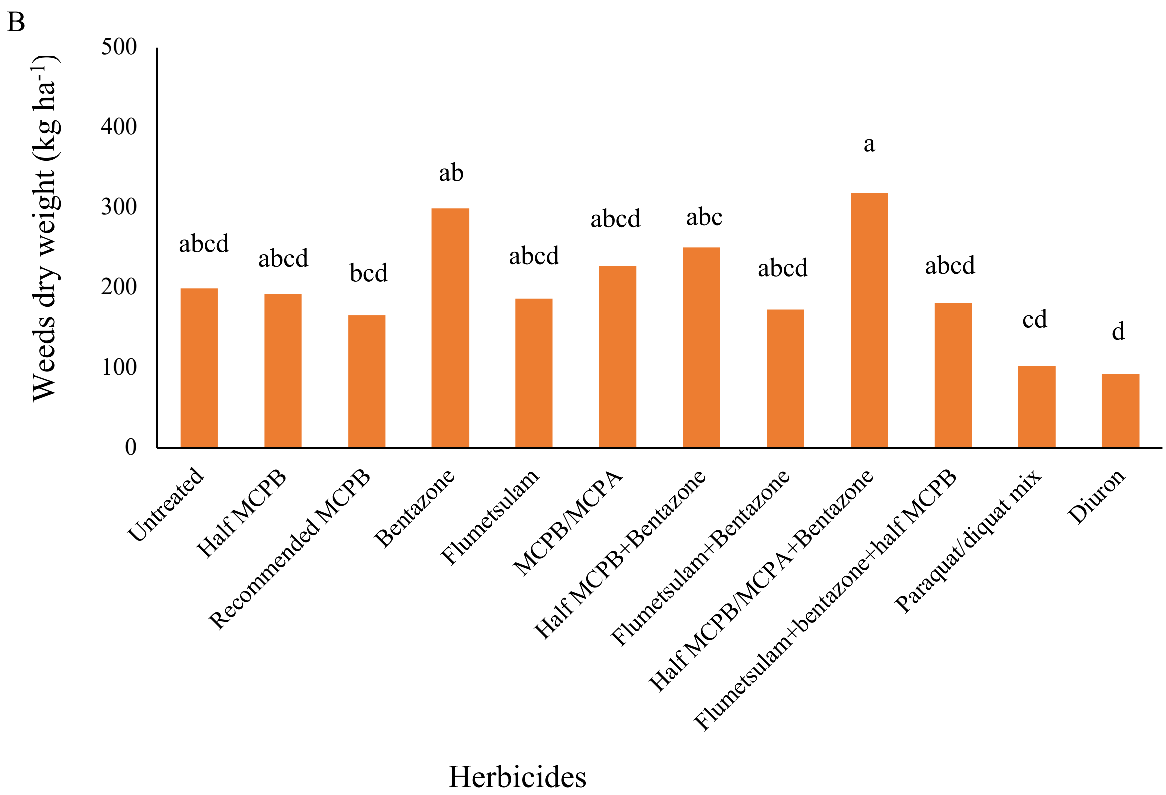
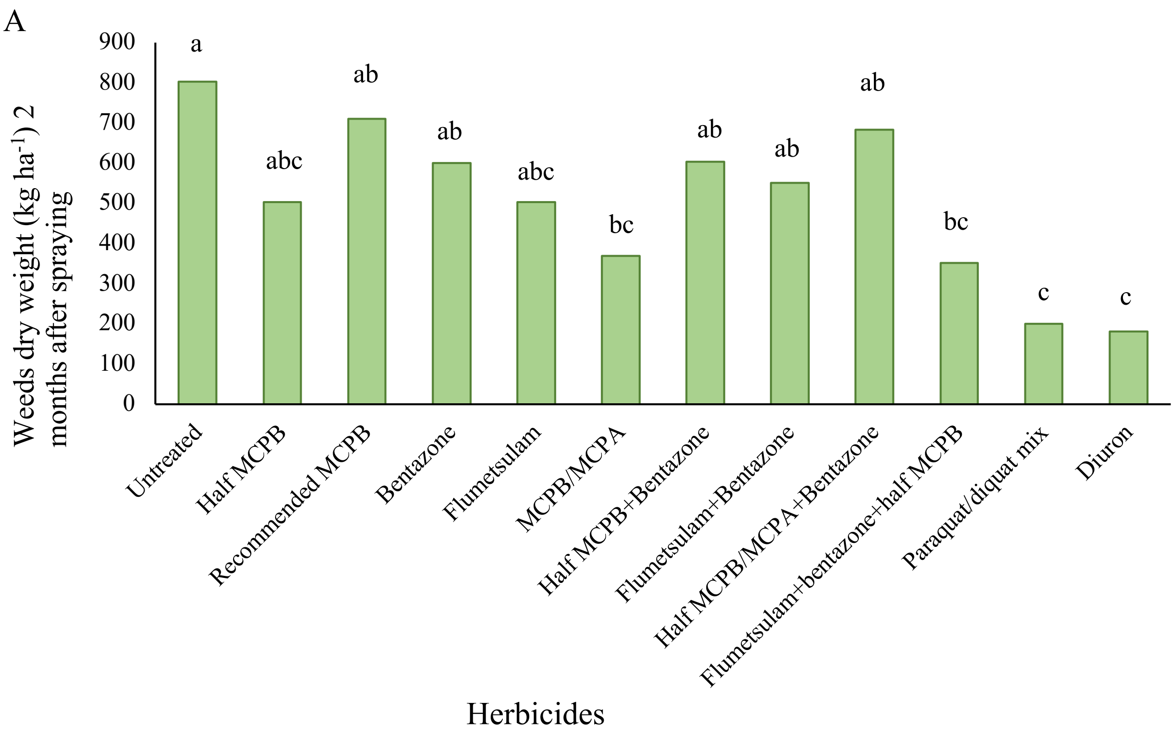


Figure 4.11 The effect of herbicide treatments on total weed dry weight averaged across all four harvests in plots with A. Agritonic plantain and B. Tonic plantain. Bars with different lowercase letters are significantly different according to LSD (p< 0.05).



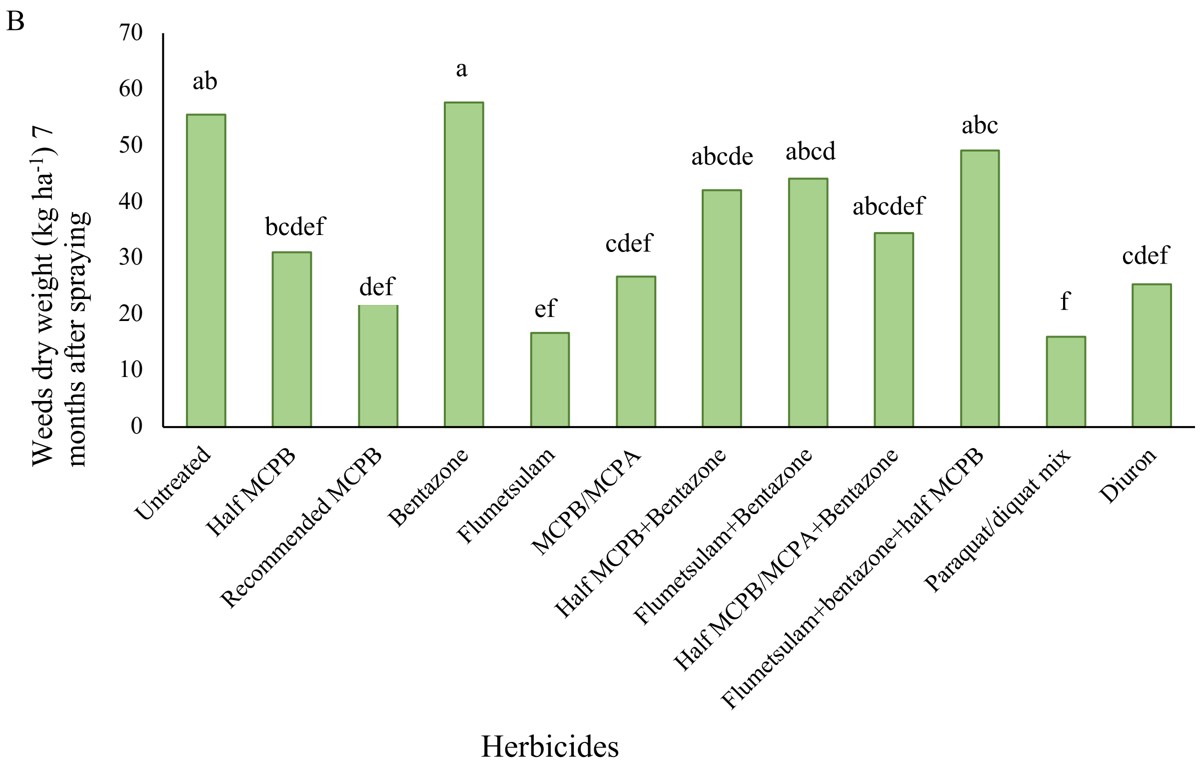


Figure 4.12 The effect of herbicides on weeds dry weight averaged across all four harvests A. 2 months and B. 7 months after spraying. Bars with different lowercase letters are significantly different according to LSD ($p < 0.05$).

The effect of herbicides on weeds changed over the four harvests. Diuron, paraquat/diquat mix, the mixture of flumetsulam+bentazone+half MCPB and the MCPB/MCPA mixture all significantly reduced the dry weight of weeds compared to the untreated control at 2 months after spraying (Figure 4.12A). By 7 months after spraying, the treatments that had significantly less weed dry weight than the untreated control were paraquat/diquat mix, diuron, flumetsulam and the recommended rates of MCPB and MCPB/MCPA (Figure 4.12B). At this final harvest, weed biomass was low in all plots and most of the weeds present were annual poa (*Poa annua*) that had established in gaps within the swards, with small amounts of creeping oxalis (*Oxalis exilis*) also present.

4.4 Discussion

The effect of diuron and paraquat/diquat on ryegrass and clover is less well known than the effect of the other herbicides in the trial which are routinely used in New Zealand pastures. The paraquat/diquat mix and diuron treatments showed potential to be used with plantain for weed control but it was decided not to assess these herbicides further because paraquat/diquat was too damaging to ryegrass despite using a low rate and diuron was too damaging to clover. The objective of this thesis was to find weed control options for ryegrass/clover/plantain swards. However, paraquat/diquat should be suitable to use in pastures with just plantain and clover, and diuron could be used with plantain and ryegrass. Gawn et al. (2012) also assessed a range of selective herbicides on establishing plantain/clover swards and found that the diuron was less damaging to clover compared to a paraquat/diquat mix 7 weeks after herbicide application whereas by end of her trial paraquat/diquat treated plots were left only with plantain and clover which was similar to the results of this experiment. Most of the significant differences shown in this chapter were due to these two herbicide treatments.

The field experiment confirmed results from the glasshouse experiments that Agritonic had more tolerance than Tonic to phenoxy herbicides. But in the glasshouse experiments, there was no competition between plantain and weeds or other pasture species, whereas this competition in the field trial made results less clear. At the end of the trial 7 months after herbicide application, plantain and clover had recovered from any adverse effects caused by the phenoxy herbicides. The mixture of flumetsulam+bentazone+half MCPB had the highest plantain dry weight apart from the paraquat/diquat mix and diuron, suggesting there was less competition from weeds against plantain in this treatment and, thus less effect on the growth of plantain. The highest total dry weight of plantain was found in the plots sprayed with the paraquat/diquat mix and diuron due to superior early growth of plantain, which was similar to the findings of Gawn et al. (2012). Flumetsulam, MCPB and bentazone were safe for ryegrass and clover. The dry weight of ryegrass was less in the plots sprayed with bentazone and was similar to untreated control which might be due to the competition exerted by the weeds that weren't damaged by bentazone. When the recommended rate of MCPB/MCPA was sprayed on young clover, there was very little detrimental effect to clover (Figure 4.8).

Mowing after each harvest helped to control the upright weeds like redroot, black nightshade and fathen and summer annual weeds like redroot, fathen and black nightshade die naturally in autumn, which were similar findings to those obtained by Gawn et al. (2012). Annual weeds, including those that germinate at any time such as twin cress and shepherd's purse, only establish when there is bare soil. So, they established initially, finished their lifecycle, died, then did not germinate again due to competition. Thus, very few weeds were found by the third and fourth harvest. The half rate of MCPB was less damaging to plantain than the recommended rate of MCPB and MCPB/MCPA but could not control most of the weeds. Plantain when sprayed with the MCPB/MCPA mix was similarly affected as by MCPB but the mixture controlled weeds better than MCPB. The redroot was suppressed by MCPB/MCPA by itself and in combination with bentazone up to 6 weeks after spraying. The fathen was diminished by MCPB and its mixtures and twin cress by the recommended rate of MCPB and MCPB/MCPA mix. But along with the suppressed redroot, new seedlings of redroot, twin cress, black nightshade and fathen started to germinate with summer grass, shepherd's purse and sow thistle (which were only minor components). None of the herbicides controlled the grasses. Bentazone alone seemed to have much less effect on the weeds. But the mixture of flumetsulam, bentazone and half MCPB gave the best result to control weeds along with the recommended rates of MCPB/MCPA which suggests flumetsulam and MCPB were helpful in controlling the weeds. MCPB/MCPA was found to be better controlling the weeds of Agritonic plots than Tonic (Figure 4.11A and 4.11B) which might be because the Tonic plantain was more affected by the herbicides and so was less competitive with the weeds than the Agritonic. Although MCPB appeared to be less effective in the Agritonic plots than the Tonic plots, this result was simply due to large numbers of weeds present in the Agritonic/MCPB plots at the first harvest which later disappeared but this skewed the overall average figure.

Getting timing correct with selective herbicide applications can often be difficult. The plantain and clover needed to be established well enough to tolerate the herbicides, yet the weeds needed to be as young as possible to be susceptible to herbicides such as bentazone, MCPB and flumetsulam (Gawn et al., 2012; Holden, 2022). Once the clover and plantain were at a suitable stage, the weather was unstable and unfortunately there was light rain immediately after the herbicide application which may have resulted in reduced effectiveness of herbicides, especially bentazone (Kudsk and Kristensen, 1992). Rainfall one hour after bentazone application generally reduces its activity on weeds (Reddy et al., 1995). On the day the herbicide treatments were applied, the plantain and clover plants were finally large enough though many weeds were too large. The winds that had prevented applications earlier had died down and no rain was forecast. However, light rain began falling soon after treatments were applied, and 0.4 mm fell over the next 8 hours, followed by 10 mm the following day, as recorded at the AgResearch weather station less than 1 km from the site. The advanced age of some weeds and this rainfall soon after application possibly affected the results obtained.

The field trial will be needed to be repeated to remove doubt over results caused by both the rainfall and age of some weeds. The main weeds we were interested in were docks and thistles, but the paddock we used for the trial had mostly redroot, fathen, black nightshade, and twin cress. This site mainly had cropping weeds because much of the history in this paddock had involved crops. So, a second reason for repeating the field trial was to try including more of the weed species that often cause problems in plantain-based pastures. A low sowing rate of ryegrass was used in this field trial so that the plantain and clover would not be affected by ryegrass competition. But the rate was probably too low and as a result, ryegrass was only a minor component of the swards during establishment, so a higher rate was needed in a repeated field trial.

4.5 Conclusion

Some useful results were obtained from this field experiment to determine the tolerance of plantain, white clover and perennial ryegrass in establishing pasture to a range of herbicides and also assessing the effectiveness of different herbicides to control weeds. The results from this experiment suggested that Agritonic plantain treated with recommended rates of MCPB and MCPB/MCPA mix was not adversely affected by these herbicides whereas Tonic plantain was affected. However, although the total dry weight of Tonic plantain was reduced significantly by these herbicides, it had recovered by the end of trial. Tonic plantain was found to have higher dry weight compared to Agritonic by the final harvest in treated plots as growth of Agritonic appears to be slower in winter.

Flumetsulam caused some initial damage to both cultivars of plantain, but they had recovered and were similar to the untreated control by the end of this trial. Bentazone appeared to be safe for the plantain but its effect was probably reduced by rainfall after application. Diuron and paraquat/diquat mix had no effect on plantain, but was damaging to clover and ryegrass, respectively. The mixture of flumetsulam+bentazone+half MCPB worked best for plantain, ryegrass and effectively controlled weeds while flumetsulam, bentazone and recommended rate of MCPB were safe on clover. The addition of MCPA to MCPB had little effect on clover and gave good control of weeds too. These herbicides were best, but we could not make firm conclusions and needed to do a second field trial.

Chapter 5

Second field experiment studying weed control in plantain-based pastures

5.1 Introduction

In the first field experiment, it was found that Agritonic plantain treated with phenoxy herbicides like MCPB and MCPB/MCPA mix was not adversely affected by these herbicides whereas Tonic plantain was affected. The dry weight of Tonic plantain had recovered by the end of the trial although it was reduced significantly over the period of the study. Flumetsulam caused some initial damage to plantain, but plants had recovered and were similar to the untreated control by the end of the trial. Bentazone appeared to be safe for the plantain, but its effect was probably reduced by rainfall after application. Diuron and paraquat/diquat had no effect on plantain, but had a severe effect on clover and ryegrass. A mixture of flumetsulam, bentazone and half the recommended rate of MCPB was safe for plantain, ryegrass and clover and controlled weeds effectively. The addition of MCPA to MCPB had little detrimental effect on clover but gave good control of weeds.

These herbicides, flumetsulam, bentazone and a low rate of MCPB, appeared to be the best herbicides for plantain-based pasture, but there was uncertainty with this finding as there were a few problems while conducting this experiment. There was rainfall within 2 hours after herbicide application. The site mainly had cropping weeds and very few docks and thistles which are the weeds more commonly found in new pasture swards. Thus, a second field trial was conducted which was mostly a repetition of first field trial to remove doubt over results caused by rainfall, age of some weeds when treated and also including more weed species that often cause problem in plantain-based pastures. The purpose of this experiment was the same as first field experiment conducted in spring 2019 which was to determine the tolerance of plantain, white clover and perennial ryegrass to a range of herbicides applied to a mixed sward of these species at an early stage of establishment in spring. The effectiveness of the herbicides was also assessed for controlling weeds that establish within the swards.

5.2 Materials and methods

This experiment had similar materials and methods to the first field experiment (Chapter 4) but with a few changes. The field trial was set up at the Sheep and Deer Research Unit of Massey University located at Sheep Farm Road, Palmerston North, on soil classified as a Tokomaru silt loam. There had been very little cropping previously at the selected site, just pasture grazing, so it was predicted that weeds would be more typical of those normally found in new pastures. The paddock was prepared by spraying with Glyphosate 540 at 4 L ha $^{-1}$ on the 30th of September 2020 as a boom application, followed by mouldboard ploughing and secondary cultivation. Then fertiliser at 250 kg ha^{-1} Cropmaster 15 (15:10:10:8 NPKS, a Ravensdown product) was applied to the soil prior to seeding to ensure adequate nutrients were present. Tonic and Agritonic cultivars of plantain (7 kg ha^{-1}) were each sown in half of the plots, both being mixed with One50 diploid perennial ryegrass (15 kg ha^{-1}) and Apex white clover (3 kg ha^{-1}). The seed rate for ryegrass was increased from the 10 kg ha^{-1} rate used in the first field trial to increase the level of competition from ryegrass with plantain to levels more realistic to rates used by farmers. The mixture of plantain, ryegrass and clover seeds were sown on the 4th of November 2020 with a seed drill. The plots got moisture from rainfall only and no separate irrigation system was installed, unlike the first trial. There was 6 mm of rainfall 1 week prior to sowing and an average temperature of 15 ºC and 24.4 mm of total rainfall within a week after sowing. Soil samples were collected from the top 15 cm from different places in the paddock and sent to Hill Laboratories to get tested for the soil characteristics (Hill Laboratories, 2022) (Table 5.1). Soil samples were also analysed by the Environmental Chemistry Laboratory of Landcare Research for particle size distribution, which showed the soil at the site had 27% clay, 66% silt, 6% fine sand (0.06-0.2 mm) and 1% medium sand (0.2-0.6 mm) (Landcare Research, 2022).

Table 5.1 Soil characteristics at the research site (Sheep and Deer Research Unit).

Soil Characteristics	Value
pH	5.9
Organic matter %	6.7
Olsen P mg/L	28
Potassium (K) me/100g	0.32
Calcium (Ca) me/100g	6.8
Magnesium (Mg) me/100g	0.92
Sodium (Na) me/100g	0.16
Cation exchange capacity (CEC) me/100g	13
% Base saturation	63
Volume weight g/mL	1.0
Total carbon %	3.9

The field trial was conducted using a randomised complete block design with four replicates and a split-plot arrangement of 10 herbicide treatments (Table 5.2) where cultivars (Agritonic and Tonic) were allocated as the main plots and herbicide treatments as subplots (Figure 5.1). In the first field trial (Chapter 4), the paraquat/diquat mix was too damaging to ryegrass and diuron was damaging to clover, so these treatments were not used in this experiment. Thus, there were 80 plots in total, and each plot was 3 m × 4 m. The treatments were applied on the 15th of December 2020 in the morning with a precision plot sprayer pressurised by carbon dioxide at 125 kPa and Teejet 730231 flat fan nozzles. At the time of herbicide application, there was very little clover present due to poor germination, plantain was 4-6 leaf stage (12-17 cm) and ryegrass 4-8 leaf stage (12-16 cm). The major weeds that were found in the plots were broad-leaved dock (*Rumex obtusifolius*) from small to 6 leaf stage, 4-10 leaf staged twin cress (*Coronopus didymus*), flowering spurrey (*Spergula arvensis*), and 4-leaf stage to flowering hawkbit (*Leontodon taraxacoides*). There were uneven distribution of flowering *Fumaria muralis* plants, flowering creeping buttercup (*Ranunculus repens*), 2-4-leaf stage black nightshade (*Solanum nigrum*), very few fathen (*Chenopodium album*), redroot (*Amaranthus powellii*), or prickly sow thistle (*Sonchus asper*) plants; and very less small to flowering dandelion (*Taraxacum officinale*), 4-6-leaf summer grass (*Digitaria sanguinalis)* and 4-7-leaf Yorkshire fog *(Holcus lanatus)*. Weather conditions during the experiment are shown in Figure

5.2. The average temperature on the day of spraying was 15.5 ºC and plants were growing actively as the soil was moist. Also, there was no rainfall for a week after spraying.

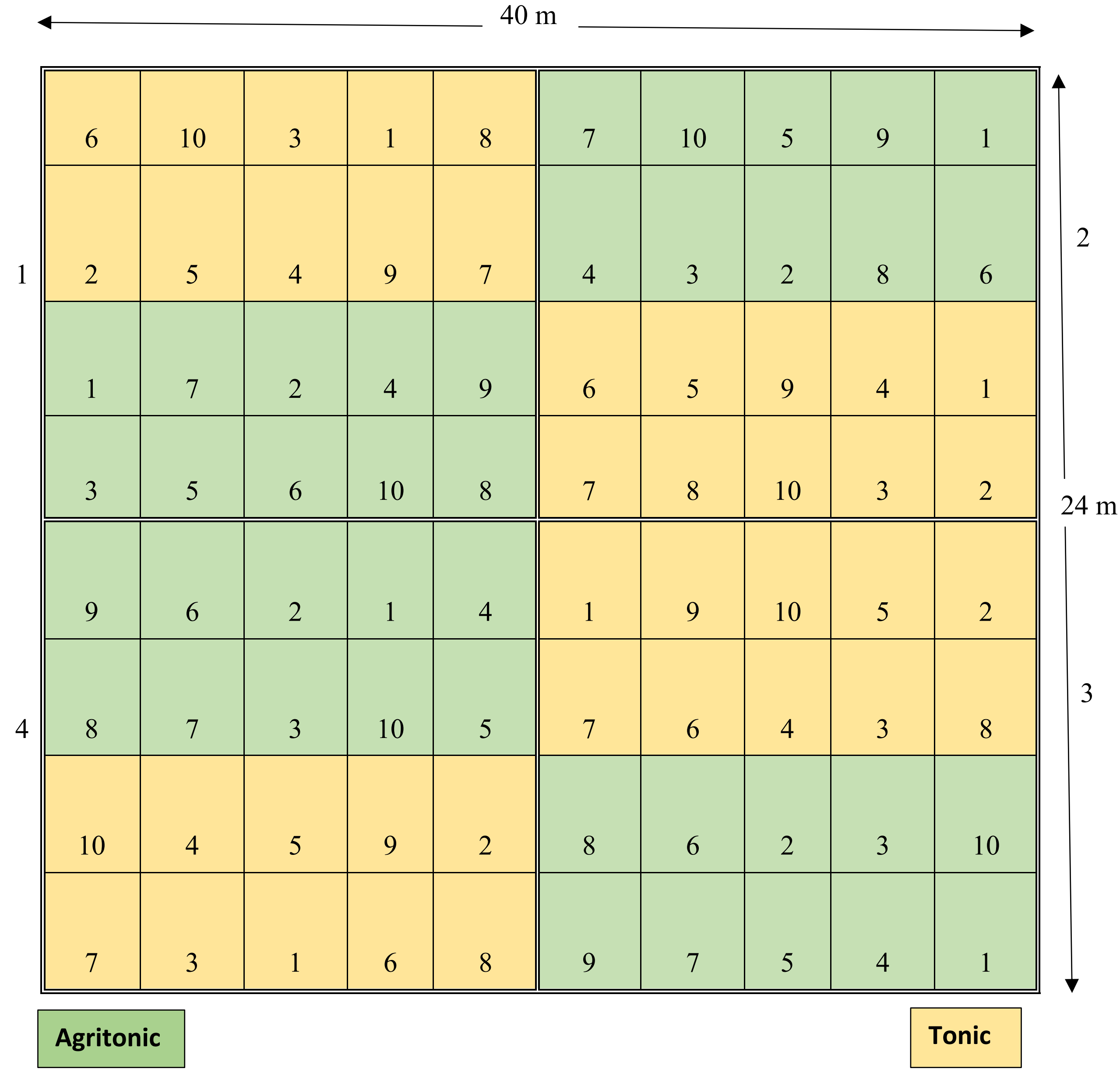


Figure 5.1 Layout of second field trial, 2020-2021. The number in each plot is the treatment number which is shown in Table 5.2, and the numbers to the sides are block numbers.

Table 5.2 Treatments applied to young plantain/clover/ryegrass sward on 15 December 2020.

Treatment	Trade name	Rates	g ai ha^{-1}
Untreated control			
Half recommended rate of MCPB	Dow AgroSciences MCPB	1.5 L ha^{-1}	577.5
Recommended rate of MCPB	Dow AgroSciences MCPB	3.0 L ha^{-1}	1155
Bentazone	Basagran	3.0 L ha^{-1}	1440
Flumetsulam	Preside	50 g ha $^{-1}$	40
MCPB/ MCPA	Thistrol Plus	3.0 L ha^{-1}	1125 + 75
Half MCPB + bentazone	Dow AgroSciences MCPB + Basagran	1.5 L ha^{-1} + 3.0 L ha^{-1}	577 + 1440
Flumetsulam + bentazone	Preside + Basagran	50 g ha $^{-1}$+ 3.0 L ha^{-1}	40 + 1440
Half MCPB/MCPA + bentazone	Thistrol Plus + Basagran	1.5 L ha^{-1} + 3.0 L ha^{-1}	562 + 37 + 1440
Flumetsulam+ bentazone + half MCPB	Preside + Basagran + Dow AgroSciences MCPB	50 g ha $^{-1}$+ 3.0 L ha^{-1} + 1.5 L ha^{-1}	40 + 1440 + 577

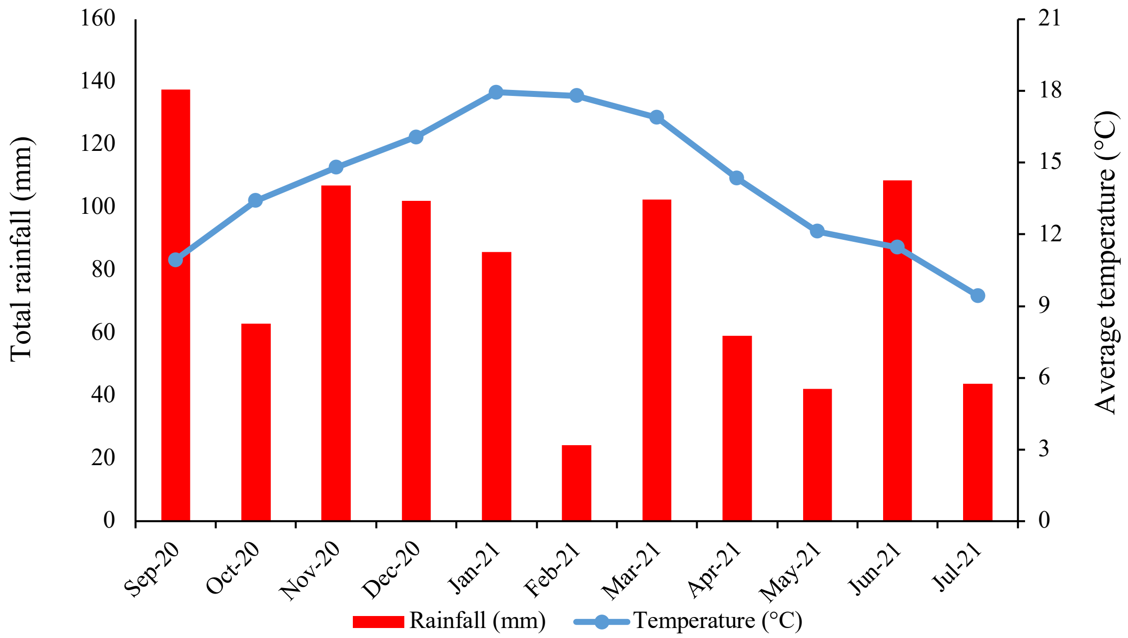


Figure 5.2 The average of maximum and minimum temperatures (°C) and total monthly rainfall (mm) for the duration of the trial, recorded at the AgResearch weather station approximately 1 km from the site.

Assessment methods were similar to those described in Chapter 4. The effects of herbicides were assessed through a scoring method for each species of pasture as well as weeds in each plot at various times after spraying. The main effects of the herbicides were assessed by taking cut quadrats from two randomly selected 0.1 m^2 (50 cm x 20 cm) quadrats per plot following the same procedure as in the first field experiment. Five harvests were conducted at 3 weeks, 2, 3, 4.5 and 6.5 months after spraying followed each time by mowing to a height of 5 cm to simulate grazing to avoid excess growth of pasture and weeds. After the second harvest (2 months after spraying), 35 kg ha^{-1} urea was applied after mowing the plots, to overcome nitrogen deficiency in the plantain.

5.2.1 Statistical analysis

An analysis of variance was performed on all data using SPSS and least significant differences were calculated to test if treatment means differed significantly. A repeated-measures mixed model was used which had three fixed factors, i.e. time, cultivars and herbicides. The random factor was block, including those nested within interactions with fixed factors and the covariance structure selected was diagonal. The repeated measures enabled comparison across the harvests.

5.3 Results

5.3.1 Plantain

There was no significant difference between the two plantain cultivars in their overall dry weight, suggesting that the cultivars responded similarly to herbicide treatments, though Agritonic had slightly higher overall dry weight than Tonic. The effect of herbicide treatments on dry weight of plantain was significantly different (Table 5.3). The dry weight of plantain was significantly less affected when treated with bentazone followed by half MCPB+bentazone and half MCPB/MCPA+bentazone. The highest reduction was recorded for recommended rates of MCPB whereas the half-recommended rate of MCPB had little effect on dry weight of plantain (Figure 5.3).

Table 5.3 ANOVA table for the effect of cultivar, herbicides, and time of harvest on plantain dry weight.

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	70.4	3231.0	<0.001
Time	4	95.2	158.1	<0.001
Plantain cultivar	1	70.4	1.6	0.212
Herbicide	9	70.4	3.4	0.002
Time * Cultivar	4	95.2	4.3	0.003
Time * Herbicide	36	95.2	1.2	0.225
Cultivar * Herbicide	9	70.4	1.4	0.208
Time * Cultivar * Herbicide	36	95.2	0.9	0.530

The total dry weight of Agritonic plantain treated with recommended rates of MCPB and MCPB/MCPA was not reduced significantly compared to the untreated control whereas a significant reduction in the dry weight of Tonic plantain was observed when treated with recommended rates of MCPB and MCPB/MCPA (Figure 5.4A and B). There was a clear difference in the tolerance of recommended rates of MCPB and MCPB/MCPA between cultivars, where Agritonic was found to be more tolerant than Tonic (Figure 5.5) as in the first field experiment.

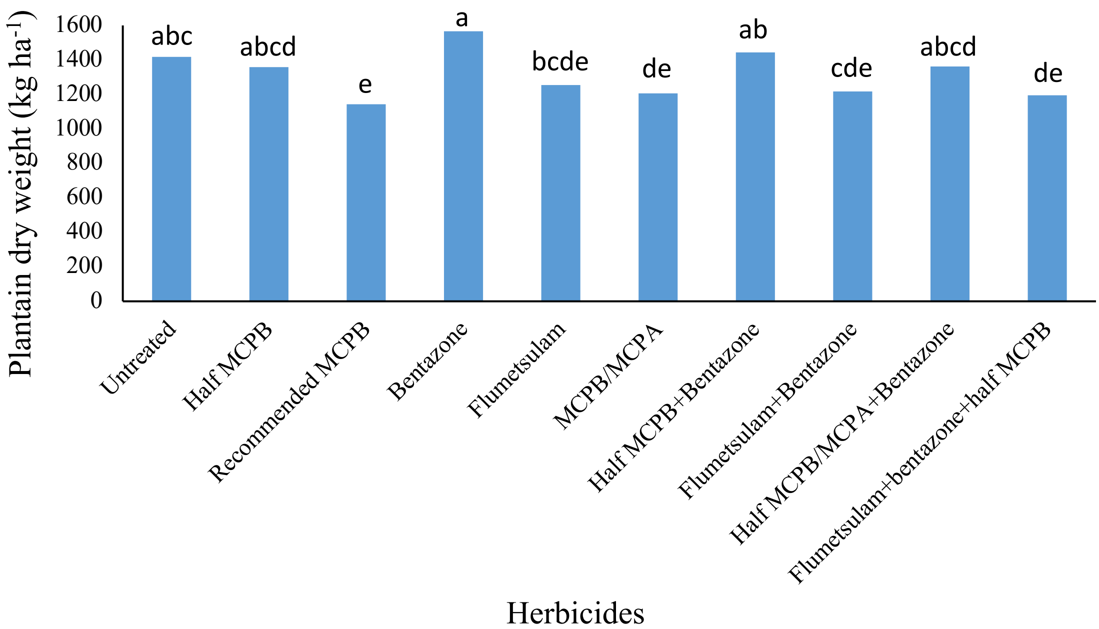
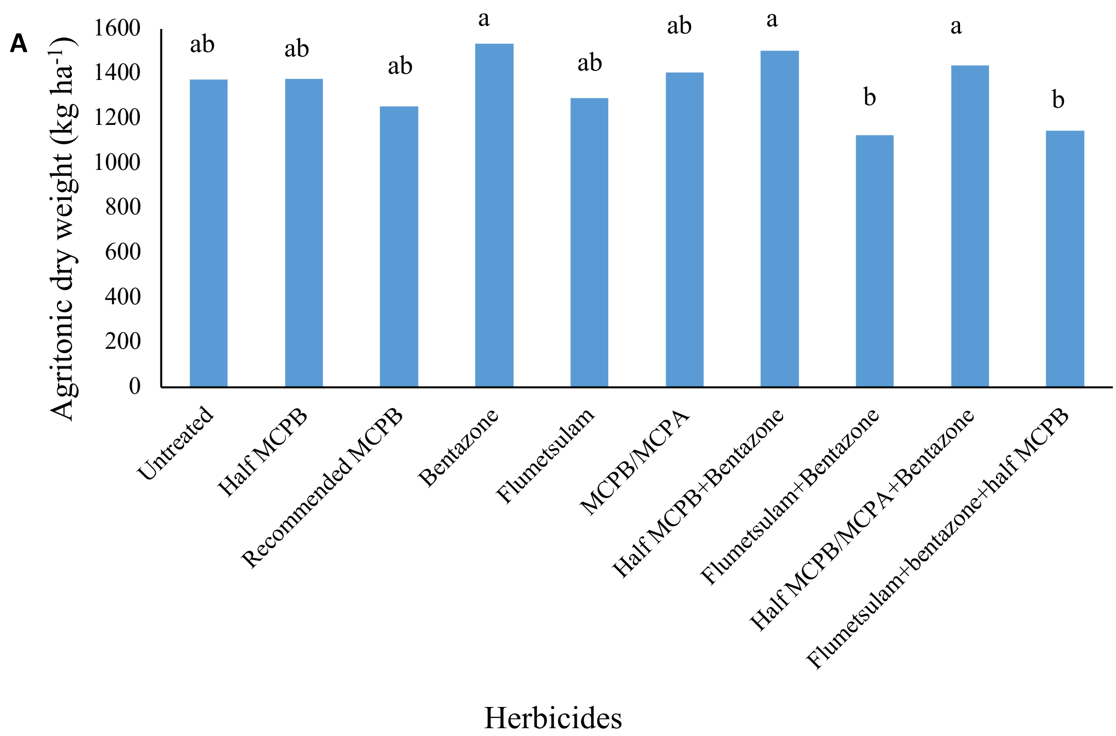


Figure 5.3 The effect of herbicide treatments on total plantain dry weight (kg ha^{-1}) averaged across all five harvests. Bars with different lowercase letters are significantly different according to LSD (p< 0.05).

Visual observations from this field experiment 2 weeks after herbicide application showed that spraying of half recommended rates of MCPB did not significantly diminish the plantain growth. Treatments involving bentazone had caused necrosis on the tips of the plantain leaves, whereas there was yellowing and twisting of plantain in the plots treated with the recommended rates of MCPB and MCPB/MCPA, much more in Tonic plots compared to Agritonic plots. Treatments that involved flumetsulam also had some adverse effect on plantain growth. The damage caused by these herbicides started to recover over time and dry weight of plantain measured at 2 months after spraying with bentazone and its mixture with half MCPB and half MCPB/MCPA were high and similar to the untreated control. There was still a lot of damage caused by the recommended rates of MCPB, MCPB/MCPA in Tonic plants 2 months after spraying whereas Agritonic plants were no different to the untreated control and those treated with flumetsulam in both cultivars which continued until the third harvest, i.e. 3 months after spraying. However, Tonic had recovered by the fourth and final harvests (4.5 and 6.5 months after spraying, respectively) and the dry weight of Tonic plantain was similar to the Agritonic plantain. Also, flumetsulam treated plots had recovered well by the final harvest (6.5 months after spraying) and there was no significant difference in plantain growth between any herbicide with good growth of the plantain at the end of the experiment in July 2021.

There was a significant interaction effect of time of harvest and cultivar on the dry weight of plantain. During the first harvest, Agritonic plantain appeared to be growing less well than Tonic plantain in plots such as MCPB and half rate of MCPB, but at 2 months after spraying, Agritonic plantain was much larger than Tonic plantain in plots treated with MCPB and MCPB/MCPA. The overall dry weight of Agritonic and Tonic plantain plants was significantly different at the second and third harvests which were 2 and 3 months after spraying, respectively, where Agritonic had greater overall dry weight than Tonic plants (Figure 5.6). By the end of the experiment, Tonic plantain appeared to have better growth and higher dry weight than Agritonic despite some having been damaged by phenoxy treatments in earlier harvests, but they were not significantly different, which is similar to what happened in the first field experiment.



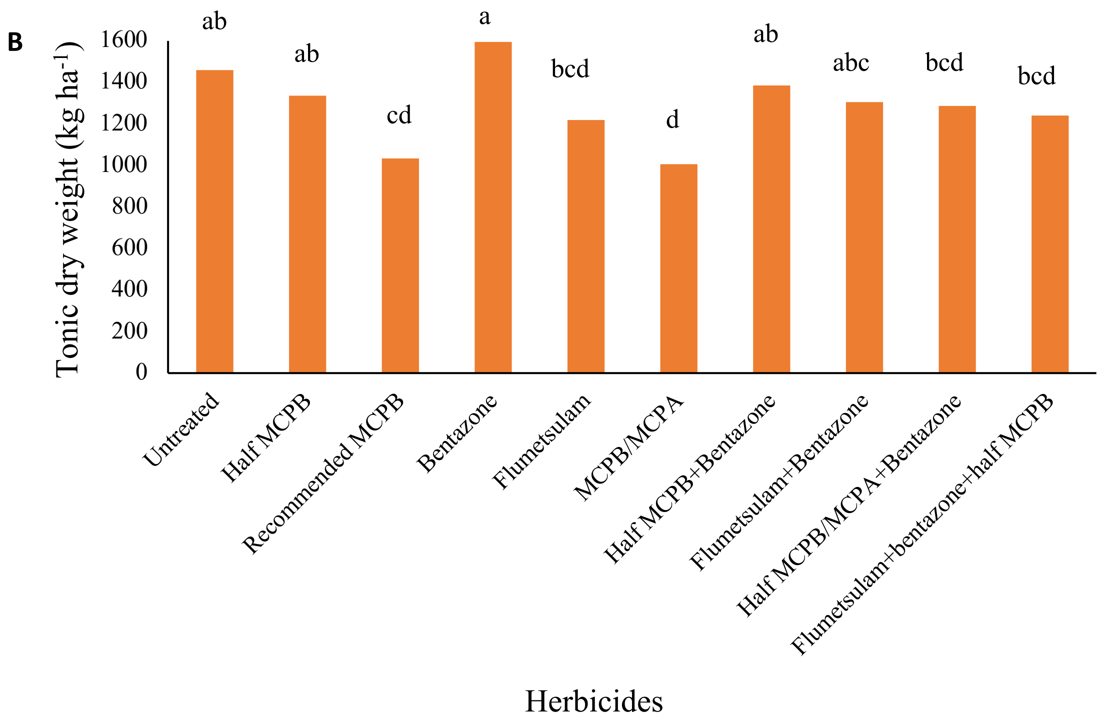


Figure 5.4 The effect of cultivar and herbicide treatments on A. Agritonic and B. Tonic dry weight averaged across all five harvests. Bars with different lowercase letters are significantly different according to LSD ($p < 0.05$).

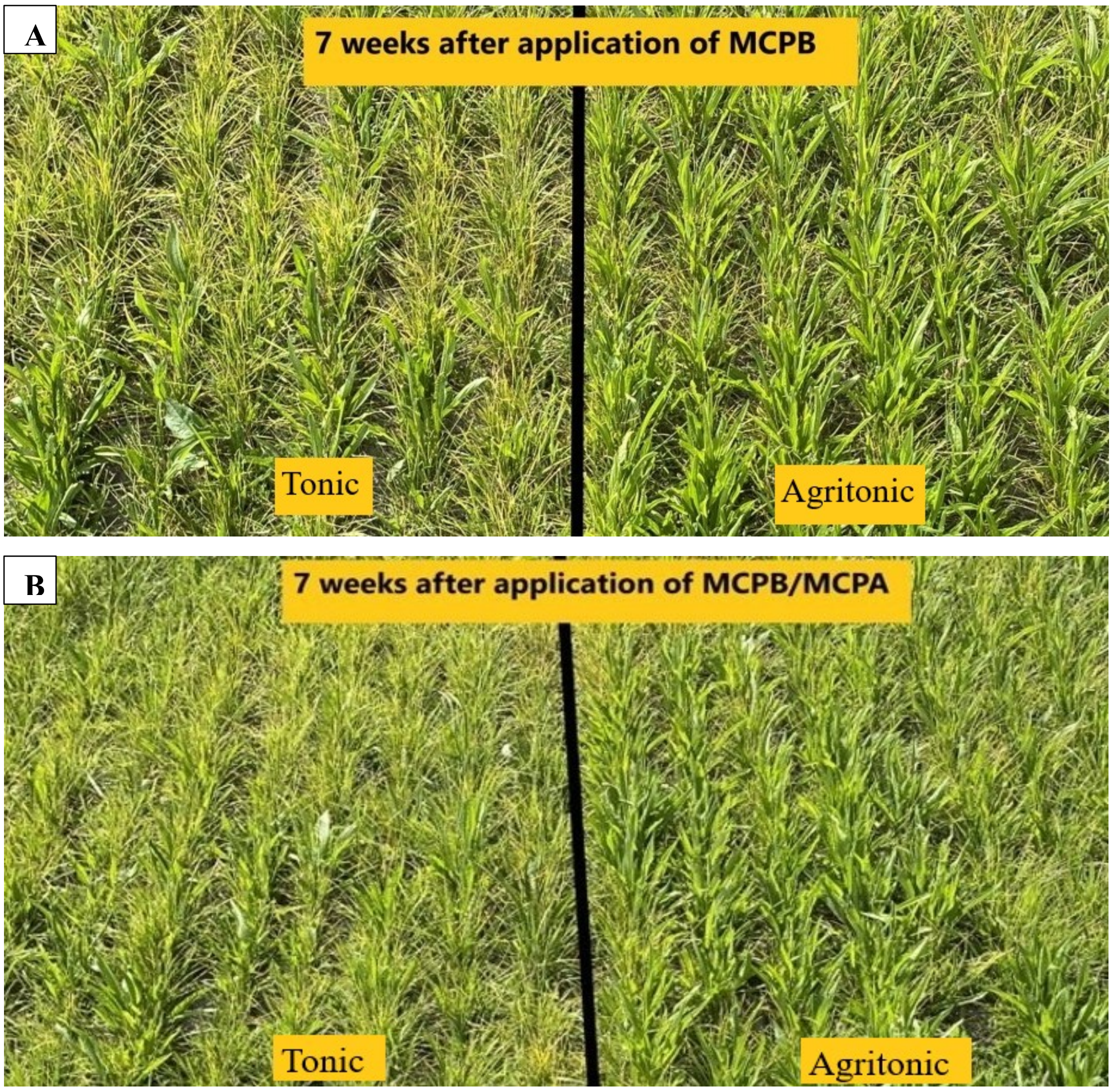


Figure 5.5 Difference in tolerance to A. MCPB and B. MCPB/MCPA of Agritonic and Tonic plantain plants, 7 weeks after application of herbicides.

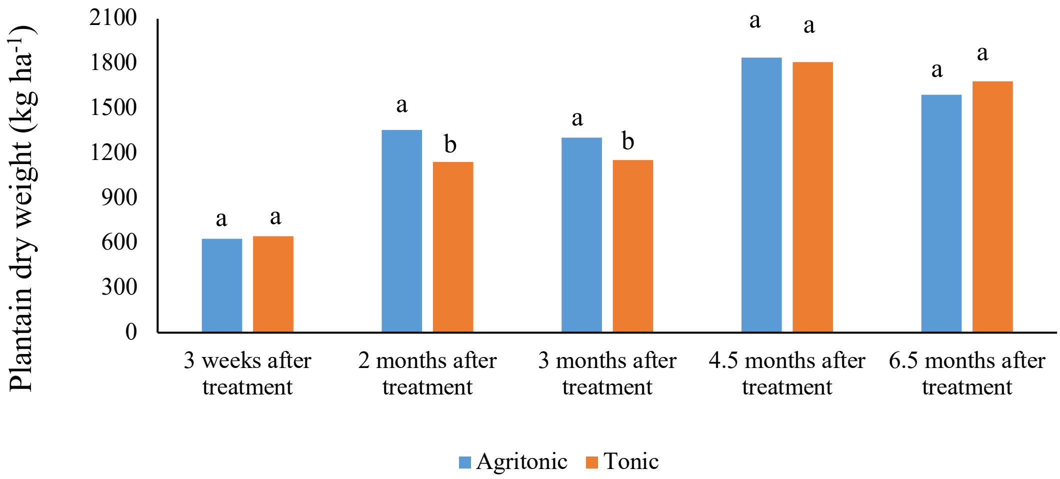


Figure 5.6 The interaction effect of plantain cultivar when harvested at different times after treatment. Bars with different lowercase letters are significantly different according to LSD ($p < 0.05$).

5.3.2 Ryegrass

Perennial ryegrass had significantly higher dry weight in plots with Agritonic plantain than plots with Tonic plantain (Table 5.4). Also, the effect of herbicide treatments on dry weight of ryegrass was significantly different (Table 5.4) which was also observed in the first field experiment. The dry weight of ryegrass was highest in the plots sprayed with flumetsulam, recommended rate of MCPB/MCPA and flumetsulam+bentazone (Figure 5.7). The recommended rate of MCPB and MCPB/MCPA had significantly higher dry weight of ryegrass compared to untreated control and the dry weight of ryegrass was less in the plots sprayed with bentazone and was similar to untreated control. These results were similar to the results from the first field experiment.

Table 5.4 ANOVA table for the effect of cultivar, herbicides, and time of harvest on ryegrass dry weight.

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	410.0	3917.9	<0.001
Time	4	200.4	108.9	<0.001
Plantain cultivar	1	410.0	10.8	0.001
Herbicide	9	410.0	5.7	<0.001
Time * Cultivar	4	200.4	0.57	0.708
Time * Herbicide	36	200.4	1.2	0.216
Cultivar * Herbicide	9	410.0	1.7	0.085
Time * Cultivar * Herbicide	36	200.4	0.5	0.990

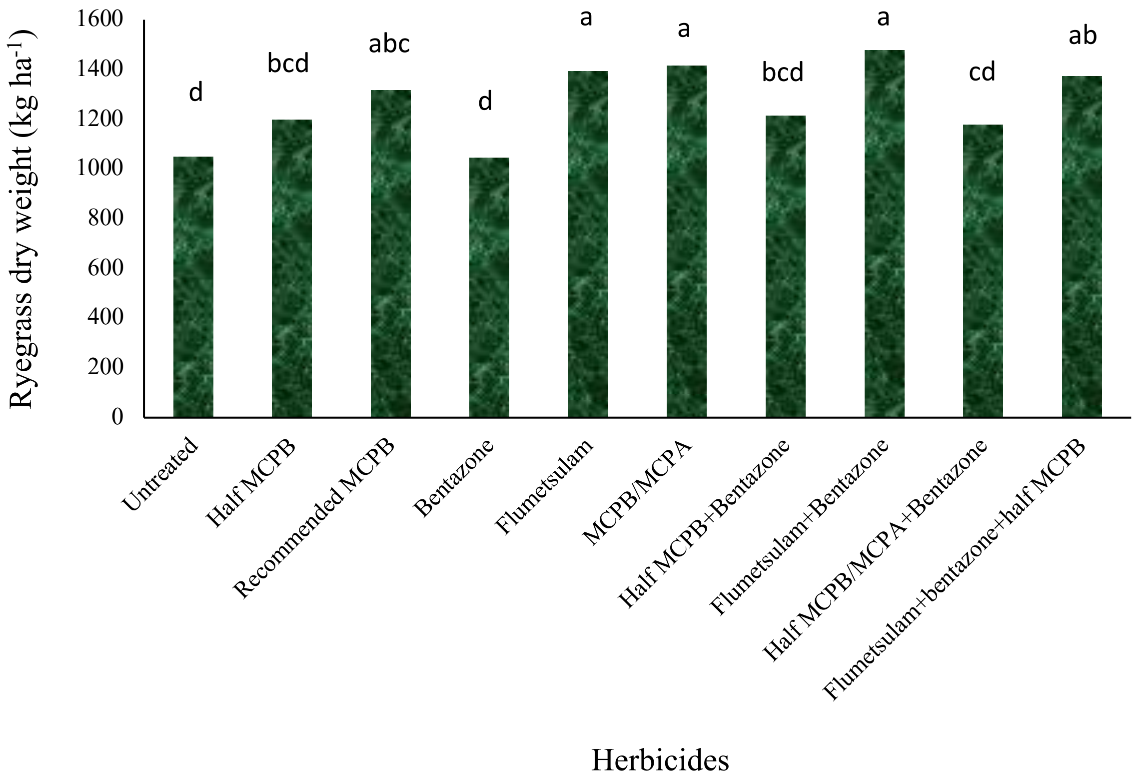


Figure 5.7 The effect of herbicide treatments on ryegrass total dry weight (kg ha^{-1}) averaged across all five harvests. Bars with different lowercase letters are significantly different according to LSD ($p < 0.05$).

5.3.3 Clover

The white clover established poorly for this trial and most of the clover was regrowth from the previous pasture. There was much less clover in all plots from the start compared with the first field trial and lots of variability among the plots in clover establishment. The dry weight of clover was significantly different between plantain cultivars (Table 5.5) with a higher dry weight of clover in Tonic plots than Agritonic plots. There was no significant difference between the different herbicide treatments (Table 5.5), suggesting that the clover responded similarly to all the herbicide treatments.

Table 5.5 ANOVA table for the effect of cultivar, herbicides, and time of harvest on clover dry weight.

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	365.1	304.3	<0.001
Time	4	226.7	38.5	<0.001
Plantain cultivar	1	365.1	5.05	0.025
Herbicide	9	365.1	1.5	0.147
Time * Cultivar	4	226.7	2.55	0.043
Time * Herbicide	36	226.7	1.05	0.467
Cultivar * Herbicide	9	365.1	1.7	0.089
Time * Cultivar * Herbicide	36	226.7	1.5	0.052

5.3.4 Total weeds

Total weeds, including the docks present in this experimental trial is considered here, but docks will also be covered separately in the next section. The weeds that were found in the plots were reported in the materials and methods section and the low initial density of weeds is shown in Figure 5.8.

Figure 5.8 Different stages of pasture and weed plants at the time of spraying of herbicides on 15 December 2020.

The most dominant weeds in the plots initially were docks and twin cress until the second harvest (2 months after spraying). Yorkshire fog, browntop (*Agrostis capillaris*) and hawkbit were dominant weeds from the third to fifth harvest. Most of the hawkbit appeared to be regrowth from the previous pasture. Redroot, black nightshade and fathen were not found after the second harvest, nor was twin cress after the third harvest. But many more new species of weeds were found like browntop, broad-leaved plantain, scarlet pimpernel (*Anagallis arvensis*), pennyroyal (*Mentha pulegium*), cudweed (*Gamochaeta* spp.) and creeping oxalis (*Oxalis exilis*) 2 months after spraying herbicides. However, there were significantly fewer weeds in the final harvest in early July than in the earlier harvests.

One week after spraying, observations of plants suggested that docks were affected by almost all herbicides; twin cress, spurrey, creeping buttercup, black nightshade, and fathen also seemed to be affected by the applied herbicides while hawkbit and scrambling fumitory did not appear to be affected. Treatments containing MCPB were helpful in controlling the seedling stage of docks, twin cress, prickly sow thistle and creeping buttercup while bentazone had little apparent effect on these weeds. After 2 weeks, flumetsulam+bentazone followed by half MCPB+bentazone, the recommended rate of MCPB, flumetsulam and flumetsulam+bentazone+half MCPB were the best treatments for controlling seedling docks and also caused some necrotic leaves for bigger docks. Similarly, twin cress was controlled by the recommended rate of MCPB, MCPB/MCPA and half MCPB+bentazone. These herbicides were effective on the seedling weeds only. There was no effect on hawkbit, scrambling fumitory and grasses as most of the hawkbit were regrowing plants and scrambling fumitory is an annual weed species not affected by many selective herbicides. Following initial effects of herbicides, many weeds started regrowing if they were large at the time of spraying and also new seedlings began germinating from the weed seeds present in the soil 3 weeks after spraying. Thus, more weeds were found in the second harvest (2 months after spraying) compared with the first harvest (3 weeks after spraying).

The herbicide treatments differed significantly in their effect on total dry weight of weeds (Table 5.6). Unlike in the first field trial, treatments containing bentazone provided good reduction of weeds overall compared to other treatments. The dry weight of weeds which had been treated with half the recommended rates of MCPB and flumetsulam was similar to the untreated ones. The plots treated with the recommended rates of MCPB and MCPB/MCPA had significantly lower dry weight of weeds compared to the untreated control (Figure 5.9).

Table 5.6 ANOVA table for the effect of cultivar, herbicides, and time of harvest on total weeds dry weight.

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	79.7	894.8	<0.001
Time	4	154.5	29.9	<0.001
Plantain cultivar	1	79.7	0.4	0.542
Herbicide	9	79.7	3.2	0.002
Time * Cultivar	4	154.5	4.2	0.003
Time * Herbicide	36	154.5	2.4	<0.001
Cultivar * Herbicide	9	79.7	0.6	0.755
Time * Cultivar * Herbicide	36	154.5	1.1	0.271

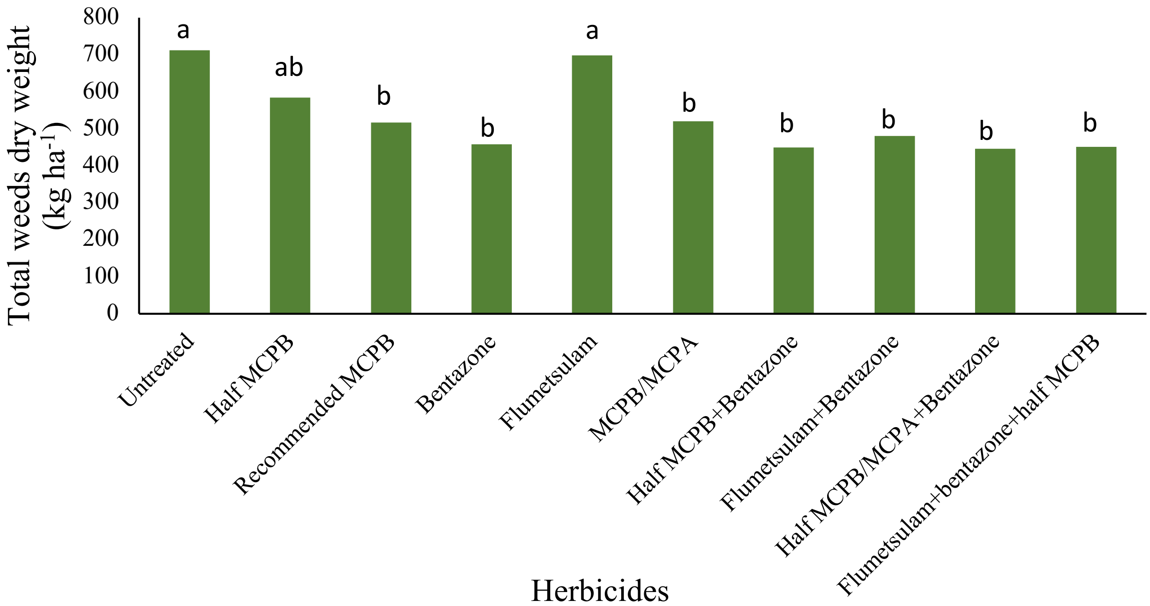


Figure 5.9 The effect of herbicide treatments on total dry weight of all weeds including docks (kg ha^{-1}) averaged across all five harvests. Bars with different lowercase letters are significantly different according to LSD ($p < 0.05$).

There was a significant interaction between the effect of harvest time and different herbicide treatments on the reduction of weeds (Table 5.6). The highest amount of weeds was found 4.5 months after spraying while the least weeds were present at the end of the trial in July 2021. The effect of herbicides on the weeds changed over the five harvests. The half-recommended rate of MCPB alone was less effective in controlling weeds than other treatments which was similar to the untreated control, while half MCPB combined with bentazone and bentazone alone significantly reduced the dry weight of weeds at 2 months after spraying (Figure 5.10A).

By 6.5 months after spraying, all the herbicides were statistically similar to the untreated control in the dry weight of weeds while plots treated with bentazone, flumetsulam+bentazone and flumetsulam+bentazone+half MCPB had less weeds than the plot treated with flumetsulam alone (Figure 5.10 B). This suggests that the most of weeds were best controlled by the combination of bentazone and half MCPB rather than by flumetsulam. At this final harvest most of the annual summer weeds and summer grass were gone and the most dominant weeds were Yorkshire fog, browntop and hawkbit.

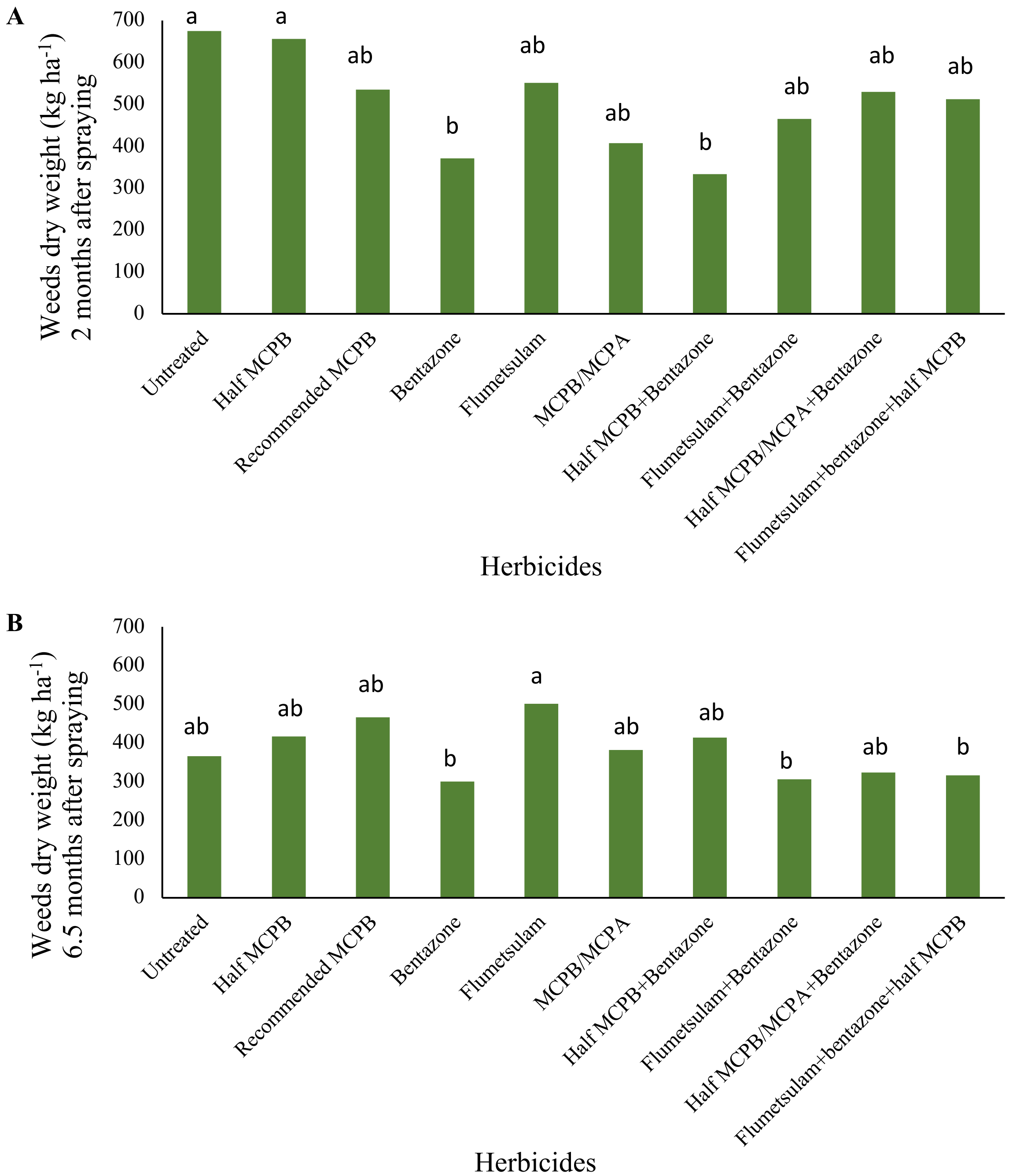


Figure 5.10 The effect of herbicides on weed dry weight A. 2 months and B. 6.5 months after spraying. Bars with different lowercase letters are significantly different according to LSD (p< 0.05).

5.3.5 Broad-leaved dock

Docks are often troublesome weeds in plantain-based pasture, and sufficient were present as seedling as well as regrowth from the previous pasture in this trial to allow analysis of them separately. The effect of the various herbicide treatments on the dry weight of docks was found to be significantly different (Table 5.7). The effect of flumetsulam on dry weight of weeds was found to be similar to the untreated control and significantly different from all other herbicides. Bentazone combined with half MCPB gave the best result in controlling seedling docks and suppressing the bigger ones, which was also similar to the combination of flumetsulam+bentazone+half MCPB (Figure 5.11). This suggests that there is no need to add flumetsulam to the bentazone and half MCPB combination as it was giving the same level of control of docks without flumetsulam.

Table 5.7 ANOVA table for the effect of cultivar, herbicides, and time of harvest on dock dry weight.

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	4.0	250.7	<0.001
Time	4	26.2	24.0	<0.001
Plantain cultivar	1	50.6	0.0	0.952
Herbicide	9	165.0	4.7	<0.001
Time * Cultivar	4	26.2	0.3	0.839
Time * Herbicide	36	66.6	1.9	0.013
Cultivar * Herbicide	9	165.06	0.7	0.746
Time * Cultivar * Herbicide	36	66.6	0.5	0.978

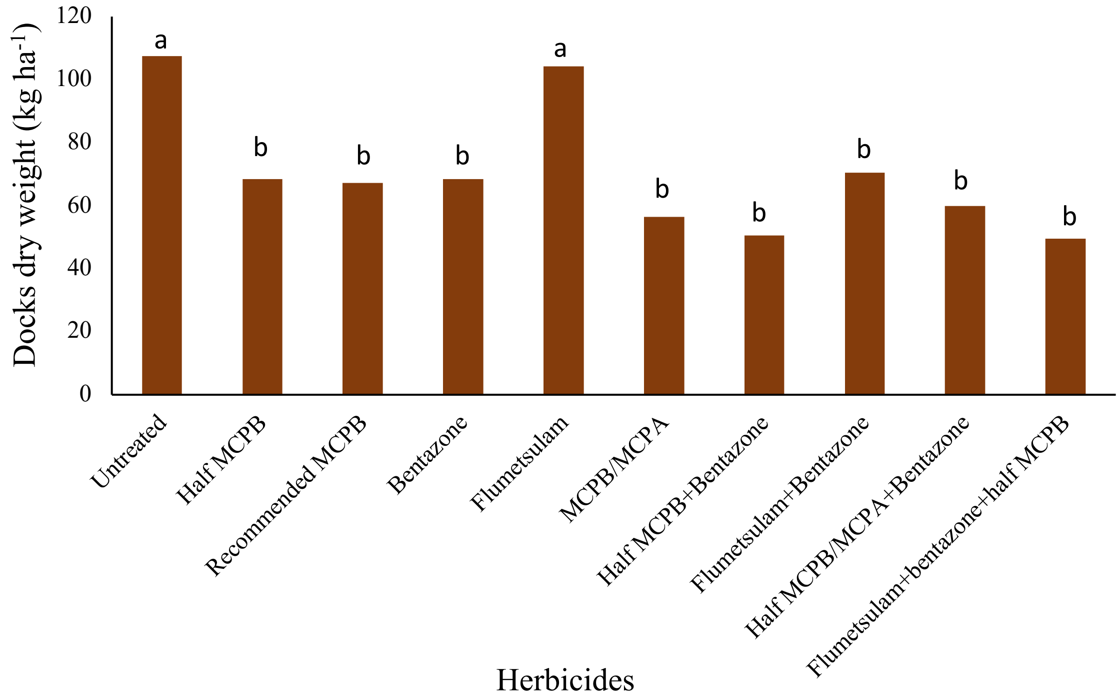


Figure 5.11 The effect of herbicide treatments on total dry weight (kg ha^{-1}) of docks averaged across all five harvests. Bars with different lowercase letters are significantly different according to LSD ($p<0.05$).

There was a significant interaction between the effect of harvest time and different herbicide treatments on the dry weight of docks (Table 5.7). The amount of docks decreased over time. The highest dock dry weight was found in the first harvest (3 weeks after spraying) where the seedlings of docks were controlled, and the bigger docks had necrotic leaves. Bentazone acted more rapidly and the phenoxy herbicides worked more slowly, thus the effect of phenoxy herbicides was not initially evident whereas the effect of bentazone and phenoxy herbicides on the dry weight of docks was clearer in the second harvest (2 months after spraying). Flumetsulam had a little effect on docks while half MCPB+bentazone and the recommended rate of MCPB/MCPA gave the best control of docks after 2 months (Figure 5.12A). By the final harvest (6.5 months after spraying), most effects of the herbicides were gone as presumably any plants then had regrown from the effects of the herbicides. Bentazone mixed with half MCPB/MCPA showed a significant effect on dry weight of docks compared to half MCPB+bentazone (Figure 5.12B). This indicated that the half MCPB+bentazone controlled the docks during the early stage while half MCPB/MCPA+bentazone gave lasting damage to the docks to the final harvest.

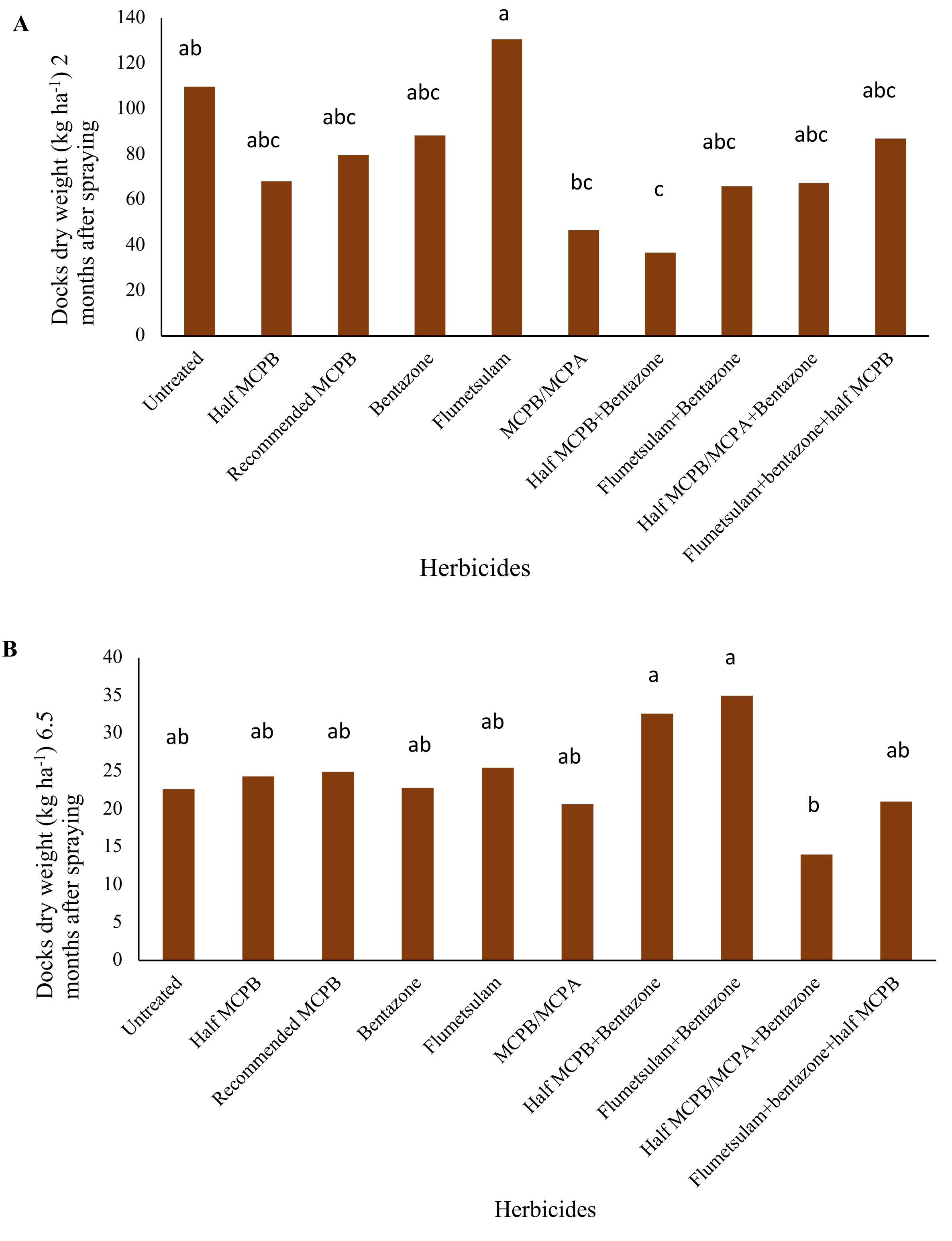


Figure 5.12 The effect of herbicides on the dry weight of docks A. 2 months and B. 6.5 months after spraying. Bars with different lowercase letters are significantly different according to LSD ($p < 0.05$).

5.4 Discussion

This experiment confirmed the results from the first field experiment that Agritonic plants were less affected by phenoxy herbicides compared with Tonic plants. At the end of the trial (6.5 months after herbicide application), Tonic plantain recovered from the adverse effects caused by phenoxy herbicides.

Plots sprayed with bentazone and half MCPB+bentazone had the highest plantain dry weight, suggesting there was less competition from weeds against plantain and less effect on the growth of plantain. However, the dry weight of ryegrass was lowest in the bentazone sprayed plot, which was not owing to the effect of bentazone on the ryegrass as it is considered a safe herbicide on ryegrass (Holden, 2022), but due to the competition created by plantain and weeds that were not controlled by bentazone in those plots. Flumetsulam affected plantain initially, but it recovered later which presumably created space for weed growth including docks. It caused higher dry weight of weeds and docks in flumetsulam sprayed plots though it was supposed to suppress docks and many other broad-leaved weeds (Gawn et al., 2012; Holden, 2022). However, flumetsulam has no effect on ryegrass (Lusk et al., 2011) and plots treated with flumetsulam had higher dry weight of ryegrass which was similar to the MCPB/MCPA and flumetsulam+bentazone (Figure 5.7).

The summer annual weeds like redroot, fathen and black nightshade died naturally in autumn and mowing also helped to control the upright weeds. Twin cress also died after completing its life cycle and did not geminate again due to competition. It was also controlled earlier by the recommended rate of MCPB and MCPB/MCPA mix. The half rate of MCPB was less damaging to plantain than the recommended rates of MCPB and MCPB/MCPA but could not control most of the weeds. Flumetsulam is mainly used for selective control of weeds in young pasture, especially weeds not controlled by MCPB such as chickweed, spurrey, mallow and stinking mayweed (Matthews et al., 1999; Bourdôt et al., 2007) but in this experiment spurrey was best controlled in the plots sprayed with half MCPB+bentazone, half MCPB/MCPA+bentazone, and bentazone. There was emergence of more weed grasses like Yorkshire fog, browntop and summer grasses along with regrowth from the previous pasture. None of these herbicides affect grasses and there are few selective herbicides available to control grass weeds in seedling ryegrass swards (Holden, 2022). Thus, pasture swards need to be established quickly to outcompete grasses like summer grass. Hawkbit seedlings probably would have died with some of the herbicides but most of the hawkbit re-established from old plants which are difficult to control using selective herbicides. The seedling docks were affected initially by bentazone and phenoxy herbicides but came back later whereas the big docks that had emerged from regrowth were difficult to control by any herbicides. Docks were reducing over time as they were affected by herbicides initially followed by mowing after each harvest and also greater competition from pasture species and other weeds. The paddock had been sprayed with Glyphosate 540 @ 4 L ha $^{-1}$ and then received full cultivation, and this combination was used to kill all the pasture and weeds present but apparently was not sufficient to control all the weeds and clover from the previous pasture. Therefore, to improve control, a higher rate of glyphosate may be required to kill all the weeds, or the addition of surfactant/penetrant to the glyphosate, adding thifensulfuron-methyl (to control docks but may have residue issues on ryegrass later) or growing a crop after coming out of pasture to allow better control of perennial weeds before going back to pasture (Harrington et al., 2013).

Treatments containing bentazone were found to have a significantly higher effect on weeds as well as docks compared to untreated control and flumetsulam alone where the mixture of bentazone+half MCPB and half MCPB/MCPA+bentazone were found to better at controlling other weeds along with docks. Although, there was no advantage in adding flumetsulam for the set of weeds found in this experiment, there might be situations where flumetsulam could be used to control other weeds. The weeds present at the time of final harvest 6.5 months after application of herbicides were partly influenced by the herbicides applied, but a much larger influence was whether the species could tolerate the mowing that had occurred after each harvest and also the time of the year as all summer annuals had died off.

As with the first field trial, not everything was ideal with this second trial. There were weeds like docks present for this trial, but there were still only small numbers of thistles. Also, the clover seedlings did not establish well. This might be due to continuous rainfall (24.4 mm) for 6 days after seed sowing which increased the moisture content in the soil and also created low light intensity which might not be favourable for clover seed emergence. Most of the clover that was present was regrowth of the clover from the previous pasture that was sprayed with glyphosate. There were several other trials where the same cultivar from the same lot of white clover seeds were sown and also had poor establishment of clover during the same time. The clover seeds were tested afterwards for germination percentage in laboratory which ensured 90% of the seeds were germinated and there was no issue with the seeds. Therefore, data regarding effects of herbicides on clover for this trial are not very useful. Much of the hawkbit present also appeared to have regrown from old root systems, and these herbicides are not likely to be effective on re-growing perennial weeds such as hawkbit.

5.5 Conclusion

The results from this experiment regarding Agritonic and Tonic plantain were similar to the results from the first field experiment, with Agritonic found to be more tolerant than Tonic plantain to MCPB and MCPB/MCPA, though Tonic recovered by 6.5 months after herbicide application.

Bentazone was safe for plantain but its effect was probably reduced by rainfall after application in the first experiment. However, in the second experiment, bentazone alone was useful in controlling weeds and when combined with other herbicides, without damaging plantain and ryegrass when there was no rainfall immediately after application. Flumetsulam caused some initial damage to both cultivars of plantain, but they had recovered and were similar to the untreated control by the end of this trial. Bentazone combined with half MCPB gave good control of weeds including docks at 2 months after spraying whereas bentazone+half MCPB/MCPA gave lasting effect on weeds and docks by the end of the trial. None of these herbicides affected the grasses. There were weeds like hawkbit and docks which were left from previous pasture, that made it very difficult for these herbicides to give lasting control as these herbicides work mainly on seedlings only. As a result of careful analysis, spraying Glyphosate 540 @ 4 L ha $^{-1}$ followed by full cultivation was not sufficient to control all the weeds and clover from previous pasture. Mowing after each harvest helped to control the upright weeds like redroot, black nightshade and fathen and also gave temporary suppression to the docks, as their growing point are at ground level similar to plantain and clover. If the plots had been grazed rather than mown, the weeds consumed might have varied depending on the type of livestock because of different diet selection behaviour. Some weeds are often inedible for cows or sheep so would have been less controlled if grazed than by mowing.

These two field experiments showed that the Agritonic is more tolerant than Tonic plantain to phenoxy herbicides, though Tonic recovered by the end of the experiments. Getting timing correct with selective herbicide applications is important as it might affect the efficiency of a few herbicides such as bentazone. Most of the herbicides are effective when the weeds are 3-4 leaf stage. Flumetsulam was fairly safe to use in the plantain-based pasture though it suppressed plantain initially but did not add much in controlling the weeds found in this experiment. Bentazone was safe to use without damaging plantain and ryegrass and supressing most of the weeds alone and in with combination of other herbicides when there was no rainfall on the same day of herbicide application. The most effective weed control strategy in establishing swards of plantain, ryegrass and white clover may be apply the combination of bentazone+half MCPB/MCPA for lasting effect on weeds. The plantain and clover should be established well enough to tolerate the herbicides and the weeds need to be as young as possible to be susceptible to herbicides such as bentazone and MCPB/MCPA.

Chapter 6

A study of tolerance mechanism to 2,4-D in Agritonic plantain

6.1 Introduction

2,4-D was the first herbicide belonging to the synthetic auxin group to be commercially developed and released worldwide in 1945 (Schulz and Segobye, 2016). It is one of the most widely used selective herbicides to control broadleaf weeds of cereal crops, corn (*Zea mays* L.), fruit, pastures, turf or ornamentals, and other crops (Zimdahl, 2007; Peterson et al., 2016). Thus, it has been broadly adopted by farmers in most countries. However, the increased reliance on 2,4-D usage has promoted the development of resistance to this herbicide, creating difficulties for weed management strategies (Shyam et al., 2022). The first resistance cases of 2,4-D were reported in wild carrot (*Daucus carota)* and spreading dayflower (*Commelina diffusa)* in North America in 1957 and in narrow-leaved plantain (*Plantago lanceolata*) was reported in Indiana state, USA in 2016 (Heap, 2022). Herbicide-resistant weeds have been reported in 98 crops in 72 countries, where 42 species are resistant to 2,4-D (Heap, 2022). In New Zealand, resistance to 2,4-D has been reported in nodding thistle (*Carduus nutans*), slender winged thistle (*Carduus pycnocephalus*) and giant buttercup (*Ranunculus acris*) (Ghanizadeh and Harrington, 2019).

Herbicide resistance mechanisms in weeds are generally categorised as either target site or non-target site (Powles and Yu, 2010). Target-site resistance (TSR) occurs by single or several mutations in the gene encoding the target-site enzyme that modifies or limits the affinity for herbicide binding, as well as increased expression of the target site gene (Yu and Powles, 2014; Vrbničanin et al., 2017). Non-target-site-resistance (NTSR) is caused by mechanisms that reduce the concentration of herbicidal active compounds reaching the site of action due to reduced herbicide absorption and translocation, increased herbicide sequestration to other plant parts (e.g. within vacuoles), or detoxification of herbicides to non-toxic metabolites (enhanced metabolism) (Ghanizadeh and Harrington, 2017c) and through root exudation (Ghanizadeh and Harrington, 2020). The TSR mechanism was reported to cause 2,4-D resistance in Indian hedge mustard (*Sisymbrium orientale*) from Australia (Figueiredo, 2022). NTSR mechanisms involving reduced 2,4-D absorption have been found in a ground ivy population (*Glechoma hederacea*) (Kohler et al., 2004), and reduced translocation has been reported in 2,4-D-resistant phenotypes of prickly lettuce (*Lactuca serriola*) (Riar et al., 2011) and wild radish (*Raphanus raphanistrum*) (Goggin et al., 2016). Enhanced metabolism caused 2,4-D resistance in common waterhemp (*Amaranthus tuberculatus*) across midwestern U.S.A (Figueiredo, 2018), field poppy (*Papaver rhoeas*) in Spain (Torra et al., 2017), nodding thistle (*Carduus nutans*) in New Zealand (Harrington and Woolley, 2006) and Palmer amaranth (*Amaranthus palmeri*) in Kansas (Shyam et al., 2022).

Although there are resistant biotypes that have developed, as discussed above, a more common phenomenon is that some plant species are tolerant to 2,4-D, and some are sensitive, which is why it can be used as a selective herbicide. The selectivity between tolerant and sensitive plants to 2,4-D is usually influenced by translocation (Pillmoor and Gaunt, 1981) or metabolism (Yu and Powles, 2014). The monocotyledons, such as grasses, are tolerant to 2,4-D and usually metabolise it through ring hydroxylation resulting in nonphytotoxic metabolites, whereas many broadleaf dicotyledons are sensitive to 2,4-D and metabolism in these plants through direct conjugation to amino acids or glucose result in metabolites that are as phytotoxic as the 2,4-D (Peterson et al., 2016). The application of synthetic auxin herbicide to sensitive dicots can cause plant death through a three-phase response: altering the plasticity of cell walls, influencing the amount of protein production and increasing ethylene production (Song, 2014). The metabolic pathways for 2,4-D degradation in plants include side-chain lengthening, side-chain degradation, conjugation, ring hydroxylation and ring cleavage (Riar et al., 2011).

Narrow-leaved plantain is a flowering plant which belongs to the family Plantaginaceae. The plant is erect, leafy with a deep, dense, branching fibrous root system spreading downwards from the crown (Reed, 2009). The flowers are generally pollinated by wind and sometimes by insects collecting pollen (Bond et al., 2007; Sharma et al., 2008). It was once regarded as a serious weed of lawns, dry grasslands and roadside. However, there has now been increased interest in plantain in New Zealand for pasture production following breeding to increase its productivity. It has become a popular pasture species sown either alone or in a mix with other pasture species (Muir, 2012). Powell et al. (2007) mentioned that a pure sward of plantain is as productive as perennial ryegrass and white clover pastures over a year.

Many herbicides are available to control broadleaf weeds in grass/clover pastures, but very few herbicides are available for mixed pastures. In New Zealand pastures, MCPA and 2,4-D are commonly preferred by farmers for boom spraying because they are effective against many troublesome weeds and cheaper than some alternative herbicides (Matthews et al., 1999). These herbicides selectively control many broadleaf plants without harming grass (Thompson and Saunders, 1984). The major problems with using MCPA and 2,4-D are that they suppress and damage clover and plantain for several months and also are not as effective as other herbicides like picloram, dicamba and clopyralid in controlling perennial and mature weeds (Matthews et al., 1999; Young, 2019). MCPB is often used in newly establishing clover-based pastures to control weed seedlings as clovers have better tolerance of this herbicide, but MCPB is not as effective on older weeds as MCPA (Young, 2019).

The glasshouse experiments and field trials covered in Chapters 3, 4 and 5 showed that Agritonic plantain has more tolerance of phenoxy herbicides compared to Tonic plantain. However, the mechanism involved in the tolerance of Agritonic plantain is unknown. Therefore, the main objective of the experiments reported in this chapter was to determine the mechanism of tolerance in Agritonic plantain to 2,4-D using radiolabeled herbicide (^{14}C-2,4-D).

Radiolabeled herbicide experiments involve putting radioactive atoms in some herbicide molecules, which are mixed with other unlabeled herbicide molecules, and the location of these radiolabeled molecules can be precisely differentiated using their radiation signal without any noise contribution from unlabeled compounds (Freund and Hegeman, 2017). The availability of radiolabeled herbicide with ^{14}C has simplified and expanded research studies of herbicide absorption, translocation and metabolism in crops and weed plants since the 1950s (Nandula and Vencill, 2015). Studies using radiolabeled herbicides may be qualitative or quantitative and can be used to determine if herbicide resistance is caused by reduced absorption and/or translocation, and/or to the accelerated metabolism of the herbicide (Mendes et al., 2017). This has been used in many experiments over the years, such as studies of glyphosate resistance in *Lolium multiflorum* (Ghanizadeh et al., 2016); 2,4-D resistance in nodding thistle (Harrington and Woolley, 2006), field poppy (Rey-Caballero et al., 2016), wild radish (Goggin et al., 2016), Palmer amaranth (Shyam et al., 2022), *Gossypium hirsutum* (Perez et al., 2022); and dicamba resistance in kochia (*Bassia scoparia*) (Cranston et al., 2001).

6.2 Materials and methods

6.2.1 Plant material

Seeds from the Agritonic plants that tolerated higher rates of phenoxy herbicides from the dose-response experiments than other Agritonic plants were saved and used to grow the Agritonic plants for this experiment to ensure they had good levels of tolerance. Tonic seedlings were also grown and compared as susceptible plants. Wheat plants were grown for the metabolism experiment as a positive control because of their natural ability to metabolise 2,4-D (Shyam et al., 2022), enabling a comparison of the metabolism of 2,4-D within wheat with Agritonic plants.

6.2.2 Growing conditions

Seeds of Agritonic and Tonic plantain were germinated in containers using propagating sand. The containers were placed in an incubation chamber at 20°C and placed below four 40-W white fluorescent tubes. After 10 days, when the seedlings were 2-3 cm tall, the plants along with containers were placed in the glasshouse at average daily minimum 17.0°C and maximum 25.5°C temperatures and relative humidity of 45-92%. After 4 weeks, when the plants were 2 to 3 leaf stage, they were uprooted from the propagating sand, and the roots were washed off using 5% sodium hypochlorite (to remove any microbial contamination) followed by four times rinsing in distilled water to remove the chemical from the roots. Then each plant was transplanted into 165 ml plastic containers coated with black and then white paint (to minimise light reaching the contents) which contained 120 ml of hydroponic solution (Hoagland and Arnon, 1950). The plants were positioned through an 8 mm diameter hole in the lid of each container, keeping it upright with cotton wool, so roots were immersed in the solution. Then the plants were kept in a glasshouse at 21.5/25.0°C average daily minimum/maximum temperature and relative humidity of 43-82% under natural light and supplemented using fluorescent lights to maintain a 14-hour day length (476 µmol m^{-2} s^{-1}) and were left to acclimatise to the solution for 7 days before treatment.

6.2.3 Absorption/translocation experiment

After a week of acclimatisation in the hydroponic solution, plants were at the 3 to 4 leaf stage. The lowest leaf of each plant was tagged using a thin wire tied loosely around the leaf, then this leaf was covered in a plastic zip-lock bag before the remainder of the leaves were sprayed with 800 g ai ha^{-1} of 2,4-D amine (1 kg ha^{-1} Baton) using a precision pot sprayer at 230 L ha^{-1} of spray solution. The plastic zip-lock bag was then removed, and seven 1 µL droplets of radiolabeled ^{14}C-2,4-D were applied using a micro syringe to this unsprayed leaf. These seven droplets contained a total of 1.717 kBq of ring-labeled ^{14}C-2,4-D (specific activity: 5.856 MBq mg^{-1}, Institute of Isotopes Co Ltd, Budapest, Hungary). Plants were then allowed to continue growing under the fluorescent lights within a glasshouse.

Plants were harvested either 24, 48, 72 or 120 hours after treatment (HAT). At the time of harvest, each plant was separated into treated leaves, untreated leaves (based on the radiolabeled 2,4-D applied), stem material and roots and placed in separate paper bags. The leaf treated had any unabsorbed ^{14}C- 2,4-D washed off with 12 mL of 50% (v/v) methanol containing 0.1% (v/v) Tween-20 (Goggin et al., 2016) and the level of radioactivity in the washings (one sample taken of the leaf wash for each plant after thorough mixing) was measured. The amount of nutrient solution left in the container in which the plant had been growing was quantified before taking one 0.5 mL sample from each container to estimate the amount of exudation of the herbicide from the roots. The radioactivity in 12 mL leaf wash solution and the remaining nutrient solution was quantified by mixing 0.5 mL of the solution with 5 mL of Ultima Gold scintillation cocktail (PerkinElmer) in 20 mL scintillation vials. The four lots of tissue from each plant were oven dried at 60°C, then combusted separately using a biological oxidiser (PerkinElmer Sample Oxidizer Model 307) for 30 seconds. The CO_2 released during the combustion of samples was trapped in 16 mL of scintillation fluid (Carbo-Sorb E: Permafluor E+, 25:75% (v/v)) (Ghanizadeh et al., 2018). The leaf wash, nutrient solution and plant sections were assayed for 14C by using a liquid scintillation counter (Tri-Carb 2900TR; PerkinElmer) (Ghanizadeh et al., 2016).

When this experiment was repeated at another time, some samples from 24 HAT were not recorded due to equipment failure while combusting the samples in the oxidiser, but otherwise, the experiment was repeated in the same way.

6.2.4 Metabolism experiment

This experiment was conducted in the same way as the absorption/translocation experiment, except the material from each tissue (treated leaf, untreated leaves, stem and roots) was frozen at -80 °C immediately after harvesting at 24, 48, 72 or 120 HAT and stored until extraction (Palma-Bautista et al., 2020) rather than combusted, and these stored samples were analysed later to determine if the radiolabeled molecules in the plant parts were intact 2,4-D molecules prior to freezing or were metabolites of 2,4-D. Wheat (*Triticum aestivum*) was also included in this experiment as it is a species known to metabolise 2,4-D successfully, so metabolites from the wheat were compared with metabolites from the plantain plants (Goggin et al., 2016). The radiolabeled material was extracted using the method described by Cranston et al. (2001) and Ghanizadeh et al. (2018) with some modifications. Liquid nitrogen (2-3 mL) was added to the individual plant sections in a mortar separately, then immediately crushed with a pestle. Methanol (8 mL of 100%) was added to homogenise the sample with the pestle. The homogenised plant material was transferred into 15 mL centrifuge tubes, then centrifuged at 16,000 rpm for 20 minutes at 4°C. The supernatant was collected, and the pellets were washed off twice with 2 mL of 100% methanol and then vortexed and centrifuged again at 16000 rpm at 4°C for 10 min. The supernatants from the initial centrifugation and pellet wash were pooled together and radioactivity of the extracted sample was measured by liquid scintillation counting (Aper et al., 2012) to determine the recovery percentage of the ^{14}C-2,4-D used. Then, mixes of supernatant were dried down using a vacuum concentrator (miVac Quattro concentrator) at 30°C. After it was dried completely, 1.5 mL of methanol was added, and this was transferred to a 1.5 mL centrifuge tube and dried down again at 30°C then 500 µL of solvent A (contained 99% water, 0.9% acetonitrile and 0.1% acetic acid) was added. This solution was transferred to 0.45µm polytetrafluoroethylene filters (Phonomenex, Milford, New Zealand) and filtered at 16,000 rpm at 4°C for 5 minutes. The filtered solution was again transferred to 0.22 µm polytetrafluoroethylene filters (Phonomenex, Milford, New Zealand), and centrifuged at 16,000 rpm at 4°C for 5 minutes. The final solution was 500 µL which was stored at -20°C until analysis by high-performance liquid chromatography (HPLC). The recovery of applied ^{14}C-2,4-D from the extraction plus leaf wash was 80-100%.

The resulting filtrate was analysed using HPLC using a 4.6*100 mm Kinetex C18, 2.6 µ particle column (Phenomenex, CO, USA) at 30°C. The mobile flow rate was 1 mL min^{-1}. Mobile phase (solvent) A contained 99% water, 0.9% acetonitrile and 0.1% acetic acid, and phase (solvent) B contained 99.9% acetonitrile and 0.1% acetic acid. The gradient used for all runs was 26 min at 30 °C starting at 100% solvent A and finishing at 100% solvent B. Samples of 100 µL were injected into the HPLC system, and 1 mL fractions were collected every minute into 20 mL scintillation vials. The amount of radioactivity in the collected fractions was measured by adding 10 mL of Ultima Gold to each vial and getting the values using a scintillation counter as in absorption/translocation experiments.

For metabolite identification, the fractions that showed metabolites corresponding to the retention times were injected into a HPLC-mass spectrometer (Thermo Scientific Q-Exactive Focus Hybrid Quadrupole-Orbitrap) operating in both positive and negative ESI modes. ESI (+) capillary voltage was 3800V and probe heater 250°C, ESI (-) capillary voltage 3300V with probe heater 250°C. 10 uL sample(s) were injected via Dionex Ultimate 3000 HPLC system directly into the mass spectrometer at a flow rate of 0.1 mL/min methanol. This was performed by the experienced technician in the lab.

This experiment was repeated at a later time, and in this second run, the nutrient solution was also analysed using HPLC.

6.2.5 Statistical analysis

The absorption/translocation and metabolism experiments were conducted using a completely randomised design with four replicates. All these experiments were repeated twice in time for each cultivar of plantain in the same glasshouse. There was no interaction between the treatments and the experiments, so the mixed model was used from the two runs for the absorption/translocation and metabolism experiments (Piepho et al., 2004). The data were tested for homogeneity of variances using the Levene test (using CAR package) and for normality using the Shapiro-Wilk normality test to examine the distribution of data. All statistical analysis was done using the software R Studio 4.1.3 (Kniss and Streibig, 2019) using mixed analysis of variance (ANOVA) and the difference between the two cultivars was compared by the Bonferroni method.

The absorption rate was calculated as the sum of the radioactivity measured in all plant sections and is expressed as a percentage of applied radioactivity. The translocation rate was the sum of radioactivity recovered in all plant sections except the treated leaf and is expressed as a percentage of absorbed radioactivity (Nandula and Vencill, 2015). The percentage of 2,4-D absorbed was calculated using the following equation:

$$\frac{\text{sum of radioactivity in all plant sections}}{\text{sum of radioactivity in all plant sections} + \text{leaf wash}} \text{ X } 100$$

6.3 Results

6.3.1 Absorption/translocation experiment

The average total recovery of applied radioactivity in the 2,4-D absorption/translocation experiment was over 92% for both plantain cultivars. The percentage of absorption of the herbicide estimated to have occurred by the four times that plants were harvested is shown in Figure 6.1. There was a significant difference between Agritonic and Tonic plants in the percentage of radiolabeled 2,4-D absorbed only at 72 HAT, where Tonic had 61.1% absorption of 2,4-D compared to Agritonic with 45.3%. For three of the harvests, there was a distinct trend showing greater absorption of the 2,4-D into Tonic plants than Agritonic plants, with the biggest difference shown for plants harvested 72 HAT. However, plants harvested 48 HAT showed the reverse trend, where Agritonic had a higher percentage of 2,4-D absorbed compared with Tonic.

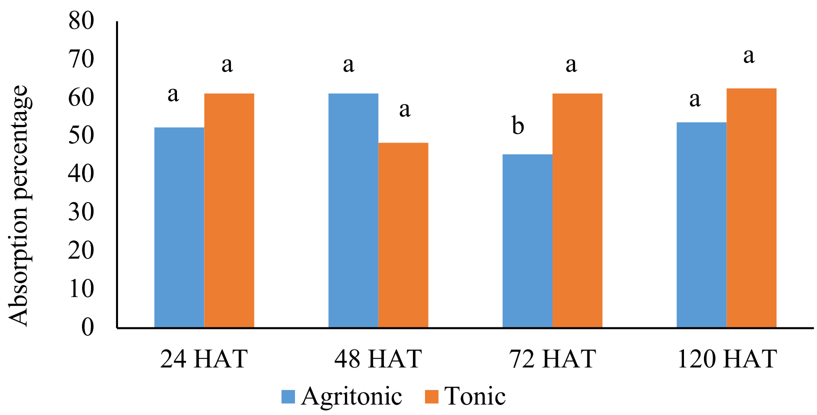


Figure 6.1 The percentage of radiolabeled 2,4-D that was estimated to have been absorbed into treated Tonic and Agritonic plantain plants 24, 48, 72 and 120 hours after treatment (HAT). Bars with different letters for a harvest are significantly different between the cultivars (p<0.05).

The percentage of absorbed herbicide that remained in the treated leaf was less in Tonic plants compared to Agritonic at all harvests and gradually decreased over time, especially for the Tonic plants (Table 6.1). However, the difference between the Agritonic and Tonic cultivars for the ^{14}C-2,4-D remaining in the treated leaf was significant only at 48 and 120 HAT. By the final harvest at 120 HAT, only 40.0% of the absorbed 2,4-D was still present in the treated leaf for Tonic compared with 56.8% for Agritonic. There was no significant difference between Agritonic and Tonic plantain in the percentage of ^{14}C-2,4-D movement into the untreated leaves at any harvests. The herbicide that moved out of the treated leaf was found in untreated leaves, the stem, and the roots and the greatest amount was found to exude out of the roots into the hydroponic solution (Table 6.1).

By 24 HAT, more than twice as much ^{14}C-2,4-D was found in the stem of Tonic plants than for the Agritonic plants, with 14% of all the ^{14}C-2,4-D in the Tonic, compared with 6% for Agritonic. Apart from this harvest, no significant differences were recorded in the amount of ^{14}C-2,4-D located in the stem for the two cultivars at 48, 72 and 120 HAT. At 120 HAT, the greatest destination for the ^{14}C-2,4-D was the nutrient solution around the roots, where the ^{14}C-2,4-D was progressively exuded over time. There was a general trend for greater amounts of ^{14}C-2,4-D being moved into the roots and being exuded from the roots of Tonic plants than Agritonic (Table 6.1), which increased over time. However, the amount located in the roots was significantly different between the two cultivars, only at 72 HAT. At 120 HAT, 31% of total absorbed radioactivity was found in the root exudates of Tonic compared to 18% in Agritonic plants.

Table 6.1 Translocation of ^{14}C-2,4-D in Agritonic and Tonic plantain at 24, 48, 72 and 120 HAT. Data are expressed as a percentage of absorbed radioactivity at each harvest time.

Tissue	Plantain cultivar	Time after application (hour)			
		24	48	72	120
Treated leaf	Agritonic	72.1	64.7a	56.0	56.8a
	Tonic	60.4	52.0b	51.5	40.3b
P values		0.11	0.009	0.34	<0.001
Untreated leaf	Agritonic	5.4	5.6	7.0	9.2
	Tonic	4.1	5.7	4.8	8.7
P values		0.57	0.98	0.17	0.79
Stem	Agritonic	6.3b	9.7	10.5	9.6
	Tonic	14.1a	13.3	10.9	11.2
P values		0.01	0.079	0.83	0.37
Root	Agritonic	4.2	6.2	5.4b	6.0
	Tonic	6.1	8.0	11.7a	8.6
P values		0.46	0.33	<0.001	0.15
Root exudates	Agritonic	12.1	13.7b	21.1	18.5b
	Tonic	15.3	21.0a	21.0	31.3a
P values		0.55	0.04	0.97	<0.001

Mean value within each column followed by the different letters indicate significant differences in each time between two plantain cultivars ($p<0.05$).

6.3.2 Metabolism experiment

The total average recovery of applied ^{14}C-2,4-D for the metabolism experiment was over 90% for both plantain cultivars. There was a general trend for more metabolism to have occurred in Agritonic plants than in Tonic plants. As shown in Figure 6.2, within each of the tissues that 2,4-D was found, the extent to which the herbicide had been metabolised into other molecules at 72 and 120 HAT varied from 1% to 19%. The differences between Agritonic and Tonic in the rate of metabolism in different plant sections appeared to be small (e.g., 15% vs 11% at 72 HAT and 18% versus 16% at 120 HAT for Agritonic and Tonic, respectively, in the treated leaf), and differences were not significant between the two cultivars for different sections, except for 72 and 120 HAT in the untreated leaves. But again, the differences were only small (4% vs 2% of all radioactivity present), despite being significant (Figure 6.2). In contrast with wheat, 26% of 2,4-D got metabolised in plantain, whereas 37% of 2,4-D was metabolised in wheat at 120 HAT (data not shown).

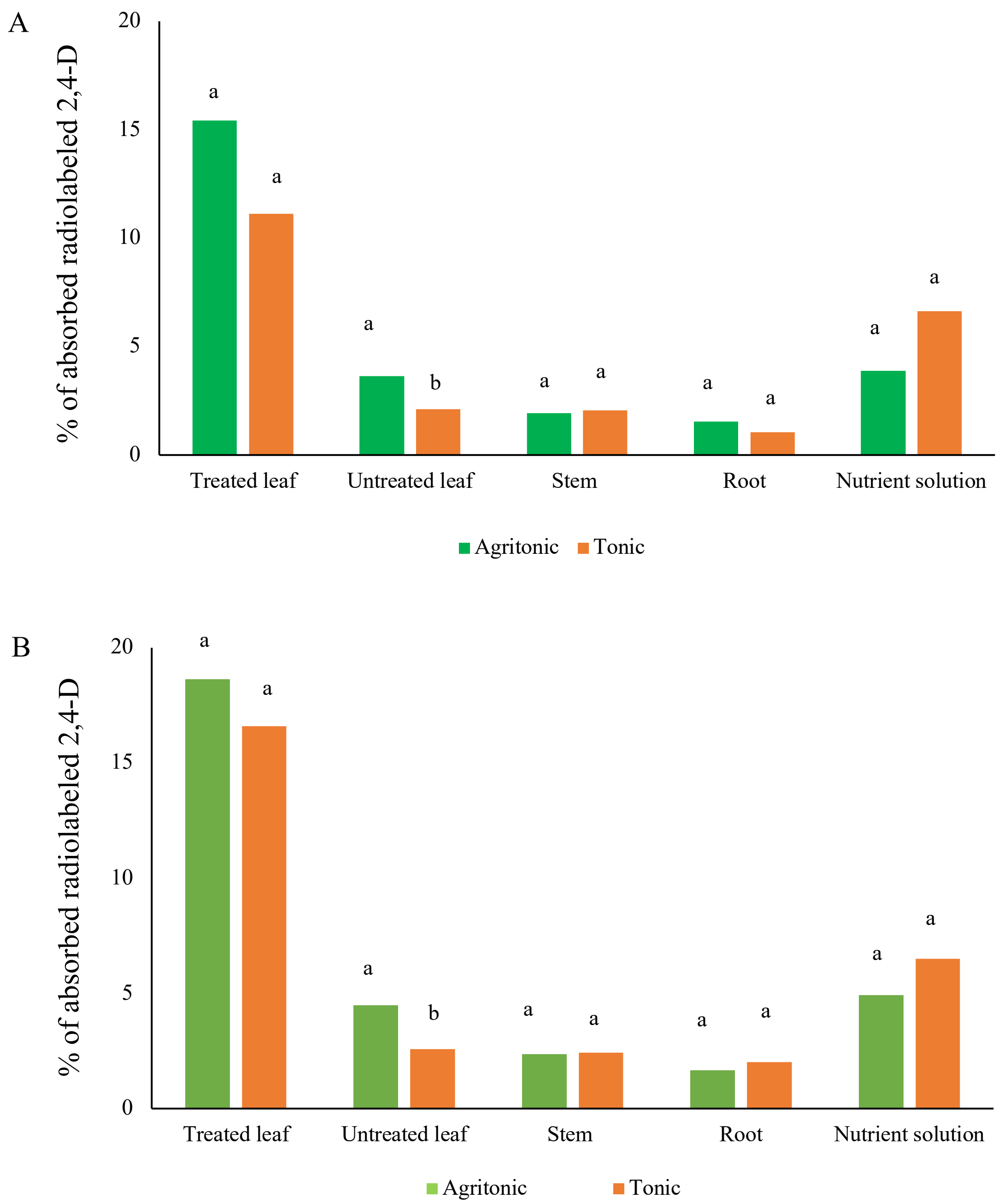


Figure 6.2 The percentage of absorbed radiolabeled 2,4-D found in the treated leaf, untreated leaves, the stem and the roots that had been metabolised into other molecules within treated Agritonic and Tonic plantain plants at (A) 72 and (B) 120 hours after treatment (HAT). Bars with different letters for plant sections are significantly different between the cultivars (p<0.05).

During the HPLC process, the various compounds present (i.e. 2,4-D and each of its metabolites) are retained on the column for different amounts of time according to their chemical composition, known as the retention time, and these different retention times can be used to differentiate the compounds (Spicer et al., 2010). The HPLC chromatographs indicated that the retention time of the standard/parent ^{14}C 2,4-D was 13.3 minutes. Apart from parent ^{14}C 2,4-D, several different metabolites were detected from the HPLC work at retention times 9-10 min, 10-11 min, 11-12 min and 12-13 min (Figure 6.3 A and B). The standard 2,4-D found at 13-14 min in Agritonic and Tonic was 71%, and the rest were the metabolites. Although the chromatograms of wheat samples at 120 HAT showed a similar pattern of metabolism to that of Agritonic and Tonic plantain (Figure 6.3.C), only 48% of the radioactivity was found at 13-14 min (i.e. was still 2,4-D), and the rest of it had been metabolised. Thus, there was the difference between the plantain cultivars and wheat. More 2,4-D was metabolised in wheat, even though it appeared to involve the same metabolites. Mass spectrometry was then used to identify these metabolites but proved unsuccessful.

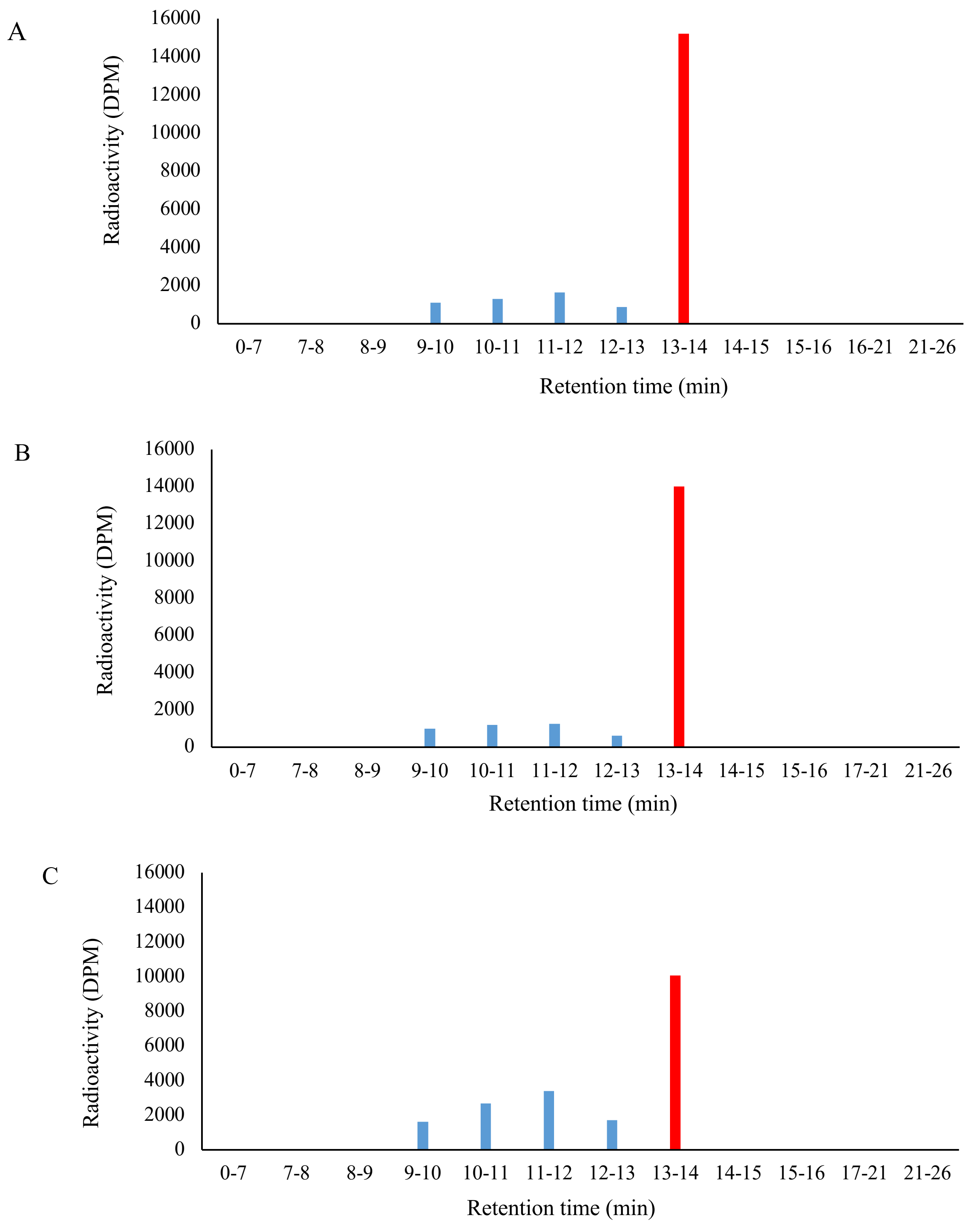


Figure 6.3 ^{14}C-2,4-D parent compound and its metabolites in (A) Agritonic plantain (B) Tonic plantain and (C) wheat at 120 hours after treatment (HAT) (radioactivity in DPM versus retention time in minutes). Data are means of four replications from two experiments and the radioactivity from different plant sections (treated leaf, untreated leaf, stem and root) were added together and averaged.

There appeared to be no difference in the metabolites found in the nutrient solution of treated Agritonic and Tonic plants (Figures 6.4 A and B), but there were differences in the metabolites in the nutrient solution of wheat compared with the plantain (Figure 6.4 C). This suggested that the 2,4-D was metabolised differently in the roots of wheat than in the roots of plantain. Apart from the apparent difference in the metabolites present, there were also more 2,4-D metabolites in the nutrient solution of wheat (63%) than that of Agritonic (16%) and Tonic (13%).

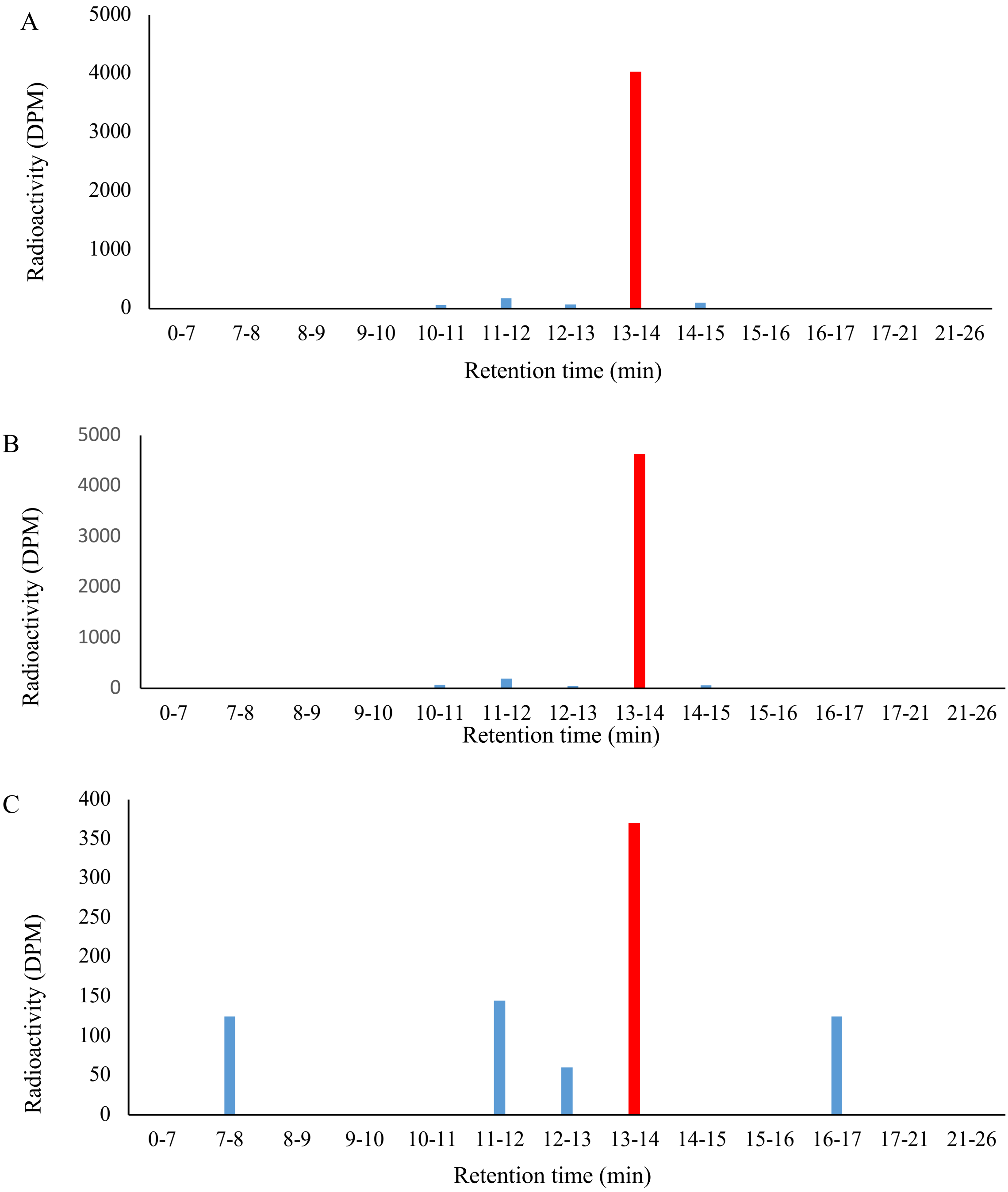


Figure 6.4 ^{14}C-2,4-D parent compound and its metabolites in (A) Agritonic plantain (B) Tonic plantain and (C) wheat found in root exudates in the nutrient solution at 120 hours after treatment (HAT) (radioactivity in DPM versus retention time in minutes). Data are means of four replications from one experiment.

6.4 Discussion

The results from this experiment showed that 2,4-D absorption was slightly less in the tolerant plantain, Agritonic, than the susceptible plantain, Tonic. Also, there was a significant difference between the Agritonic and Tonic cultivars for the ^{14}C-2,4-D remaining in the treated leaf at 48 and 120 HAT only, but the amount of herbicide remaining in the treated leaf of Agritonic was greater than Tonic at all harvests. Although the difference between Agritonic and Tonic was not always significant the consistent, but small difference, suggested the effect probably had biological significance (Cousens, 2002). There was much variability between individual Agritonic plants suggesting that not all of the Agritonic plants were as tolerant as each other.

In this study, different patterns of ^{14}C-2,4-D translocation was observed between the 2,4-D tolerant (Agritonic) and susceptible (Tonic) plantain populations. Agritonic had more ^{14}C-2,4-D retained in the treated leaf, and less ^{14}C-2,4-D was translocated to the stem and root. In contrast, a greater percentage of ^{14}C-2,4-D was translocated to the stem and root at 24 and 72 HAT, respectively, in Tonic. Thus, more 2,4-D accumulation in the meristematic region in the Tonic than in Agritonic plantain could be a possible explanation for differences in tolerance occurring. Reduced translocation can be due to the sequestration of the herbicide or its conjugates in the vacuole or the apoplast, away from both the site of herbicide action and the vascular system (Yuan et al., 2007; Ghanizadeh and Harrington, 2017c). If less herbicide is being moved out of the leaf in Agritonic, that could affect herbicide absorption into the leaf, which relies on a concentration gradient to move from outside the leaf to the inside.

Reduced 2,4-D translocation had been previously reported for resistant populations of prickly lettuce (Riar et al., 2011), wild radish (Goggin et al., 2016), oriental mustard(Dang et al., 2018), and *Gossypium hirsutum* (Perez et al., 2022). Goggin et al. (2016) found that reduced herbicide translocation in 2,4-D resistant wild radish was due to an ABCB-type auxin transporter. Rey-Caballero et al. (2016) also suggested that reduced 2,4-D translocation was involved in the resistance mechanism within corn poppy, leading to less ethylene production and greater survival in the resistant plants. These results contrasted with other reports where no difference in total 2,4-D translocation was found between 2,4-D tolerant and susceptible ground ivy (Kohler et al., 2004).

The biggest difference between Agritonic and Tonic in the current study was the amount of 2,4-D found in the nutrient solution, with more for Tonic. Tonic plants translocated more 2,4-D to roots than Agritonic plants, even in the first 24 hours, and also significantly more 2,4-D was exuded in the nutrient solution of Tonic at 48 and 120 HAT. Similar results were observed by Harrington and Woolley (2006) where significantly more radioactive 2,4-D was released from the roots of the susceptible nodding thistle plants than resistant ones. It is well known that 2,4-D can be exuded naturally from the roots of weeds in an unmetabolised form (Reid and Hurtt, 1970), which seems to be the case in our study. So rather than root exudation being the mechanism of resistance, it appeared to be a result of more herbicide being translocated to the roots in Tonic than Agritonic. In contrast, Jugulam et al. (2013) found that the MCPA-resistant wild radish population translocated MCPA more rapidly to roots than the susceptible ones.

The HPLC analysis found no evidence of differences in 2,4-D metabolism between tolerant or susceptible plantain harvested at 72 and 120 HAT. Thus, enhanced metabolism appears not to be associated with the tolerance mechanism. Although the 2,4-D appeared to be undergoing some metabolism, this was occurring in similar ways in the two cultivars. This result was similar to the work by Goggin et al. (2016), where 2,4-D was metabolised in wild radish, but there was no difference in the metabolism between the susceptible and resistant populations. Conversely, enhanced 2,4-D metabolism mediated by cytochrome P450 played an important role in the mechanism of resistance in corn poppy resistant populations (Torra et al., 2017) and common waterhemp (Figueiredo 2018). Figueiredo (2022) found that the metabolites produced by susceptible *Amaranthus tuberculatus* plants had aspartic acid conjugation, and the resistant plants produced 5-OH-2,4-D-related metabolites as a detoxification reaction.

There was a similar pattern of metabolism in wheat and the two plantain cultivars, but there was no significant difference between the cultivars in the extent of metabolism. More of the 2,4-D had been metabolised in wheat compared with the plantain. Metabolites with similar retention times were found in wheat as well as in Agritonic and Tonic plantain (Figure 6.3), suggesting that these plantain cultivars might metabolise 2,4-D through the same biochemical processes as wheat. The metabolites from wheat have been identified in other work as being predominantly carboxylic glucose esters and phenolic glycosides (Hamburg et al., 2001).

There was little difference between the Agritonic and Tonic in the tolerance of 2,4-D. Also, there was much variability between Agritonic plants as some were tolerant and some were not, so traits that were selected for when creating this cultivar appear not to be uniformly present in all plants. As a result, this variability in the plants has probably masked some differences in physiology during statistical analysis in these preliminary findings.

6.5 Conclusion

The results from this experiment suggest that tolerance to 2,4-D in Agritonic plantain may be based on the reduced translocation when comparing the 2,4-D-tolerant Agritonic plants with the susceptible Tonic plants even though the level of tolerance was small. This research has revealed that the 2,4-D-tolerant Agritonic plantain exhibited slightly different patterns of translocation and probably absorption compared with Tonic plantain, whereas differences in metabolism do not appear to be involved. Although the differences in translocation appear quite small, they might be sufficient to create increased tolerance in plantain with further selection.

Chapter 7

Susceptibility of plantain to herbicides wiped onto seed-heads

7.1 Introduction

The wiping of herbicide through weed wipers is a technique used in agricultural management to selectively control upright weed species in pastures and low-growing crops (Harrington and Ghanizadeh, 2017). A detailed description of weed wipers, the history of wiper applicators, uses of weed wipers, and the herbicides used for this experiment were reviewed in Section 2.3.3. From earlier experiments conducted in Chapters 3, 4, 5 and 6, it was found that Agritonic plantain can tolerate some selective herbicides in both glasshouse and field conditions when applied to young weeds when plantain is also young. Once plantain-based pastures are well-established, many of these herbicides are unlikely to be very effective on older weeds (Holden, 2022). One possibility to control such weeds is to use a wiper applicator to apply herbicides to weed species such as Californian thistle (*Cirsium arvense*) or broad-leaved dock (*Rumex obtusifolius*) when they grow taller than the surrounding sward. However, at many times of the year when these species are taller than the pasture, plantain has seed-heads that also grow taller than the surrounding pasture. Although pastures are usually grazed prior to using a wiper applicator to increase the height differential between the unpalatable weeds and the sward (Moyo et al., 2022), the plantain seed-heads can often also remain ungrazed.

There are limited published data on the effectiveness of weed wipers for controlling taller weeds in pastures using different herbicides and its effect on pastures. In addition, there is also very limited information on the damaging effects of herbicides wiped on pasture plant species, especially plantain. Thus, glasshouse experiments were conducted to test the tolerance of plantain (Agritonic and Tonic) with seed-heads to the application of some herbicides suitable for use in weed wipers (glyphosate, clopyralid, aminopyralid, dicamba, picloram and triclopyr) to the seed-heads simulating potential contact during weed wiping of pasture weeds. Evaluating the tolerance to the different herbicides involved measurements of plantain production over time to evaluate the time it takes before the plantain was no longer affected.

7.2 Materials and Methods

7.2.1 First wiping experiment

Plantain seeds were sown in 128 planter bags, i.e. PB¾ (64 each for Agritonic and Tonic), each filled with 0.4 L of potting mix (Dalton Base mix, which was comprised of 50 % Fines A grade *Pinus radiata* bark size 0-12 mm with added calcium ammonium nitrate [a common additive to raw bark as a nitrogen source for the microbial activity during composting], 30% coco fibre and 20% Pacific pumice 7 mm) and long-term Osmocote, i.e. 500 g, 50 g Osmoform and 150 g dolomite per 100 L of potting mix, in a heated glasshouse with sub-irrigation on 8 June 2020. When the plants had fully established and developed seed-heads, the number and average height of stems were measured for each plant, and all the leaves were cut to 7 cm to have a uniform length on 29 November 2020, though seed-heads were not cut. Canes were used to tie the seed-heads loosely upright so they did not bend over neighbouring plants. A range of herbicides was applied to the seed-heads of both plantain cultivars on 1 December 2020, using concentrations recommended for wiper application to weeds (Table 7.1 and 7.2). A paper towel was moistened with each solution and wrapped around all parts of the seed-heads above the height of 12 cm from the ground level for about 5 seconds, with care being taken to ensure no dripping occurred on to leaves of the plants. The plants were spread out after applying herbicides to allow the herbicide to dry without dripping from the stems. After 24 hours, half of the treated plants were exposed to overhead irrigation for 8 minutes to simulate 2 mm of rainfall. Since the plants were in small planter bags for so long, Osmocote Pro (3-4 months controlled release) fertiliser granules were applied at 1.6 g per bag on 24 December 2020 to provide additional nutrition to the plants.

Table 7.1 List of treatments applied to the plantain seed-heads (see Table 7.2 for details of herbicides used).

Herbicides	Product concentration (g ai L^{-1})	Concentration applied (ml L^{-1})	Rate (Herbicide: Water)
untreated control			
glyphosate (low rate)	360	50	1:20
glyphosate/metsulfuron	360+200 g kg^{-1}	25 (+ 1.5 g)	1:40 (+0.5 g L^{-1})
glyphosate (high rate)	360	200	1:5
clopyralid	300	25	1:40
aminopyralid	30	25	1:40
dicamba	500	10	1:40
triclopyr/picloram/ aminopyralid	300+100+8	25	1:40

Table 7.2 Trade names and formulation details of herbicides used.

Herbicide	Trade name	Formulations
glyphosate	Nufarm Weedmaster G360	360 g ai L^{-1} glyphosate (isopropylamine salt)
metsulfuron	Answer	200 g kg^{-1} metsulfuron (methyl ester)
clopyralid	Dow AgroSciences Versatill	300 g L^{-1} clopyralid (amine salt)
aminopyralid	Dow AgroSciences Tordon Max	30 g L^{-1} aminopyralid (trisopropylamine salt)
dicamba	Nufarm Kamba 500	500 g L^{-1}dicamba (dimethylamine salt)
triclopyr/picloram/ aminopyralid	Tordon Brushkiller XT	300 g L^{-1} triclopyr (butoxyethyl ester) and 100 g L^{-1} picloram and 8 g L^{-1} aminopyralid as amine salts, also contains 367 g L^{-1} diethylene glycol

The experiment was conducted using a randomised complete block design with four replicates of two cultivars of plantain (Agritonic and Tonic), eight different herbicidal treatments, and two rainfall scenarios (with and without overhead irrigation). As the plants varied quite substantially in the number of stems present at treatment, the block design allowed each herbicide to be applied both to plants with few stems and plants with many stems. The daily maximum and minimum temperatures during the 2 weeks after spraying averaged 23.5 ºC and

17.0 ºC, respectively. Scoring of plants to quantify the effect of herbicides on plants was done every fortnight after herbicide application starting from 17 December (2 weeks after rainfall simulation). Scores were allocated based on a 0-10 scale where 0 signified no effect and 10 signified total death. Two months after herbicide treatment, all plants with leaves and stems were cut to a height of 7 cm, and the cut material was oven dried at 70ºC for 48 hours and weighed. Another dry weight assessment was done using the same method 3 months after herbicide treatment. On 30 March 2021 (4 months after treatment), all above-ground parts of each plant were cut to ground level, and dry weight was measured. The final scoring was done immediately before this final harvest for dry weight. As dry weight results showed the same trends as the scores, only dry weight data are presented below.

7.2.2 Second wiping experiment

The experiment was repeated to confirm the results from the first experiment, using the same procedure as described above in the same glasshouse one year later at the same time of year.

7.2.3 Statistical analysis

Four-way repeated measures ANCOVA was performed with one within-subject factor (time) and three between-subject factors (cultivar, herbicide and rainfall scenario) with one covariate (number of seed-heads in the individual plant). The covariate effect was removed to adjust for the variation among individuals within each block. The covariates appeared in the model was evaluated at the number of seed-heads equals 6.66 and 8.19 in the first and second wiping, respectively. The Greenhouse-Geisser correction was used to compensate for the violation of the sphericity assumptions, and adjustment for multiple comparisons was made through Bonferroni. Means were separated using LSD tests at 5% probability.

7.3 Results

7.3.1 First wiping experiment

The herbicide treatments differed significantly in their effect on the total dry weight of plantain. Rainfall scenarios had no significant effect overall on the total dry weight of plantain, though there was a significant interaction between rainfall and herbicides (Table 7.3). There was a significant difference between the two plantain cultivars in their overall dry weight, where Agritonic had a higher total dry weight (8.0 g plant^{-1}) than Tonic (7.4 g plant^{-1}) but there was no significant interaction for cultivars and herbicides, suggesting there were no differences between the cultivars in their response to herbicides (Table 7.3).

The overall dry weight of plantain when treated with dicamba, the low rate of glyphosate or aminopyralid was less affected than other herbicide treatments, and these were not significantly different from the untreated control plants (Figure 7.1). The greatest reduction was recorded for the high rate of glyphosate, despite the low rate of glyphosate not reducing plantain dry weight significantly. The glyphosate/metsulfuron mix and the triclopyr/picloram/aminopyralid treatment were not significantly different from the high rate of glyphosate in reducing plantain growth (Figure 7.1).

Table 7.3 ANCOVA table for Test of Between-Subjects effects (cultivar, rainfall and herbicides) and the number of seed-heads used as a covariate for plantain dry weight for first wiping experiment.

Source	df	Mean Square	F	Sig.
Intercept	1	3412.0	453.8	<0.001
Number of seed-heads	1	123.3	16.4	<0.001
Cultivar	1	58.8	7.8	0.006
Rainfall	1	11.7	1.6	0.216
Herbicides	7	59.7	7.9	<0.001
Cultivar * Rainfall	1	0.7	0.1	0.766
Cultivar * Herbicides	7	5.9	0.8	0.601
Rainfall * Herbicides	7	15.9	2.1	0.049
Cultivar * Rainfall * Herbicides	7	6.2	0.8	0.568
Error	95	7.5		

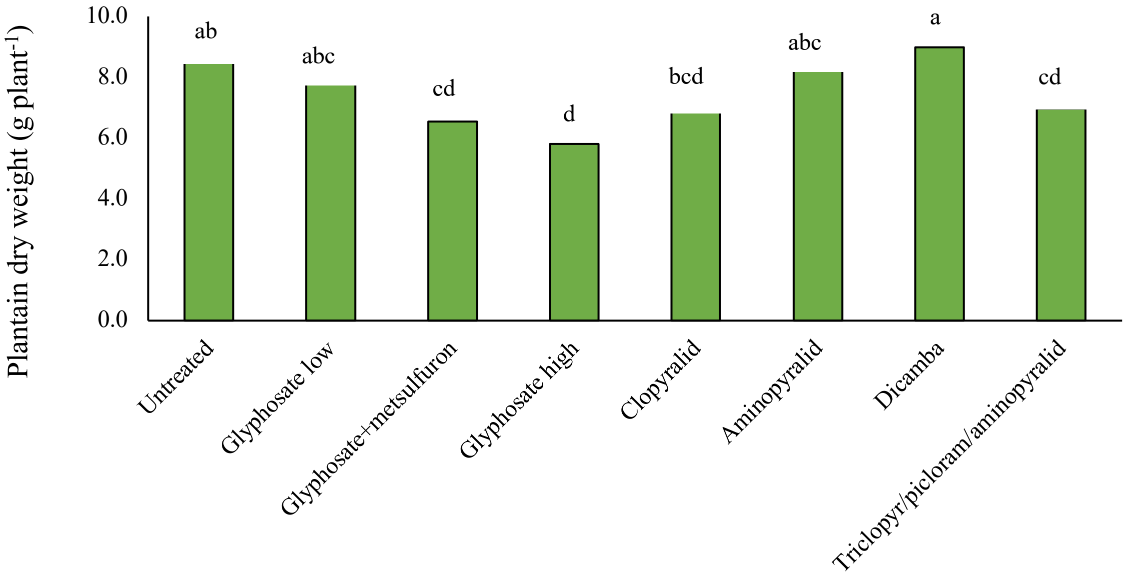
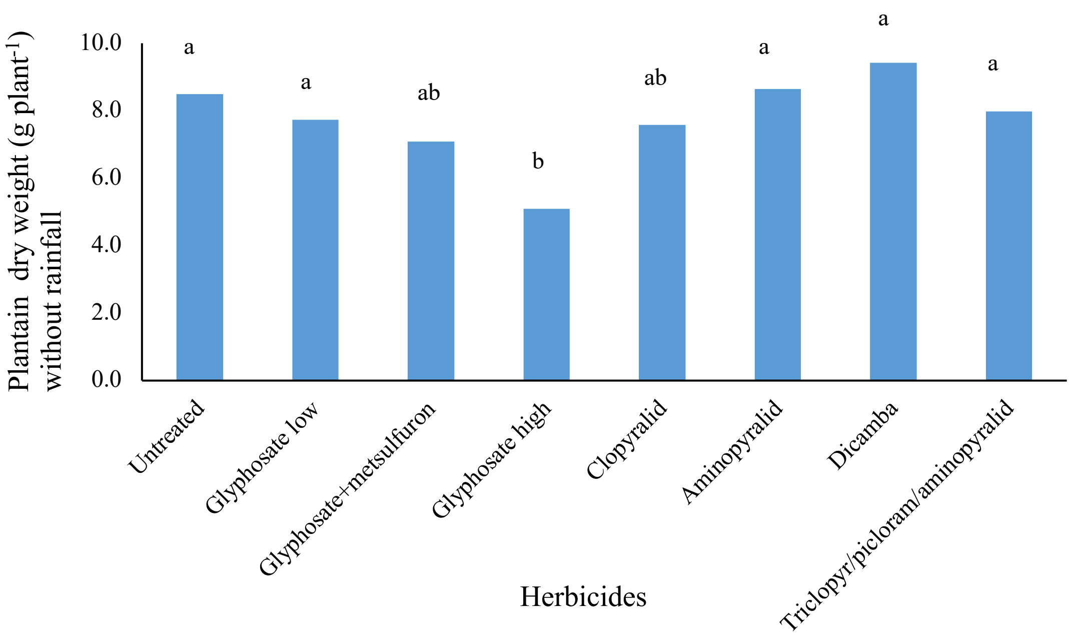


Figure 7.1 The effect of herbicide treatments on total dry weight of plantain averaged across all three harvests and both cultivars and both rainfall scenarios. Bars with different lowercase letters are significantly different according to LSD ($p<0.05$).

The visual observations from this glasshouse experiment at 2 weeks after herbicide wiping on the seed-heads of plantain showed that clopyralid had caused the greatest adverse effect to plantain followed by the high rate of glyphosate. These herbicides caused necrosis and twisting of leaves in both rainfall scenarios, i.e. with or without rainfall. Plantain appeared to have recovered from any adverse effects caused by dicamba by 1 month after wiping.

When no rainfall was applied, none of the herbicide treatments caused significant reductions in the total dry weight of plantain measured from the four harvests when compared to the untreated control except for the high rate of glyphosate (Figure 7.2A). However, when rainfall was simulated 1 day after the herbicides were applied, this caused a severe effect on the dry weight of plantain treated with the glyphosate/metsulfuron mix and triclopyr/picloram/aminopyralid, in addition to the adverse effects of the high rate of glyphosate (Figure 7.2 B). Dicamba had the least effect following rainfall, with a similar dry weight as the untreated control.

A.



B.

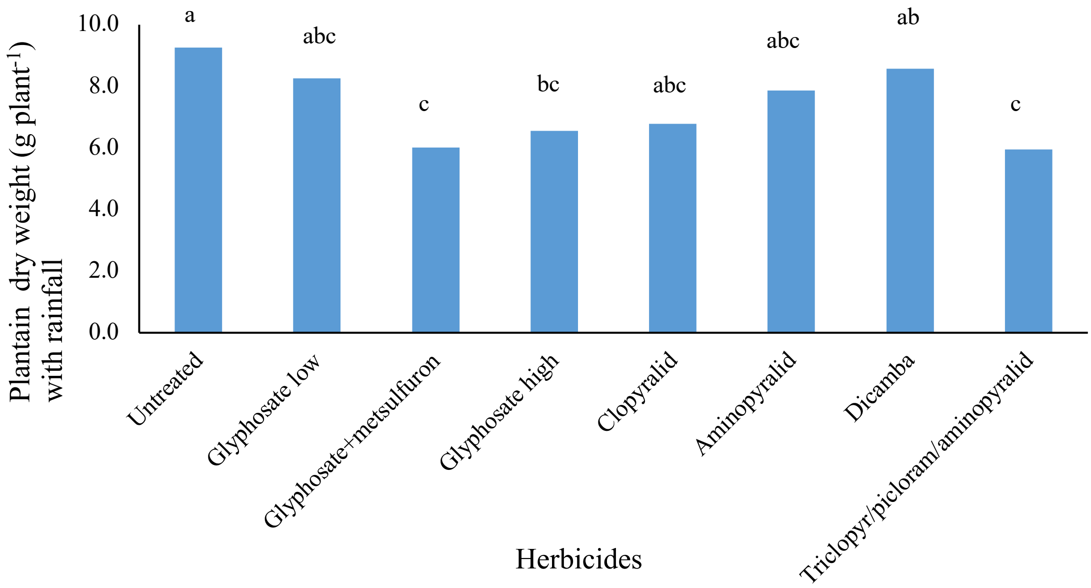


Figure 7.2 The effect of rainfall and herbicide treatments A. without rainfall and B. with rainfall on the total dry weight of plantain averaged across all three harvests and both Agritonic and Tonic cultivars. Bars with different lowercase letters are significantly different according to LSD ($p<0.05$).

There was a significant interaction between the effect of harvest time, cultivars and herbicide treatments on plantain's dry weight (Table 7.4). The effect of herbicides on the dry weight of plantain changed over the three harvests. Unfortunately, an error was made when nutrients were applied 1 month after treatment, as the untreated plants and those treated with the low rate of glyphosate did not receive the additional fertiliser. These plants were treated with 1.6 g pot^{-1} Osmocote immediately after the first harvest, and they then recovered well. At this first harvest, plants treated with dicamba and aminopyralid had the highest dry weights. The necrotic and twisted leaves caused by clopyralid started to recover over time, and the dry weight of plantain measured at 2 months after wiping with clopyralid was similar to the untreated control. The plants treated with clopyralid had produced many new leaves, though they still showed some distortion.

Table 7.4 ANCOVA table for Tests of Within-Subjects effects (time) with cultivar, rainfall and herbicides and the number of seed-heads used as a covariate for plantain dry weight for first wiping experiment.

Source	df	Mean Square	F	Sig.
Time	1.2	535.2	395.8	<0.001
Time * Number of seed-heads	1.2	0.2	0.2	0.723
Time * Cultivar	1.2	19.9	14.7	<0.001
Time * Rainfall	1.2	0.5	0.4	0.563
Time * Herbicides	8.3	48.4	35.8	<0.001
Time * Cultivar * Rainfall	1.2	0.8	0.6	0.458
Time * Cultivar * Herbicides	8.3	2.8	2.0	0.045
Time * Rainfall * Herbicides	8.3	2.8	2.1	0.041
Time * Cultivar * Rainfall * Herbicides	8.3	1.0	0.7	0.691
Error(Time)	112.8	1.4		

At the second harvest (3 months after herbicide wiping), plants treated with dicamba still showed the least effect, with those treated with aminopyralid and clopyralid having a minimal effect and were similar to the untreated control. There was no significant difference between all other herbicides and the untreated control except the high rate of glyphosate, which had a significantly lower dry weight of plantain compared to untreated plants (Figure 7.3 B). By the final harvest (4 months after wiping), the low rate of glyphosate and dicamba showed the least effect on plantain, and was not significantly different from the untreated control in the dry weight of plantain. The high rate of glyphosate still showed the highest adverse effect on plantain, though reduced dry weights were also recorded for triclopyr/picloram/aminopyralid, clopyralid and the glyphosate/metsulfuron mix (Figure 7.3 C).

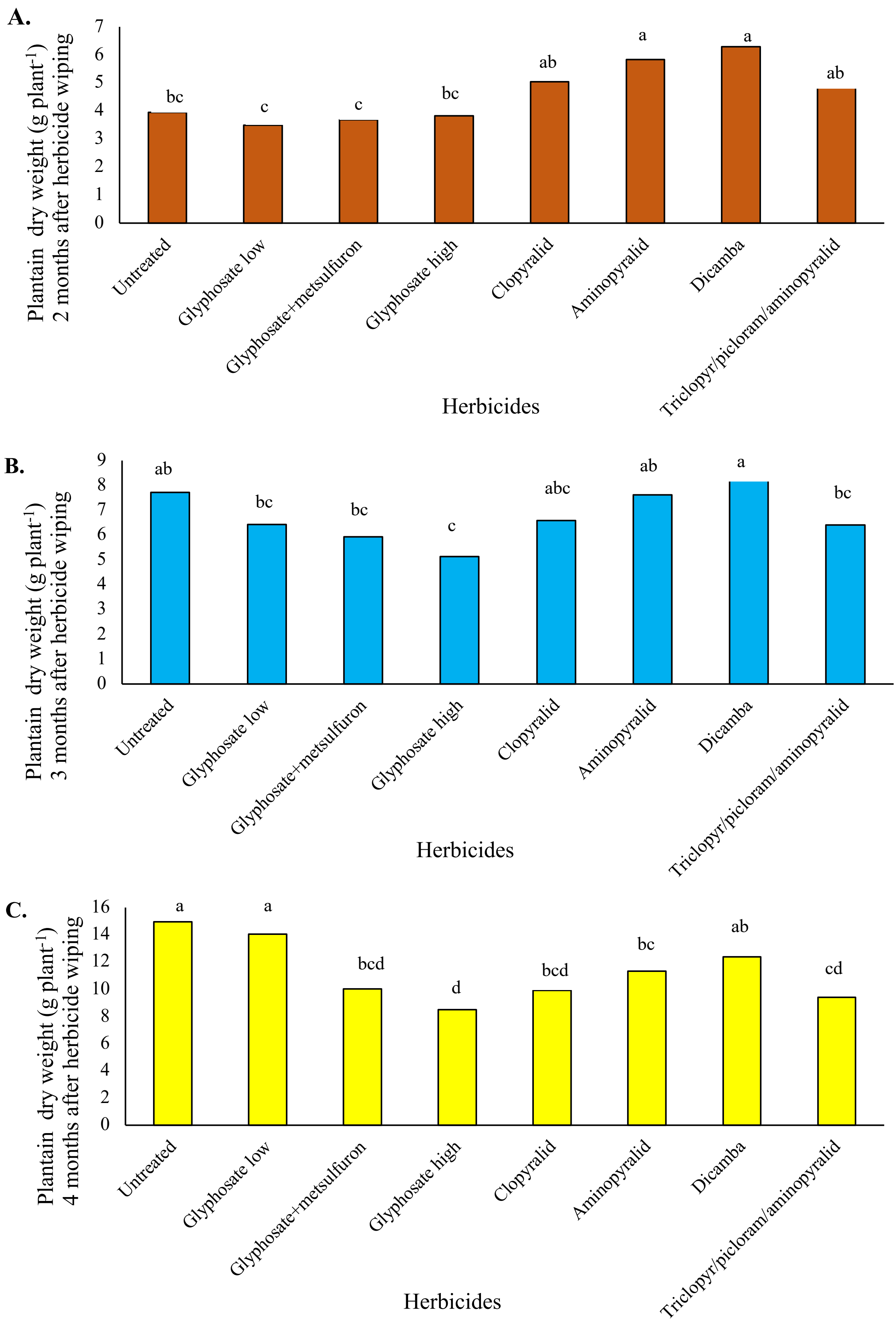


Figure 7.3 The effect of herbicides on plantain dry weight A. 2 months B. 3 months and C. 4 months after treatment averaged across the two cultivars and both rainfall scenarios. Bars with different lowercase letters are significantly different according to LSD ($p<0.05$).

7.3.2 Second wiping experiment

Most of the results were similar to the first wiping experiment. However, one big difference was that the untreated control and low rate of glyphosate were not compromised by lack of nutrients as in the first experiment. In contrast to the first wiping experiment, although rainfall scenarios had a significant effect overall on the total dry weight of plantain (plants with no rainfall were 13.7 g plant^{-1} and rainfall simulated plants were 11.4 g plant^{-1}), there was not a significant interaction between rainfall and herbicides (Table 7.5). The greatest reduction in the total dry weight of plantain was found for the high rate of glyphosate and glyphosate/metsulfuron mix. Dicamba-wiped plants had significantly less dry matter compared to the untreated control, which contrasted with the first wiping experiment. The low rate of glyphosate, when wiped to plantain seed-heads, reduced the dry weight of plantain significantly compared to the untreated control but was not significantly different from clopyralid and dicamba (Figure 7.4).

Table 7.5 ANCOVA table for Test of Between- Subjects effects (cultivar, rainfall and herbicides) and the number of seed-heads used as a covariate for plantain dry weight for second wiping experiment.

Source	df	Mean Square	F	Sig.
Intercept	1	5216.6	206.9	<0.001
Number of seed-heads	1	233.9	9.3	0.003
Cultivar	1	234.6	9.3	0.003
Rainfall	1	494.1	19.6	<0.001
Herbicides	7	647.2	25.7	<0.001
Cultivar * Rainfall	1	1.1	0.0	0.835
Cultivar * Herbicides	7	20.9	0.8	0.564
Rainfall * Herbicides	7	36.5	1.4	0.196
Cultivar * Rainfall * Herbicides	7	4.7	0.2	0.987
Error	95	25.2		

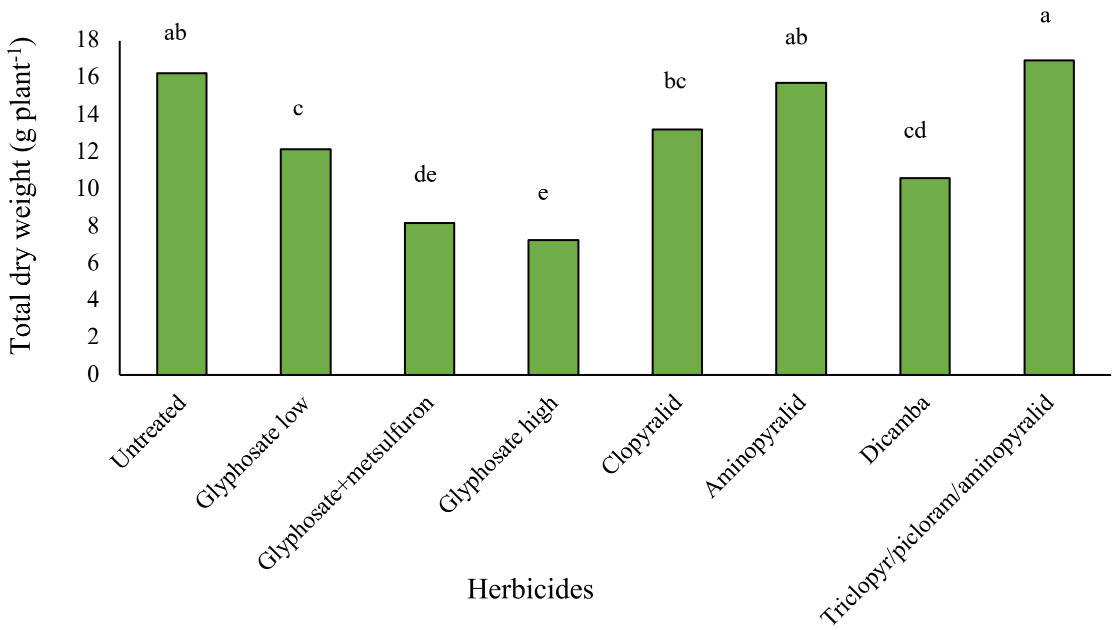


Figure 7.4 The effect of herbicide treatments on total dry weight of plantain averaged across all three harvests for the second wiping experiment. Bars with different lowercase letters are significantly different according to LSD ($p<0.05$).

The visual observations at 2 weeks after herbicide wiping on the seed-heads of plantain showed that aminopyralid-wiped plants for both Agritonic and Tonic and also in both rainfall scenarios with or without rainfall, had the least effect of any herbicidal treatments, which was similar to triclopyr/picloram/aminopyralid (data not shown). Half of the plantain (both Agritonic and Tonic) plants wiped with dicamba and glyphosate/metsulfuron mix died when rainfall was applied afterwards. In contrast, there was much less effect on plants not exposed to post-spraying rainfall for these herbicides (data not shown) at the time of final harvest.

There was a significant interaction between the effect of harvest time and herbicide treatments on the dry weight of plantain (Table 7.6). Two months after the herbicide wiping, the adverse effect on the dry weight of plantain was significantly greater for the high rate of glyphosate and the glyphosate/metsulfuron mix than other herbicides (Figure 7.5 A). The plants treated with a low rate of glyphosate grew very slowly and produced new leaves later than other plants. Plants treated with triclopyr/picloram/aminopyralid had dry weights similar to those treated with aminopyralid and untreated plants. They showed some symptoms initially but recovered rapidly. The effects on plantain from the low rate of glyphosate were not significantly different to the effects of dicamba and clopyralid. The results from 3 months after wiping were similar to 2 months after wiping (Figure 7.5 B). During the third harvest (3 months after wiping), triclopyr/picloram/aminopyralid and aminopyralid had the least effect on plantain and the dry weight was not significantly different to the untreated control (Figure 7.5 C). Plants wiped with clopyralid, and the low rate of glyphosate seemed to recover well by the time of final harvest and were similar to the untreated control. Plants treated with glyphosate/metsulfuron suffered the greatest effect, which were not significantly different from those treated with the high rate of glyphosate or dicamba (Figure 7.5 C).

Table 7.6 ANCOVA table for Tests of Within-Subjects effects (time) with cultivar, rainfall and herbicides and the number of seed-heads used as a covariate for plantain dry weight for second wiping experiment.

Source	df	Mean Square	F	Sig.
Time	1.1	726.5	136.1	<0.001
Time * Number of seed-heads	1.1	3.2	0.6	0.452
Time * Cultivar	1.1	19.5	3.7	0.056
Time * Rainfall	1.1	49.3	9.2	0.002
Time * Herbicides	7.6	43.3	8.1	<0.001
Time * Cultivar * Rainfall	1.1	0.3	0.1	0.821
Time * Cultivar * Herbicides	7.6	4.3	0.8	0.600
Time * Rainfall * Herbicides	7.6	7.0	1.3	0.250
Time * Cultivar * Rainfall * Herbicides	7.6	1.2	0.2	0.984
Error(Time)	102.8	5.3		

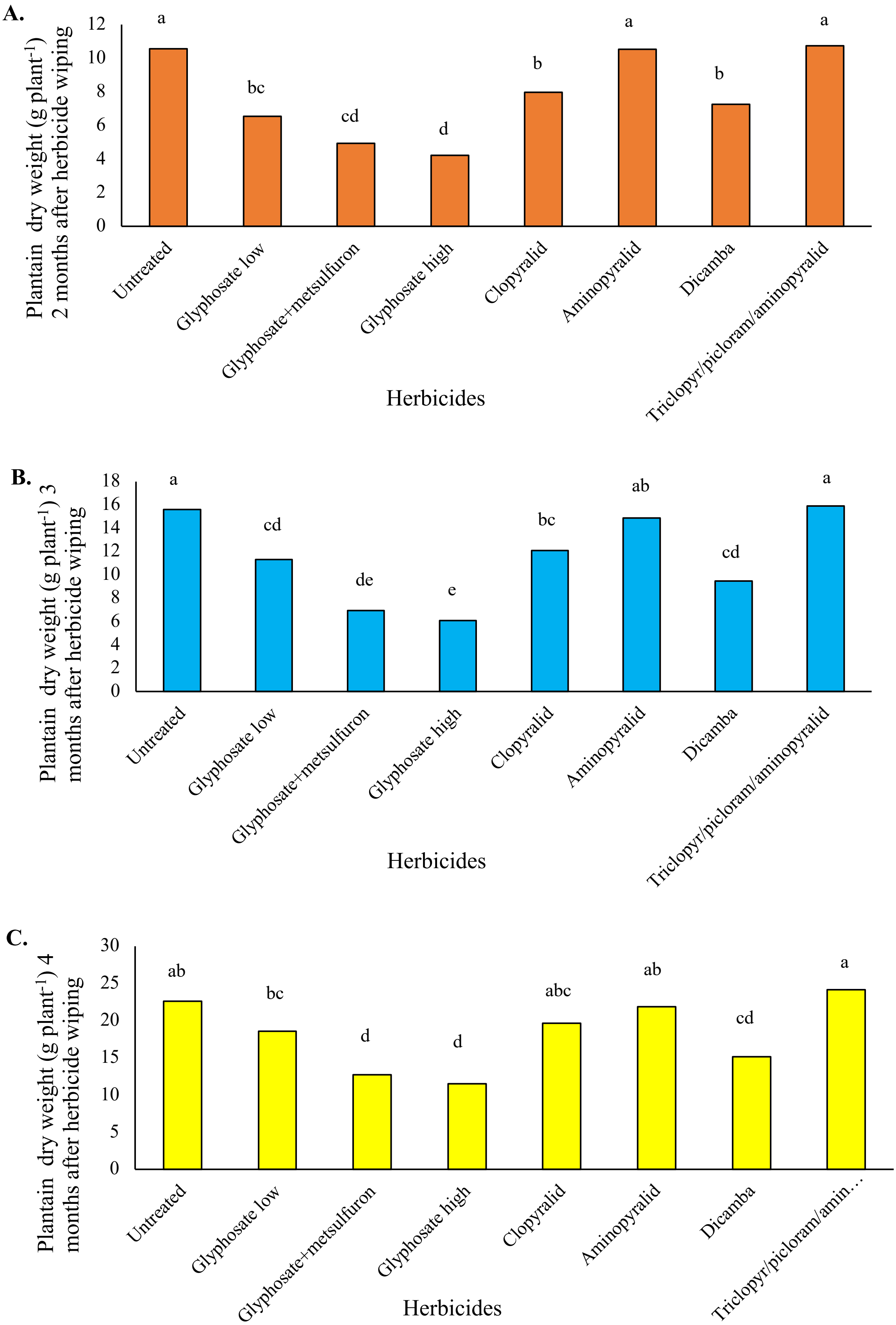


Figure 7.5 The effect of herbicides on plantain dry weight A. 2 months B. 3 months and C. 4 months after treatment for the second wiping experiment. Bars with different lowercase letters are significantly different according to LSD ($p<0.05$).

7.4 Discussion

Direct application of herbicides to plantain seed-heads in the two experiments showed most herbicides had little effect on plantain growth indicating there is potential to use herbicides in weed wipers to control weeds in plantain pastures. Weed wipers provide an effective tool for perennial weed control in permanent pastures as there are only a few selective herbicides available to manage weeds using broadcast applications that will not damage white clover (Ghanizadeh & Harrington, 2019; Grekul et al., 2005). Two problematic upright weeds for plantain are Californian thistle and broad-leaved dock, which are poorly controlled by selective herbicides. It is important for the herbicides to translocate sufficiently to kill the root system to achieve effective control of the weeds. The application of herbicides to the seed-heads of plantain might not be harmful, even though if the herbicides were applied to the foliage (or washed down there by rain) they would damage the plantain. Sugars produced by the cells in the stem are likely to send sugars up to the seed-heads, and also all phloem movement up the stem from the rosette also tends to be upwards. Thus, the application of herbicides to upper leaves and seed-heads results in limited translocation to the roots (Grekul et al., 2005; Wilson et al., 2006).

Dicamba and aminopyralid are safe for plantain (Harrington, 2023). But they can't be boom-sprayed, otherwise, they would kill the clover. This effect on clover may also be a problem with using them for wiping if they get washed off the stems or weeds to the clover below. Harrington et al (2016) reported that when rain was simulated, and clover was placed under treated Californian thistle that had herbicide on the foliage, herbicide was washed off the weed and on to the clover, causing damage. Aminopyralid is also a useful herbicide for spot-spraying thistles in pastures where improved cultivars of plantain have been planted (Harrington, 2023). The systemic herbicides used in this experiment, including glyphosate, dicamba, clopyralid, metsulfuron, triclopyr and picloram work well to control Californian thistle as they can translocate downwards if applied at the correct growth stage and kill the root system (Grekul et al., 2005; Martin et al., 1990; Moyo et al., 2016; Van Toor, 1994). However, for docks, there is almost nothing published on effective herbicides for use in weed wipers, though the manual for operating the Rotowiper maintains that a glyphosate/metsulfuron mix is the only effective treatment for docks (Rotowiper, 2004), which is why it was included in the experiment. The mixture of triclopyr, picloram and aminopyralid, and also dicamba, are recommended for control of docks when applied conventionally, but both treatments kill clover (Holden, 2022). Thus, the thistles will be easier to target than docks due to the herbicides that appear to be safe are effective on thistles, yet the only treatment known to control docks with wipers appears to be risky, and it is unclear whether dicamba or triclopyr triple mix will work or not. Both low and high rates of glyphosate have been shown to give excellent control of rushes when applied using a rotary weed wiper (Martin et al., 1990).

The high rate of glyphosate resulted in a significantly lower overall dry weight of plantain than the low rate of glyphosate. The two application rates of glyphosate were assessed as high rates of glyphosate appear on recommendations on many labels, which are a carry-over from the days when wick-boom applicators were used that wipe much less herbicides on each weed. Moyo et al. (2022) found that lower rates of glyphosate (1:20) gave good results for controlling Californian thistle, despite many labels saying to use 1:2 concentrations. Very low rates of glyphosate applied using spray application have been found to be ineffective against both white clover and perennial ryegrass and result in improved nutritional characteristics of pasture due to increasing the clover content (Casey et al., 2000). With wiper application of herbicides to Californian thistle in a field experiment conducted by Moyo (2008), metsulfuron and triclopyr/picloram were highly damaging to the pasture following subsequent rainfall resulting in the removal of clovers and suppression of ryegrass while the effect of glyphosate was minimal.

The results from Experiments 1 and 2 suggested that the effect of most of the herbicides on plantain was similar to the untreated control except for the high rate of glyphosate when no rainfall was provided. Dicamba had the least effect following rainfall in the first experiment, while triclopyr/picloram/aminopyralid had the least effect on plantain growth in the second experiment. Although the effects of the herbicides like dicamba and glyphosate/metsulfuron mix were sufficient to kill some plants in the second experiment, those that didn’t die managed to recover. This plant mortality only happened in one of the two experiments, and the reason for the differences between experiments is unknown. The herbicide treatments that appeared consistently least damaging to plantain following rainfall across the two experiments were aminopyralid and the low rate of glyphosate.

The possible movement of herbicide from plants treated using wipers after rainfall to non-target plants has been outlined in previous studies (Campbell & Nicol, 1998). Harrington et al. (2016)

found that clopyralid, metsulfuron and triclopyr/picloram caused significant levels of damage to white clover plants when placed under Californian thistle and artificial rain was applied 1 day after herbicide application. Under normal field conditions, the rainfall may occur much sooner than 24 hours after application and can be heavier than 2 mm, so some of these herbicides could affect both plantain and clover if herbicide has been applied to weeds and plantain seedheads.

Moyo et al. (2016) found that the amount of herbicide applied to weeds varies according to the type of wiper used. Some wipers apply more herbicide than other wipers. Spectrophotometry was used to measure herbicide output which allowed a comparison of the performance of three different wiper applicators: Rotowiper, C-Dax Eliminator and WeedSwiper. In the Rotowiper, the herbicide is dripped onto a rotating roller covered in the carpet; the C-Dax Eliminator has a series of parallel wiping pads arranged at a 45-degree angle to the direction of travel mounted on a steel frame; and the WeedSwiper has a single wiping pad on a folding horizontal beam and has a moisture sensor in the wiping pads to control the rate of herbicide deposition on the pads. The WeedSwiper produced a more uniform output of herbicide than the other two for which the operator had to rewet the pads manually, which caused the variable moisture level (Moyo et al., 2016). However, the WeedSwiper wiped less herbicide overall to weeds than the other two applicators. This variability in output makes it difficult to give precise recommendations on herbicide concentrations required to control weeds.

The relative safety to plantain/clover swards and efficacy to weeds of treatments assessed in these glasshouse experiments were supposed to be assessed in the field, but there was no time left for a field assessment. Therefore, we can only speculate on whether these herbicides are as safe in the field as they were in the glasshouse under a controlled environment.

7.5 Conclusion

Although it appears that herbicide damage can occur due to wiping herbicide on the seed-heads of plantain, especially when the herbicides get washed off by simulated rainfall, observations from these experiments suggested that most of these herbicides showed good potential to be used for weed wiping in older swards after grazing despite plantain seed-heads being present. However, a high rate of glyphosate and the glyphosate/metsulfuron mix caused the greatest damage to the plantain while aminopyralid and a low rate of glyphosate had the least effect on the plantain growth. Despite that rainfall within 24 hours after herbicide wiping might be the reason for an effect on plantain, a huge knowledge gap still exists on the amount of rainfall that is likely to cause any damage. Over-application of herbicides should be avoided as higher rates are more damaging and for some herbicides, can increase residual effects of herbicides on soil and causes difficulty to re-establish the clover. These experiments show that applying herbicides by wiping could have a place in selective removal of tall weeds which grow alongside plantain. However, it will be necessary to test it in the field to formulate a practical and economic technique that will eliminate tall weeds through a wiping process. Thus, a well-designed trial should be conducted in the future to focus on use of weed wipers in the field including tall weeds along with plantain, ryegrass and clover in the paddock.

Chapter 8

General discussion and conclusions

8.1 Introduction

The traditional pastoral system of New Zealand is based predominantly on perennial ryegrass with white clover which can result in feed deficits throughout summer and autumn when dry. Thus, plantain is mixed with these pasture which provides greater herbage quality and yield throughout the year. It also helps with reducing nitrate leaching from pastures. When adding extra species to a grass/clover sward, it becomes more difficult to control weeds within these swards. Herbicides play an important role in weed management systems but less herbicide options are currently available for plantain mixed swards compared with simpler forage or legume swards (Lockley & Wu, 2008). Thus, the main objective of this thesis was to improve weed control options for ryegrass/clover pastures in New Zealand that contain plantain. This included determining the tolerance of pasture species to different herbicides and identifying the herbicide options most effective for weeds. The results from experiments discussed in each chapter in this thesis provided further understanding of some aspects of tolerance to phenoxy herbicides by plantain in New Zealand. In this chapter, the major findings will be discussed, including implications of the results, and priorities for future research are suggested.

8.2 Tolerance of Agritonic compared to Tonic to phenoxy herbicides

2,4-D-resistant plantain was first reported from the USA in cemetery turf where this herbicide had been used continuously for 30 years (Patton et al., 2018), but has never been reported in New Zealand. The authors noted that the resistant biotype was 6.2 times more resistant to 2,4-D compared with the susceptible one, while it was still susceptible to triclopyr. In another 2,4-D-resistant biotype of plantain in USA, Russell et al.(2021) recorded 4-fold resistance, which was slightly lower than that reported by Patton et al. (2018). In this thesis, phenoxy herbicide tolerance that has been bred into the Agritonic cultivar of plantain was evaluated by comparing it with the Tonic cultivar, which provided a better understanding of the level of tolerance to phenoxy herbicides like MCPB, MCPA, MCPB/MCPA mix, 2,4-D and 2,4-DB in Agritonic plantain. In this research, a lower level of tolerance was recorded to phenoxy herbicides than reported from USA, which varied from 1.33 to 3.45-fold, depending on the phenoxy herbicides. All plants treated with these phenoxy herbicides were hit hard at the start, but those treated with the recommended rates of MCPB and MCPB/MCPA recovered very well by the end of experiments. Some plants even survived higher rates of these two herbicides that were evaluated.

The results from dose-response experiments showed that Tonic plantain could also survive the recommended rates of MCPB and the MCPB/MCPA mixture, but were more damaged than Agritonic. Some of the Agritonic plantain also survived the recommended rates of MCPA, 2,4-DB and 2,4-D, however Tonic plantain did not survive these treatments. The half rates of MCPA, 2,4-DB and 2,4-D amine were enough to kill the Tonic plants. Therefore, these herbicides would need to be used at a very low rate (i.e. below half recommended rate) to control weeds if used in a field that contains plantain. Also, the low rates of MCPB/MCPA mix may be enough to kill weeds, which was studied in the field trials later, but generally such low rates are unlikely to control many weed species.

8.3 Weed control in plantain-based pastures

In Chapter 3, Agritonic plantain was found to be tolerant to some selective herbicides under glasshouse conditions when grown alone. Thus, field experiments were conducted to assess how tolerant this plantain was to a variety of herbicides relative to clover and weeds (Chapters 4 and 5). The main objectives of these experiments were to determine the tolerance of plantain, white clover and perennial ryegrass to a range of herbicides applied to a mixed sward of these species at an early stage of establishment in spring. The effectiveness of the herbicides was also assessed for controlling weeds that establish within the swards.

These two field studies showed that Agritonic was more tolerant than Tonic plantain to phenoxy herbicides, though Tonic recovered by the end of the experiments. The paraquat/diquat treatment should be suitable to use in pastures with just plantain and clover, and diuron could be used with just plantain and ryegrass. Flumetsulam was safe to use in the plantain-based pasture though it suppressed plantain initially, but this recovered by the end of the trials. Bentazone appeared to be safe for the plantain but its effect was probably reduced by rainfall after application in the first field experiment. When there was no rainfall in the same day of herbicide application in the second field experiment, it suppressed most of the weeds both when used alone and in combination with other herbicides, without damaging plantain, clover or ryegrass. The half rate of MCPB was less damaging to both cultivars of plantain than the recommended rates of MCPB and MCPB/MCPA, but could not control most of the weeds present. Both Agritonic and Tonic plantain when sprayed with the MCPB/MCPA mix was similarly affected as by MCPB but the mixture controlled weeds better than MCPB, and the recommended rate of MCPB/MCPA had very little detrimental effect on young clover (Figure 4.8). The mixture of flumetsulam + bentazone + half MCPB had least effect on total dry weight of plantain, ryegrass and effectively controlled weeds while flumetsulam, bentazone and recommended rate of MCPB were safe on clover in the first field experiment. The most effective weed control strategy found in the second field experiment for establishing swards of plantain, ryegrass and white clover was to apply the combination of bentazone + half MCPB/MCPA which gave lasting effects on weeds including docks.

Mowing after each harvest helped to control the upright weeds like redroot, black nightshade and fathen and also gave temporary suppression to the docks. Summer annual weeds like redroot, fathen and black nightshade also died naturally in autumn. The effects of mowing and time of year were similar to findings of Gawn et al. (2012). The plantain and clover needed to be established well enough to tolerate the herbicides, yet the weeds needed to be as young as possible to be susceptible to herbicides such as bentazone, MCPB and flumetsulam (Gawn et al., 2012; Holden, 2022).

8.4 Mechanism of 2,4-D tolerance in Agritonic plantain

In Chapter 3, Agritonic plantain showed a small level of tolerance (1.33 to 3.45-fold) to phenoxy herbicides compared to Tonic plantain, which suggested the herbicide behaves differently within Agritonic than Tonic plants. The mechanism involved in this tolerance of Agritonic was unknown before this project. Therefore, the main objective of the experiments reported in Chapter 6 was to investigate the mechanism of tolerance in Agritonic plantain to 2,4-D using radiolabeled herbicide (^{14}C-2,4-D).

In these studies, the possible mechanisms of 2,4-D tolerance were investigated based on previously published work overseas and in New Zealand, namely non-target-site-resistance (NTSR) which included absorption, translocation and metabolism. The results from this experiment showed that 2,4-D absorption was slightly less in the tolerant plantain (Agritonic) than the susceptible plantain (Tonic), and the amount of herbicide remaining in the treated leaf of Agritonic was greater than for Tonic at all harvests. Similarly, different patterns of ^{14}C-2,4-D translocation were observed between the 2,4-D tolerant and susceptible plantain populations. Agritonic had more ^{14}C-2,4-D retained in the treated leaf, and less ^{14}C-2,4-D was translocated to the stem and root. Thus, less 2,4-D accumulation in the meristematic region in Agritonic than in Tonic plants could be a possible explanation for differences in tolerance. The HPLC analysis found no evidence of differences in 2,4-D metabolism between tolerant and susceptible plantain harvested at 72 and 120 hours after treatment. Thus, enhanced metabolism appears not to be associated with the tolerance mechanism. Therefore, the tolerance to 2,4-D in Agritonic plantain appeared to be based on the reduced translocation when comparing the 2,4-D-tolerant Agritonic plants with the susceptible Tonic plants, even though the level of tolerance was small, and reduced absorption may have also contributed.

8.5 Herbicide susceptibility of plantain when wiped on to seed-heads

The earlier chapters suggested that Agritonic plantain can tolerate some selective herbicides when applied to young plantain and also young weeds. These selective herbicides become less effective when weeds are well-established. Thistles and docks are some of the major tall weeds found in well-established plantain pastures, which were hoped could be controlled by wiping experiment. Information is scarce on the effectiveness of weed wipers for controlling taller weeds in pasture and their effect on pastures, especially with respect to potential damage of plantain which might have some herbicide wiped on to their upright seed-heads. In Chapter 7, glasshouse experiments were conducted to test the tolerance of mature plantain plants (both Agritonic and Tonic) to application of some herbicides suitable for use in weed wipers (glyphosate, clopyralid, aminopyralid, dicamba and triclopyr) to the seed-heads simulating potential contact during weed wiping of pasture weeds.

Some herbicides like dicamba and aminopyralid are safe for plantain but they cannot be boom sprayed as they are not safe for clover. Dicamba had the least effect following rainfall in the first experiment while triclopyr/picloram/aminopyralid had the least effect on the plantain growth in the second experiment. The herbicide treatments that appeared consistently least damaging to plantain following rainfall across the two experiments were aminopyralid and a low rate of glyphosate. However, a high rate of glyphosate and the glyphosate/metsulfuron mix caused a greater level of damage to the plantain.

8.6 Steps to develop strategies to improve weed control options for ryegrass/clover pastures that contain plantain

The first step in developing strategies to improve weed control options is to identify the problem and provide information regarding it. Farmers should be informed about the tolerance of selective herbicides by the plantain and how they can adopt them. The second step is to find the solutions to manage the problem and develop the strategies for controlling the weeds in the pasture. For this, biochemical, molecular and physiological characteristics of herbicide tolerance should be investigated. After developing the management strategies, it is crucial to inform the farmers through field days, meetings and seminars.

8.6.1 Identifying the problem

This PhD study was initiated because better weed control options are required for ryegrass/clover pasture containing plantain. Following consultation with farmers and researchers working with pastures containing plantain, weeds such as redroot, docks and thistles were identified as particular problems in these pastures. Although it is possible to selectively control these weeds when plantain is grown alone, it becomes difficult when plantain is grown together with ryegrass and clover.

8.6.2 Possible strategies for controlling weeds in mixed pastures containing plantain

Strategies for controlling weeds should consist of both chemical and non-chemical methods. Herbicides are commonly used for weed control due to their effectiveness. Farmers growing mixed swards containing plantain would be particularly interested in knowing the best rates of application of herbicides to give good weed control with minimal damage to the plantain, and also whether the difference between the cultivars is large enough to make it worth using Agritonic rather than Tonic (Figure 3.3). MCPB and MCPB/MCPA appear to be the most promising phenoxy herbicide treatments with respect to tolerance by Agritonic overall while giving good weed control. The tolerance in Agritonic plantain was very variable however, partly due to how seeds are produced from bred plants (i.e. cross-pollinated by susceptible plants), so there is potential to breed for higher levels of tolerance. Plantain is self-sterile, cross fertilized and pollinated by wind (Bond et al., 2007; Warwick & Briggs, 1979), which increases the chances of being pollinated by a susceptible plant. Thus, plant breeders can work more on how to get rid of the variability in Agritonic and make it more tolerant.

There was only a small difference between the Agritonic and Tonic cultivars in the tolerance of phenoxy herbicides, but sufficient to give reduced damage in Agritonic when using MCPB. Also, there was much variability between Agritonic plants as some were tolerant and some were not, so traits that were selected for when creating this cultivar appear not to be uniformly present in all plants. As a result, this variability in the plants has probably masked some differences in physiology when investigating the mechanism of tolerance.

Getting timing correct with selective herbicide applications is important as it can affect the efficiency of selective herbicides such as bentazone. The plantain and clover should be established well enough to tolerate the herbicides yet the weeds need to be as young as possible to be susceptible to herbicides such as bentazone and MCPB (Holden, 2022; Gawn et.al., 2012). Most of the herbicides are effective when the weeds are 3-4 leaf stage. Some of the weeds in the field trials got older than that before being sprayed, and the oldest ones did not die. Flumetsulam was fairly safe to use in the plantain-based pasture though it suppressed plantain initially but did not add much in controlling the weeds found in this experiment. Bentazone was safe to use without damaging plantain and ryegrass and supressed most of the weeds alone and in with combination of other herbicides when there was no rainfall on the same day of herbicide application. The safest and most effective weed control strategy in establishing swards of plantain, ryegrass and white clover was a combination of bentazone and half MCPB/MCPA followed by mowing for a lasting effect on weeds.

Most of the work in this thesis looked at herbicide use for seedling weeds in new swards. The herbicide wiping experiment showed that applying herbicides by wiping could have a place in selective removal of tall weeds which grow alongside plantain in more established swards. However, it will be necessary to test it in the field to formulate a practical and economic technique that will eliminate tall weeds through a wiping process. Thus, a well-designed trial should be conducted in future to focus on use of weed wipers in the field for tall weeds growing among plantain, ryegrass and clover in the paddock.

References

Agricom. (2018). Seed guide Christchurch, New Zealand.Retrieved on March 2020.

Aper, J., Mechant, E., Rubin, B., Heyerick, A., Callebaut, G., Mangelinckx, S., . . . Reheul, D. (2012). Absorption, translocation and metabolism of metamitron in *Chenopodium album*. *Pest Management Science*, *68*(2), 209-216. doi:10.1002/ps.2246.

Ash, G. J. (2010). The science, art and business of successful bioherbicides. *Biological Control*, *52*(3), 230-240. doi: :https://doi.org/10.1016/j.biocontrol.2009.08.007

Bond, W., Davies, G., & Turner, R. (2007). The biology and non- chemical control of Ribwort Plantain (*Plantago lanceolata* L.). *Garden Organic*, 1-8. Retrieved from http;//www.gardenorganic.org.uk/organicweeds

Bourdôt, G. W., Fowler, S. V., Edwards, G. R., Kriticos, D. J., Kean, J. M., Rahman, A., & Parsons, A. J. (2007). Pastoral weeds in New Zealand: Status and potential solutions. *New Zealand Journal of Agricultural Research, 50*(2), 139-161. doi:10.1080/00288230709510288

Bourdot, G. W., & Hurrel, G. A. (1988). Differential tolerance of MCPA among giant buttercup (*Ranunculus acris*) biotypes in Takaka, Golden Bay. *New Zealand Plant Protection Society, 41*, 231-234.

Bourdôt, G. W., & Lamoureaux, S. (2002). Giant buttercup (*Ranunculus acris* L.) management in dairy pastures—Current problems and future solutions. *Proceedings of the New Zealand Grassland Association, 64*, 61-65.

Bourdot, G. W., & Saville, D. J. (2002). Estimating economic losses due to pasture weeds. *New Zealand Plant Protection Society, 55*, 106-110.

Bowcher, A. J. (2002). *Competition between temperate perennial pasture species and annual weeds: the effect of pasture management on population dynamics and resource use.* (Doctor of Philosophy). Charles sturt University

Box, L. A., Edwards, G. R., & Bryant, R. H. (2016). Milk production and urinary nitrogen excretion of dairy cows grazing perennial ryegrass-white clover and pure plantain pastures. *Proceedings of the New Zealand Society of Animal Production, 76*, 18-21.

Box, L. A., & Judson, A. G. (2018). The concentration of bioactive compounds in *Plantago lanceolata* is genotype specific. *Journal of New Zealand Grasslands, 80*, 113- 118.

Bradley, K. W., Hagood, J. E. S., Love, K. P., & Heidel, R. D. (2004). Response of Biennial and Perennial Weeds to Selected Herbicides and Prepackaged Herbicide Combinations in Grass Pastures and Hay Fields. *Weed Technology, 18*(3), 795-800.

Bryant, R. H., Dodd, M. B., Moorhead, A. J. E., Edwards, P., & Pinxterhuis, I. J. B. (2019). Effectiveness of strategies used to establish plantain in existing pastures. *Journal of New Zealand Grasslands, 81*, 131-138. Retrieved from https://www.nzgajournal.org.nz/index.php/JoNZG/article/view/406/63

Bukun, B., Shaner, D. L., Nissen, S. J., Westra, P., & Brunk, G. (2010). Comparison of the Interactions of Aminopyralid vs. Clopyralid with Soil. *Weed Science, 58*(4), 473-477. Retrieved from www.jstor.org/stable/40891264

Campbell, M. H., & Nicol, H. I. (1998). Effects of wiping herbiicdes on serrated tussock (*Nassella trichotoma* (Nees) Arech.) and African lovegrass (*Eragrostis curvula* (Shrad.) Nees). *Plant protection Quarterly, 13*(1), 36-38.

Carlton, A. J., Cameron, K. C., Di, H. J., Edwards, G. R., & Clough, T. J. (2019). Nitrate leaching losses are lower from ryegrass/white clover forages containing plantain than from ryegrass/white clover forages under different irrigation. *New Zealand Journal of Agricultural Research, 62*(2), 150-172. doi:10.1080/00288233.2018.1461659

Casey, M., Brown, C., & Stevens, D. (2000). A summary of research into the use of low rates of glyphosate as a pasture management tool. *Proceedings of the New Zealand Grassland Association, 62*, 123-128. doi:10.33584/jnzg.2000.62.2384

CAST. (1975). The Phenoxy Herbicides. *Weed Science, 23*(3), 253-263. Retrieved from www.jstor.org/stable/4042283

Cavers, P. B., Bassett, I. J., & Crompton, C. W. (1980). The Biology Of Canadian WeedS.: 47. *Plantago lanceolata* L. *Canadian Journal of Plant Science, 60*(4), 1269-1282. doi:10.4141/cjps80-180

Chalak-Haghighi, M., Van Ierland, E. C., Bourdôt, G. W., & Leathwick, D. (2008). Management strategies for an invasive weed: A dynamic programming approach for

Californian thistle in New Zealand. *New Zealand Journal of Agricultural Research, 51*(4), 409-424. doi:10.1080/00288230809510471

Champion, P., James, T. K., Popay, A. I., & Ford, K. (2012). *An Illustrated Guide to Common Grasses, Sedges and Rushes of New Zealand*. Christchurch, New Zealand: New Zealand Plant Protection Society.

Charlton, J. F. L., & Stewart, A. V. (1999). *Pasture species and cultivars used in New Zealand – a list.* Paper presented at the *Proceedings of the New Zealand Grassland Association* Hawkes Bay.

Christian, R., & Jens, C. S. (2005). Bioassay Analysis using R. *Journal of statistical software, 12*(5). doi:http://dx.doi.org/10.18637/jss.v012.i05

Cobb, A. H., & Reade, R. H. (2010). The Inhibition of Amino Acid Biosynthesis. In *Herbicides and Plant Physiology, Second Edition* (pp. 176-199). UK: John Wiley and Sons Ltd.

Cousens, R. D. (2020). A question of logic: experiments cannot prove lack of an herbicide-resistance fitness penalty. *Weed Science*, *68*(3), 197-198.

Cranston, H. J., Kern, A. J., Josette, L. H., Erica, K. M., Maxwell, B. D., & Dyer, W. E. (2001). Dicamba Resistance in Kochia. *Weed Science, 49*(2), 164-170. Retrieved from http://www.jstor.org/stable/4046498

Cranston, L. M. (2014). *Chicory (Cichorium intybus) and plantain (Plantago lanceolata); physiological and morphological responses to water stress, defoliation, and grazing preference with implications for the management of the Herb and Legume Mix : a thesis presented in partial fulfilment of the requirements for the degree of* Doctor of Philosophy in Plant Science*, Massey University, Te Kunenga ki Pūrehuroa, New Zealand.* (Thesis). Massey University, Retrieved from http://hdl.handle.net/10179/6002 Available from EBSCOhost ir00033a database.

Curran, W. S., & Lingenfelter, D. (2001). *Weed Management in Pasture Systems*. USA: The Pennsylvania State University

DairyNZ. (n.d). Plantain. Retrieved from https://www.dairynz.co.nz/feed/crops/plantain/#

DairyNZ. (October 2013a). *Plantain establishment*. Retrieved from https://www.dairynz.co.nz/media/253815/1-78a_Plantain_establishment.pdf

DairyNZ. (October 2013b). *Plantain management* Retrieved from https://www.dairynz.co.nz/media/253818/1-78b_Plantain_management.pdf

Dang, H. T., Malone, J. M., Boutsalis, P., Krishnan, M., Gill, G., & Preston, C. (2018). Reduced translocation in 2,4-D-resistant oriental mustard populations (*Sisymbrium orientale* L.) from Australia. *Pest Management Science, 74*(6), 1524-1532. doi:10.1002/ps.4845

Department of Primary Industries/ NSW Government. (n.d.). Narrow leaf plantain. Retrieved from https://www.dpi.nsw.gov.au/agriculture/pastures-and-rangelands/species-varieties/pf/factsheets/narrow-leaf-plantain-396

Derrick, R. W., Moseley, G., & Wilman, D. (1993). Intake, by sheep, and digestibility of chickweed, dandelion, dock, ribwort and spurrey, compared with perennial ryegrass. *The Journal of Agricultural Science, 120*(1), 51-61. doi:10.1017/S0021859600073585

Derting, C. W. (1987). Wiper application. In C. G. McWhorter & M. R. Gebhardt (Eds.), *Methods of Applying Herbicides* (pp. 207-227). Illinois, USA: *Weed Science Society of America.*

DiTomaso, J. M., Kyser, G. B., Miller, J. R., Garcia, S., Smith, R. F., Nader, G., . . . Orloff, S. B. (2006). Integrating prescribed burning and clopyralid for the management of yellow starthistle (*Centaurea solstitialis*). *Weed Science, 54*, 757–767.

Dodd, M., Dalley, D., Wims, C., Elliott, D., &Griffin, A. (2019). A comparison of temperate pasture species mixtures selected to increase dairy production and reduce urinary nitrogen excretion. *New Zealand Journal of Agricultural Research, 62*(4), 504-527.

Donaldson, S., & Bowers, G. (1998). Weed Identification and control guide. *University of Nevada Cooperative Extension*, 1-32.

Dowling, P. M., Michalk, D. L., & Sindel, B. M. (2000). Weed Management in Pasture Systems. In RG and FJ Richardson (Ed.), *Australian Weed Management Systems* (pp. 307-328). Melbourne, Australia.

Duke, S. O., & Powles, S. B. (2009). Glyphosate-resistant crops and weeds: Now and in the future. *AgBioForum, 12*(3-4), 346-357. Retrieved from http://ezproxy.massey.ac.nz/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=edselc&AN=edselc.2-52.0-77954361485&site=eds-live&scope=site

Eerens, J. P. J., Rahman, A., & James, T. K. (2002). Optimising pasture production to minimise weed growth. *Proceedings of the New Zealand Grassland Association, 64*, 143-146.

Farmers Weekly. (21st April, 2016). Weeding out the problem in plantain and clover. *Farmers Weekly*. Retrieved from https://farmersweekly.co.nz/topic/crop-and-stock-management/weeds/weeding-out-the-problem-in-plantain-and-clover

Figueiredo, M. R. A., Leibhart, L. J., Reicher, Z. J., Tranel, P. J., Nissen, S. J., Westra, P., . . . Jugulam, M. (2018). Metabolism of 2,4-dichlorophenoxyacetic acid contributes to resistance in a common waterhemp (*Amaranthus tuberculatus*) population. *Pest Management Science, 74*(10), 2356-2362. doi:https://doi.org/10.1002/ps.4811

Figueiredo, M. R. A. d., Barnes, H., Boot, C., Figueiredo, A. d., Nissen, S., Dayan, F., & Gaines, T. (2022). Identification of a Novel 2,4- D Metabolic Detoxification Pathway in 2,4- D -Resistant Waterhemp (*Amaranthus tuberculatus*). *Journal of Agricultural and Food Chemistry, 70*. doi:10.1021/acs.jafc.2c05908

Figueiredo, M. R. A. d., Küpper, A., Malone, J. M., Petrovic, T., Figueiredo, A. B. T. B. d., Campagnola, G., . . . Gaines, T. A. (2022). An in-frame deletion mutation in the degron tail of auxin coreceptor IAA2 confers resistance to the herbicide 2,4-D in Sisymbrium orientale. *Proceedings of the National Academy of Sciences of the United States of America, 119*(9), e2105819119. doi:10.1073/pnas.2105819119

Fowler, S. V., Gourlay, A. H., & Hill, R. (2016). Biological control of ragwort in the New Zealand dairy sector: an ex-post economic analysis. *New Zealand Journal of Agricultural Research, 59*(3), 205-215. doi:10.1080/00288233.2016.1170050

Franz, J. E., Mao, M. K., & Sikorski, J. A. (1997). *Glyphosate : a unique global herbicide*: American Chemical Society.

Fraser, T. J., & Rowarth, J. S. (1996). Legumes, herbs or grass for lamb performance? *Proceeding of the New Zealand Grassland Association, 58*, 49-52.

Fraser, T. J., Stevens, D. R., Scholfield, R. W., Nelson, B. J., Nelson, A. J., & Shortland, S. M. (2016). Improved forages to enhance hill country sheep production. *Grassland Research and Practice Series, 16*, 225-232.

Freund, D. M., & Hegeman, A. D. (2017). Recent advances in stable isotope-enabled mass spectrometry-based plant metabolomics. *Current Opinion in Biotechnology, 43*, 41-48. doi:10.1016/j.copbio.2016.08.002

Gabruck, D. T., Bork, E. W., Hall, L. M., King, J. R., & Hare, D. D. (2013). Interspecific relationships between white Clover, Kentucky bluegrass, and Canada thistle during establishment. *Agronomy Journal, 105*, 1467-1474.

Gawn, T. L., Harrington, K. C., & Matthew, C. (2012). Weed control in establishing mixed swards of clover, plantain and chicory. *New Zealand Plant Protection, 65*, 59-63.

Gerard, P., Schwendel, B. H., Fraser, K., & Eden, T. (2018). Effect of narrow-leaved plantain cultivar on development of two geometrid pests, *Scopula rubraria* and *Epyaxa rosearia*. *New Zealand Journal of Agricultural Research, 61*(4), 403-413. doi:10.1080/00288233.2017.1398763

Ghanizadeh, H., & Harrington, K. C. (2017b). Cross-resistance to auxinic herbicides in dicamba-resistant *Chenopodium album*. *New Zealand Journal of Agricultural Research, 60*(1), 45-53. doi:10.1080/00288233.2016.1238397

Ghanizadeh, H., & Harrington, K. C. (2017c). Non-target Site Mechanisms of Resistance to Herbicides. *Critical Reviews in Plant Sciences, 36*(1), 24-34. doi:10.1080/07352689.2017.1316134

Ghanizadeh, H., & Harrington, K. C. (2019a). Herbicide resistant weeds in New Zealand: state of knowledge. *New Zealand Journal of Agricultural Research*, 12. doi:10.1080/00288233.2019.1705863

Ghanizadeh, H., & Harrington, K. C. (2019b). Weed Management in New Zealand Pastures. *Agronomy, 9*(8), 448. Retrieved from https://www.mdpi.com/2073-4395/9/8/448

Ghanizadeh, H., & Harrington, K. C. (2020). Perspective: root exudation of herbicides as a novel mode of herbicide resistance in weeds. *Pest Management Science, 76*, 2543-2547. doi:https://onlinelibrary.wiley.com/doi/epdf/10.1002/ps.5850

Ghanizadeh, H., Harrington, K. C., & James, T. K. (2018). A comparison of dicamba absorption, translocation and metabolism in *Chenopodium album* populations resistant and susceptible to dicamba. *Crop Protection, 110*, 112-116. doi:https://doi.org/10.1016/j.cropro.2018.04.007

Ghanizadeh, H., Harrington, K. C., James, T. K., & Woolley, D. J. (2015). Quick tests for detecting glyphosate-resistant Italian and perennial ryegrass. *New Zealand Journal of Agricultural Research, 58*(2), 108-120. doi:10.1080/00288233.2014.981344

Ghanizadeh, H., Harrington, K. C., James, T. K., Woolley, D. J., & Ellison, N. W. (2016). Restricted herbicide translocation was found in two glyphosate-resistant italian ryegrass (*Lolium multiflorum* lam.) populations from New Zealand. *Journal of Agricultural Science And Technology, 18*(4), 1041-1051. Retrieved from https://www.sid.ir/en/journal/ViewPaper.aspx?ID=538421

Glassey, C. B., Clark, C. E. F., Roach, C. G., & Lee, J. M. (2012). Herbicide application and direct drilling improves establishment and yield of chicory and plantain. *Grass and Forage Science, 68*(1), 178-185. doi:10.1111/j.1365-2494.2012.00885.x

Goggin, D. E., Cawthray, G. R., & Powles, S. B. (2016). 2,4-D resistance in wild radish: reduced herbicide translocation via inhibition of cellular transport. *Journal of Experimental Botany, 67*(11), 3223-3235. doi:10.1093/jxb/erw120

Golding, K. P., Wilson, E. D., Kemp, P. D., Pain, S. J., Kenyon, P. R., Morris, S. T., & Hutton, P. G. (2011). Mixed herb and legume pasture improves the growth of lambs post-weaning. *Animal Production Science, 51*(8), 717-723. doi:https://doi.org/10.1071/AN11027

Grekul, C. W., Cole, D. E., & Bork, E. W. (2005). Canada thistle (*Cirsium arvense*) and pasture forage responses to wiping with various herbicides. *Weed Technology, 19*, 298-306. Retrieved from http://ezproxy.massey.ac.nz/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=edswsc&AN=000229922600013&site=eds-live&scope=site

Haggar, R. J., Oswald, A. K., & Richardson, W. G. (1986). A review of the impact and control of creeping thistle (*Cirsium arvense* L.) in grassland. *Crop Protection, 5*(1), 73-76. doi:https://doi.org/10.1016/0261-2194(86)90040-2

Hamburg, A., Puvanesarajah, V., Burnett, T. J., Barnekow, D. E., Premkumar, N. D., & Smith, G. A. (2001). Comparative degradation of [14C]-2,4-dichlorophenoxyacetic acid in wheat and potato after Foliar application and in wheat, radish, lettuce, and apple after soil application. *Journal of Agricultural and Food Chemistry, 49*(1), 146-155. doi:10.1021/jf000120q

Harker, K. N., Baron, V. S., Chanasyk, D. S., Naeth, M. A., & Stevenson, F. C. (2000). Grazing intensity effects on weed populations in annual and perennial pasture systems. *Weed Science, 48*(2), 231-238. doi:10.1614/0043-1745(2000)048[0231:GIEOWP]2.0.CO;2

Harrington, K. C. (2018). *Controlling Weeds- Administrating Guide*. Palmerston North, New Zealand: Massey University.

Harrington, K. C. (2023). New Zealand Weeds database. Retrieved on November 2022 from https://www.massey.ac.nz/about/colleges-schools-and-institutes/college-of-sciences/our-research/themes-and-research-strengths/plant-science-research/new-zealand-weeds-database/

Harrington, K. C., & Ghanizadeh, H. (2017). Herbicide application using wiper applicators - A review. *Crop Protection, 102*, 56-62. doi:10.1016/j.cropro.2017.08.009

Harrington, K. C., Ghanizadeh, H., Moyo, C., Kemp, P., & Eerens, H. (2016). Are root exudation or rainfall on treated plants likely causes of pasture damage after wiper application of herbicides? *Proceedings of the 20th Australasian Weeds Conference, 20*, 72-75.

Harrington, K. C., Ghanizadeh, H., Moyo, C., Kemp, P. D., & Eerens, J. P. J. (2017). Pasture damage from spot-sprayed herbicides. *New Zealand Plant Protection, 70*, 1-7. doi:10.30843/nzpp.2017.70.47

Harrington, K. C., Hood, M. Z., & McKinnon, K. C. (2000). Assessment of two herbicide wiping devices. *New Zealand Plant Protection, 53*(0). doi:10.30843/nzpp.2000.53.3707

Harrington, K. C., Kemp, P. D., Horne, D. J., & He, X. Z. (2013). Weed population dynamics for contrasting organic pasture establishment techniques. *New Zealand Plant Protection, 66*, 110-117.

Harrington, K. C., Thatcher, A., & Kemp, P. D. (2006). Mineral composition and nutritive value of some common pasture weeds. *New Zealand Plant Protection, 59*, 261-265.

Harrington, K. C., & Woolley, D. J. (2006). Investigations of how phenoxy-resistant *Carduus nutans* biotypes survive herbicide spraying. *New Zealand Journal of Agricultural Research, 49*(4), 465-474. doi:10.1080/00288233.2006.9513737

Harrington, K. C., Ghanizadeh, H., Moyo, C., Kemp, P. D., & Eerens, J. P. J. (2017). Pasture damage from spot-sprayed herbicides. *New Zealand Plant Protection*, *70,* 1-7.

Harrison, H. F., Jr. (1983). Hoeing and hand-held wiper application of glyphosate for weed control in vegetables. *Horticultural Science, 18*, 333-334.

Heap, I. (2022). The International Survey of Herbicide Resistant Weeds. Retrieved from www.weedscience.com. Available from Internet Retrieved 23 January, 2022 www.weedscience.com

Heap, I., & Morrison, I. N. (2002). Resistance to auxin-type herbicides in wild mustard (*Sinapis arvensis* L.) populations in western Canada. *Weed Science Society of America, Abstr. , 32*, 55.

Hill Laboratories. (2022). technical notes in Soil analysis and sampling. Retrieved on November 2022 from https://www.hill-laboratories.com/client-resources-2/technical-notes/

Hoagland, D. R., & Arnon, D. I. (1950). The water-culture method for growing plants without soil (Publication no. https://archive.org/details/waterculturememe3450hoag). from Berkeley, California: College of Agriculture, University of California, California Agricultural Experimental Station

Holden, P. (2022). *New Zealand Novachem Agrichemical Manual*. doi:https://www.novachem.co.nz/Home.aspx

Hutton, P. G., Kenyon, P. R., Bedi, M. K., Kemp, P. D., Stafford, K. J., West, D. M., & Morris, S. T. (2011). A herb and legume sward mix increased ewe milk production and ewe and lamb live weight gain to weaning compared to a ryegrass dominant sward. *Animal Feed Science and Technology, 164*(1), 1-7. doi:https://doi.org/10.1016/j.anifeedsci.2010.11.014

Johnson, J. (July 2011). Weed wiper technology and usage. *The Samuel Roberts Noble Foundation,* 1-8.

Judson, H. G., Fraser, P. M., & Peterson, M. E. (2019). Nitrification inhibition by urine from cattle consuming *Plantago lanceolata*. *Journal of New Zealand Grasslands, 81*, 110-116. Retrieved from https://www.nzgajournal.org.nz/index.php/JoNZG/article/view/413/60

Jugulam, M., DiMeo, N., Veldhuis, L. J., Walsh, M., & Hall, J. C. (2013). Investigation of MCPA (4-Chloro-2-ethylphenoxyacetate) Resistance in Wild Radish (*Raphanus raphanistrum* L.). *Journal of Agricultural and Food Chemistry, 61*(51), 12516-12521. doi:10.1021/jf404095h

Kay, S. H. (1995). Efficacy of wipe-on applications of glyphosate and imazapyr on common reed in aquatic sites. *Journal of Aquatic Plant Management, 33*, 25-26. Retrieved from https://www.scopus.com/inward/record.uri?eid=2-s2.0-0029137893&partnerID=40&md5=470031efd6ad4d9f2821c29717c82a4b

Kemp, P., Matthew, C., & Lucas, R. (1999). Pasture species and cultivars. In J. White & J. Hodgson (Eds.), *New Zealand pasture and crop science* (pp. 83-99). Auckland,New Zealand: Oxford University Press.

Kenyon, P. R., Morel, P. C. H., Corner-Thomas, R. A., Perez, H. L., Somasiri, S. C., Kemp, P. D., & Morris, S. T. (2017). Improved per hectare production in a lamb finishing system using mixtures of red and white clover with plantain and chicory compared to ryegrass and white clover. *Small Ruminant Research, 151*, 90-97. doi:10.1016/j.smallrumres.2017.04.019

Kniss, A., & Streibig, J. C. (2019). Statistical Analaysis of Agricultural Experiments using R. Retrieved from https://Rstats4ag.org. https://Rstats4ag.org.

Kohler, E. A., Throssell, C. S., & Reicher, Z. J. (2004). 2,4-D Rate Response, Absorption, and Translocation of Two Ground Ivy (*Glechoma hederacea*) Populations. *Weed Technology, 18*(4), 917-923. doi:10.1614/WT-03-089R1

Kudsk, P., & Kristensen, J. L. (1992). Effect of environmental factors on herbicide performance. Paper presented at the *First International Weed Control Conference*, Melbourne, Australia.

Landcare Research. (2022). Particle size analysis. Retrieved on November 2022 from https://www.landcareresearch.co.nz/partner-with-us/laboratories-and-diagnostics/soil-physics-laboratory/laboratory-tests/

Laws, D., & Genever, L. (2013). *Using Chicory and Plantain in Beef and Sheep Systems*. Retrieved on November 2021 from Stoneleigh Park Kenilworth Warwickshir, England.

Lingenfelter, D. D., & Hartwig, N. L. (2007). *Introduction to Weeds and Herbicides*. USA: The Pennsylvania State University.

Lockley, P., & Wu, H. (2008). Herbicide tolerance in pasture legumes and herbs. Paper presented at the *16th Australian weeds conference proceedings*: Weed management 2008 hot topics in the tropics, Queensland Weeds Society: Cairns, Qld.

Lusk, C. S., Bourdot, G. W., Harrington, K. C., & Hurrell, G. A. (2011). Pasture tolerance and efficacy of three herbicides used against giant buttercup (*Ranunculus acris* subsp *acris* L). *New Zealand Plant Protection, 64*, 86-92. doi:10.30843/nzpp.2011.64.5967

Lusk, C. S., Hurrell, G. A., Harrington, K. C., Bourdôt, G. W., & Saville, D. J. (2015). Resistance of *Ranunculus acris* to flumetsulam, thifensulfuron-methyl and MCPA in New Zealand dairy pastures. *New Zealand Journal of Agricultural Research, 58*(3), 271-280. doi:10.1080/00288233.2015.1016539

Makepeace, W., & Thompson, A. (1982). Ragwort control using a rope wick applicator. *Proceedings of New Zealand Weed and Pest Control Conference, 35*, 256-260.

Marchesan, M., Paper, D. H., Hose, S., & Franz, G. (1998). Investigation of the Antiinflammatory Activity of Liquid Extracts of *Plantago lanceolata* L. *Phytotherapy Research, 12*, S33- S34.

Martin, P., Fullerton, D. K., & James, T. K. (1990). Weed trials using a rotary weed wiper. *Proceedings of the New Zealand Weed and Pest Control Conference, 43*, 262-265. doi:https://doi.org/10.30843/nzpp.1990.43

Matthew, L. J. (1982). Pasture weeds of New Zealand. In W. Holzner & M. Numata (Eds.), *Biology and ecology of weeds* (pp. 387-394). Dordrecht: Springer Netherlands.

Matthews, P. N. P., Harrington, K. C., & Hampton, J. G. (1999). Management of Grazing Systems. In J. White & J. Hodgson (Eds.), *New Zealand pasture and crop science*. Auckland, New Zealand: Oxford University Press.

McWhorter, C. G., & Derting, C. W. (1985). Methods of application of glyphosate. In E. Grossbard & D. Atkinson (Eds.), *The Herbicide Glyphosate* (pp. 241-259). London, UK: Butterworths.

Mendes, K. F., Martins, B. A. B., Reis, F. C., Dias, A. C. R., & Tornisielo, V. L. (2017). Methodologies to study the behaviour of herbicides on plants and the soil using radioisotopes. *Planta Daninha, 35*.

Minneé, E. M. K., Clark, C. E. F., & Clark, D. A. (2013). Herbage production from five grazeable forages. *Proceedings of the New Zealand Grassland Association, 75*, 245-250.

Monaco, T. J., Weller, S. C., & ashton, F. M. (2002). *Weed Science: Principles and Practices* (Fourth ed.). New, York, USA.: John Wiley & Sons, Inc.

Moorhead, A. J. E., Judson, H. G., & Stewart, A. V. (2002). Liveweight gain of lambs grazing 'Ceres Tonic' plantain (*Plantago lanceolata*) or perennial ryegrass (*Lolium perenne*). *Proceedings of the New Zealand Society of Animal Production, 62*, 171-173.

Moorhead, A. J. E., & Piggot, G. J. (2009). The performance of pasture mixes containing 'Ceres Tonic" plantain *(Plantago lanceolata)* in Northland. *Proceedings of the New Zealand Grassland Association, 71*, 195-199.

Morita, H. (2002). *Handbook of Arable Weeds of Japan*. Tokyo, Japan: Kumiai Chemical Industry Co. Ltd.

Moyo, C. (2008). *Improving the efficiency of herbicide application to pasture weeds by weed-wiping and spot-spraying : a thesis presented in partial fulfillment of the requirements for the degree of Doctor of Philososphy in Plant Science at Massey University, Palmerston North, New Zealand.* (Doctor of Philosophy (Ph.D.) Doctoral). Massey University, Retrieved from http://hdl.handle.net/10179/779

Moyo, C., Harrington, K. C., Ghanizadeh, H., Kemp, P. D., & Eerens, J. P. J. (2016). Spectrophotometric technique for measuring herbicide deposition from wiper applicators. *New Zealand Journal of Agricultural Research, 59*(4), 412-421. doi:10.1080/00288233.2016.1229681

Moyo, C., Harrington, K. C., Kemp, P., Eerens, J., & Ghanizadeh, H. (2022). Wiper Application of Herbicides to *Cirsium arvense*. *Agronomy, 12*, 2262. doi:10.3390/agronomy12102262

Moyo, C., Harrington, K. C., Kemp, P. D., & Eerens, J. P. J. (2006a). Wiper application of herbicides to Californian thistle. *New Zealand Plant Protection, 59*, 361. Retrieved from http://www.nzpps.org/journal/59/nzpp_593610.pdf

Moyo, C., Harrington, K. C., Kemp, P. D., & Eerens, J. P. J. (2006b). Wiper application of herbicides to Californian thistle. Paper presented at the *New Zealand Plant Protection Conference*, Marlborough Centre and Brancott Winery, Blenheim.

Muir, P. (2012). *Plantain- A brief literature review*. beef + lamb, New Zealand : Hawkes Bay Regional Council.

Nandula, V. K., & Vencill, W. K. (2015). Herbicide Absorption and Translocation in Plants using Radioisotopes. *Weed Science, 63*(SP1), 140-151. doi:10.1614/WS-D-13-00107.1

Nie, Z. N., Miller, S., Moore, G. A., Hackney, B. F., Boschma, S. P., Reed, K. F. M., . . . Dear, B. S. (2008). Field evaluation of perennial grasses and herbs in southern Australia. 2. Persistence, root characteristics and summer activity. *Australian Journal of Experimental Agriculture, 48*(4), 424-435. doi:https://doi.org/10.1071/EA07136

Ozkan, H. E. (1995). Herbicide application equipment. In A. E. Smith (Ed.), *Handbook of Weed Management System.* (pp. 155- 216). New York; USA.: Marcel Dekker Inc.

Palma-Bautista, C., Delgado, A. M. R., Dellaferrera, I., Rosario, J., Vigna, M., Torra, J., & Prado, R. (2020). Resistance Mechanisms to 2,4-D in Six Different Dicotyledonous Weeds Around the World. *Agronomy, 10*, 566.

Patton, A. J., Weisenberger, D. V., & Schortgen, G. P. (2018). 2,4-D–Resistant Buckhorn Plantain (*Plantago lanceolata*) in Managed Turf. *Weed Technology, 32*(2), 182-189. doi:10.1017/wet.2017.98

Perez, L. M., Yue, Z., Saha, S., Dean, J. F. D., Jenkins, J. N., Stelly, D. M., & Tseng, T.-M. (2022). Absorption and translocation of [14C]2,4-dichlorophenoxyacetic acid in herbicide-tolerant chromosome substitution lines of *Gossypium hirsutum* L. *Frontiers in Agronomy, 4*. doi:10.3389/fagro.2022.936119

Peri, P. L., Brown, H. E., & McKenzie, B. A. (2000). The effect of sowing depth on the emergence and early development of six pasture species. *Agronomy New Zealand, 30*, 45-53.

Peterson, M. A., McMaster, S. A., Riechers, D. E., Skelton, J., & Stahlman, P. W. (2016). 2,4-D Past, Present, and Future: A Review. *Weed Technology, 30*(2), 303-345. Retrieved from http://www.jstor.org/stable/24856067

Piepho, H. P., Büchse, A., & Richter, C. (2004). A Mixed Modelling Approach for Randomized Experiments with Repeated Measures. *Journal of Agronomy and Crop Science, 190*(4), 230-247. doi:https://doi.org/10.1111/j.1439-037X.2004.00097.x

Pillmoor, J. B., & Gaunt, J. K. (1981). Behaviour and mode of action of the phenoxy-acetic acids in plants. *Progress in Pesticide Biochemistry*, 147-218.

Popay, I. (2008a). Weeds of agriculture - Introduction of weeds. Retrieved from https://teara.govt.nz/en/weeds-of-agriculture/page-1

Popay, I. (2008b). Weeds of Agriculture- Weeds in pasture, crops and forestry. Retrieved from https://teara.govt.nz/en/weeds-of-agriculture/page-2

Popay, I., Champion, P., & James, T. (2010). *An Illustrated Guide to Common Weeds of New Zealand*. Christchurch, New Zealand: New Zealand Plant Protection Society.

Popay, I., & Field, R. (1996). Grazing Animals as Weed Control Agents. *Weed Technology, 10*(1), 217-231. doi:10.1017/S0890037X00045942

Powell, A. M., Kemp, P. D., Jaya, I. K. D., & Osborne, M. A. (2007). Establishment, growth and development of plantain and chicory under grazing. *Proceedings of the New Zealand Grassland Association, 69*, 41-45.

Powles, S. B., & Yu, Q. (2010). Evolution in action: plants resistant to herbicides. *Annual Review of Plant Biology, 61*, 317-347. doi:10.1146/annurev-arplant-042809-112119

Pyne Gould Guiness Ltd. (1996). Variety: 'Ceres Tonic' syn 'PG 30'. Application no: 96/017. *Plant Varieties Journal*. Retrieved from https://www.cabi.org/isc/abstract/19971601274

Ramsfield, T. D. (2006). Risk assessment of indundative biological control with *Chondrostereum purpureum* in New Zealand. *New Zealand Journal of Forestry Science, 36*(1), 11-20.

Rao, V. S. (2000). *Principles of weed science*. Enfield, USA.: Science Publishers Inc.

Reddy, K. N., Locke, M. A., & Howard, K. D. (1995). Bentazon Spray Retention, Activity, and Foliar Washoff in Weed Species. *Weed Technology, 9*(4), 773-778. Retrieved from http://www.jstor.org/stable/3988358

Reed, K. (2009a). *Plantain*: pastures australia : A collaboration between AWI, GRDC, MLA, RIRDC and Dairy Australia.

Reed, K. (2009b). *Plantain [Factsheet]*: Pastures Australia : A collaboration between AWI, GRDC, MLA, RIRDC and Dairy Australia.

Reid, C. P. P., & Hurtt, W. (1970). Root Exudation of Herbicides by Woody Plants: Allelopathic Implications. *Nature, 225*.

Rey-Caballero, J., Menéndez, J., Giné-Bordonaba, J., Salas, M., Alcántara, R., & Torra, J. (2016). Unravelling the resistance mechanisms to 2,4-D (2,4-dichlorophenoxyacetic acid) in corn poppy (*Papaver rhoeas*). *Pesticide Biochemistry and Physiology, 133*, 67-72. doi:https://doi.org/10.1016/j.pestbp.2016.03.002

Riar, D. S., Burke, I. C., Yenish, J. P., Bell, J., & Gill, K. (2011). Inheritance and physiological basis for 2,4-D resistance in prickly lettuce (*Lactuca serriola* L.). *Journal of Agricultural and Food Chemistry, 59*(17), 9417-9423. doi:10.1021/jf2019616

Ritz, C., Baty, F., Streibig, J. C., & Gerhard, D. (2015). Dose-Response Analysis Using R. *PLoS ONE, 10*(12). doi:10.1371/journal.pone.0146021

Ritz, C., Jensen, S. M., Gerhard, D., & Streibig, J. C. (2019). *Dose-Response Analysis Using R* (1st edition ed.): CRC Press.

Rotowiper. (2004). *Rotowiper- assembly and operators manual*. Retrieved from Ashburton, New Zealand:

Rowarth, J. S. (1998). Plantain. In J. Rowarth (Ed.), *Practical herbage seedcrop management* (pp. 189-193): Lincoln University Press: Lincoln, New Zealand.

Rumball, W., Keogh, R. G., Lane, G. E., Miller, J. E., & Claydon, R. B. (1997). 'Grasslands Lancelot' plantain (*Plantago lanceolata* L.). *New Zealand Journal of Agricultural Research, 40*(3), 373-377. doi:10.1080/00288233.1997.9513258

Russell, T. R., Lulis, T. T., Aynardi, B. A., Tang, K. T., & Kaminski, J. E. (2021). Buckhorn plantain (*Plantago lanceolata*) resistant to 2,4-D in Pennsylvania and alternative control options. *Weed Technology, 35*(2), 297-303. doi:10.1017/wet.2020.116

Sanderson, M. A., Labreveux, M., Hall, M. H., & Elwinger, G. F. (2003). Forage Yield and Persistence of Chicory and English Plantain. *Crop Science, 43*(3), 995-1000. doi:10.2135/cropsci2003.9950

Sanderson, M. A., Soder, K. J., Muller, L. D., Klement, K. D., Skinner, R. H., & Goslee, S. C. (2005). Forage Mixture Productivity and Botanical Composition in Pastures Grazed by Dairy Cattle. *Agronomy Journal, 97*, 1465-1471. doi:10.2134/agronj2005.0032

Saunders, J. T., Greer, G., Bourdôt, G., Saunders, C., James, T., Rolando, C., . . . Watt, M. S. (2017). The economic costs of weeds on productive land in New Zealand. *International Journal of Agricultural Sustainability, 15*(4), 380-392. doi:10.1080/14735903.2017.1334179

Schepers, J. S., & Burnside, O. C. (1979). Electronic Moisture Sensor for Maintaining Herbicide Solution on a Roller Applicator. *Weed Science, 27*(5), 559-561. doi:10.1017/S004317450004460X

Schulz, B., & Segobye, K. (2016). 2,4-D transport and herbicide resistance in weeds. *Journal of Experimental Botany, 67*(11), 3177-3179. doi:10.1093/jxb/erw199

Schuster, M. Z., Pelissari, A., de Moraes, A., Harrison, S. K., Sulc, R. M., Lustosa, S. B. C., . . . Carvalho, P. C. F. (2016). Grazing intensities affect weed seedling emergence and the seed bank in an integrated crop–livestock system. *Agriculture, Ecosystems & Environment, 232*, 232-239. doi:https://doi.org/10.1016/j.agee.2016.08.005

Sellers, B. A. (2019). Weed Management in Pastures and Rangeland- 2019. In F. A. Jason, B. J. Barry, & M. Gregory (Eds.), *Forage management (Florida Forage Handbook)*. University of Florida IFAS Extension: University of Florida.

Sharma, N., Koul, P., & Koul, A. K. (2008). Pollination biology of some species of genus *Plantago L. Botanical Journal of the Linnean Society, 111*(2), 129-138. doi:10.1111/j.1095-8339.1993.tb01895.x

Shyam, C., Peterson, D. E., & Jugulam, M. (2022). Resistance to 2,4-D in Palmer amaranth (*Amaranthus palmeri*) from Kansas is mediated by enhanced metabolism. *Weed Science, 70*(4), 390-400. doi:10.1017/wsc.2022.29

Sinhadipathige, S. C. S., Kenyon, P. R., Kemp, P. D., Morris, S. T., & Morel, P. C. H. (2012). Can herb-clover mixes increase lamb liveweight gains in spring? Paper presented at the *Proceedings of the New Zealand Grassland Association, 74,* 137-142.

Somasiri, S. C., Kenyon, P. R., Kemp, P. D., Morel, P. C. H., & Morris, S. T. (2016). Effect of herb-clover mixes of plantain and chicory on yearling lamb production in the early spring period. *Animal Production Science, 56*(10), 1662-1668. doi:10.1071/an14796

Song, Y. (2014). Insight into the mode of action of 2,4-dichlorophenoxyacetic acid (2,4-D) as an herbicide. *Journal of Integrative Plant Biology, 56*(2), 106-113. doi:10.1111/jipb.12131

Spicer, V., Grigoryan, M., Gotfrid, A., Standing, K. G., & Krokhin, O. V. (2010). Predicting Retention Time Shifts Associated with Variation of the Gradient Slope in Peptide RP-HPLC. *Analytical Chemistry, 82*(23), 9678-9685. doi:10.1021/ac102228a

Stewart, A. V. (1996). Plantain *(Plantago lanceolata)* - a potential pasture species. Paper presented at the *Proceedings of the New Zealand Grassland Association*.

Stewart, A. V., & Charlton, D. (2006). *Pasture and forage plants for New Zealand.* (3rd ed.): New Zealand Grassland Association, New Zealand Grassland Trust, Wellington, N.Z.

Thompson, A., & Saunders, A. (1984). A comparison of 2,4-D and MCPA, alone and in combination, for the control of ragwort. *Proceedings of the New Zealand Weed and Pest Control Conference, 37*, 33-36. Retrieved from http://www.nzpps.org/journal/37/nzpp_370330.pdf

Torra, J., Rojano-Delgado, A. M., Rey-Caballero, J., Royo-Esnal, A., Salas, M. L., & Prado, R. D. (2017). Enhanced 2,4-D Metabolism in Two Resistant *Papaver rhoeas* Populations from Spain. *Frontiers in Plant Science, 8*(1584). doi:10.3389/fpls.2017.01584

Tozer, K. N., Bourdot, G. W., & Edwards, G. R. (2011). What factors lead to poor pasture persistence and weed ingress? *Proceedings of International Grassland Conference, 15*, 129-138. Retrieved from https://www.grassland.org.nz/publications/nzgrassland_publication_2245.pdf

USDA-Agricultural Research Service. (2019). Germplasm Resources Information Network (GRIN- Taxonomy). Retrieved from https://npgsweb.ars-grin.gov/gringlobal/taxonomydetail.aspx?id=28787. Retrieved 10 October, 2019, from National Germplasm Resources Laboratory. https://npgsweb.ars-grin.gov/gringlobal/taxonomydetail.aspx?id=28787

Van Toor, R. F. (1994). Effect of applying glyphosate and clopyralid by rotary weed wiper on *Californian thistle* in Southland. *Proceedings of New Zealand Plant Protection Conference, 47*, 91-92. doi:https://doi.org/10.30843/nzpp.1994.47.11037

Van Toor, R. F., & Brewster, D. S. (1995). Automatic herbicide dispenser for rotary wipers. *Applied Engineering in Agriculture, 11*(3), 377-380. Retrieved from https://www.scopus.com/inward/record.uri?eid=2-s2.0-76549109763&partnerID=40&md5=d224680b69d6677448638cf549c34d58

Verkaaik, M. L., Hurrell, G. A., Bourdôt, G. W., & Saville, D. (2004). Evaluation of *Sclerotinia sclerotiorum* for giant buttercup control in dairy pastures. *New Zealand Plant Protection, 57*, 286-291.

Vrbničanin, S., Pavlović, D., & Božić, D. (2017). Weed Resistance to Herbicides. In Z. Pacanoski (Ed.).

Wardle, D. A., Nicholson, K. S., Ahmed, M., & Rahman, A. (1994). Interference effects of the invasive plantCarduus nutans L. against the nitrogen fixation ability of Trifolium repens L. *Plant and Soil, 163*(2), 287-297. doi:https://doi.org/10.1007/BF00007978

Warwick, S. I., & Briggs, D. (1979). The genecology of lawn weeds. III. Cultivation experiments with *Achillea millefolium* L., *Bellis perennis* L., *Plantago lanceolata* L., *Plantago major* L. and *Prunella vilgaris* L. collected from lawns and contrasting grassland habitats. *New Phytologist, 83*, 509-536. doi:https://doi.org/10.1111/j.1469-8137.1979.tb07616.x

Whitcomb, C. E. (1999). An introduction to ALS-inhibiting herbicides. *Toxicology and Industrial Health, 15*(1-2), 232-240. doi:10.1177/074823379901500120

Wilson, R. G., Martin, A. R., & Kachman, S. D. (2006). Seasonal Changes in Carbohydrates in the Root of Canada thistle (*Cirsium arvense*) and the Disruption of these Changes by Herbicides. *Weed Technology, 20*(1), 242-248. doi:10.1614/WT-05-052R1.1

Woodward, S. L., Waugh, C. D., Roach, C. G., Fynn, D., & Philips, J. (2013). Are diverse species mixtures better pastures for dairy farming? *Proceedings of the New Zealand Grassland Association, 75*, 79-84.

Young, S. (2019). *New Zealand Novachem Agrichemical Manual*. Retrieved from https://www.novachem.co.nz/Subscribers/Quick-Search.aspx?term=glyphosate

Yu, Q., & Powles, S. (2014). Metabolism-Based Herbicide Resistance and Cross-Resistance in Crop Weeds: A Threat to Herbicide Sustainability and Global Crop Production. *Plant Physiology, 166*(3), 1106-1118. Retrieved from http://www.jstor.org/stable/43191532

Yuan, J. S., Tranel, P. J., & Stewart, C. N., Jr. (2007). Non-target-site herbicide resistance: a family business. *Trends Plant Science, 12*(1), 6-13. doi:10.1016/j.tplants.2006.11.001

Zimdahl, R. L. (2007). *Fundamental of Weed Science* (Third ed.). USA: Elsevier Inc.

Appendix

Appendix 3.1: Two-way ANOVA of percentage of reduction in dry weight compared to untreated control at recommended rates of different herbicides for the first dose-response experiment.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	18749.8a	9	2083.3	3.1	0.006
Intercept	283254.6	1	283254.6	425.4	<0.001
Cultivar	2432.1	1	2432.1	3.6	0.063
Herbicide	15192.2	4	3798.0	5.7	0.001
Cultivar * Herbicide	1125.5	4	281.4	0.4	0.791
Error	26633.0	40	665.8		
Total	328637.4	50			
Corrected Total	45382.8	49			

a. R Squared = 0.413 (Adjusted R Squared = 0.281)

Appendix 3.2: Two-way ANOVA of percentage of reduction in dry weight compared to untreated control at recommended rates of different herbicides for the second dose-response experiment.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	701.7[a]	9	78.0	1.2	0.297
Intercept	546768.2	1	546768.2	8647.8	<0.001
Cultivar	38.6	1	38.6	0.6	0.438
Herbicide	480.0	4	120.0	1.9	0.125
Cultivar * Herbicide	183.1	4	45.8	0.7	0.580
Error	3161.3	50	63.2		
Total	550631.2	60			
Corrected Total	3863.0	59			

a. R Squared = .182 (Adjusted R Squared = .034)