Variation in susceptibility of giant buttercup (*Ranunculus acris* L. subsp*. acris*) populations to herbicides

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ABSTRACT

Giant buttercup (*Ranunculus acris* L.) is a serious weed of dairy pastures throughout New Zealand causing substantial economic losses from lost pasture productivity. It has developed resistance to the phenoxy herbicides (MCPA and MCPB) at many sites around New Zealand, particularly in Golden Bay. Since the discovery of resistance in the 1980s, two newer herbicides from a different mode-of-action group (acetolactate synthase inhibitor, ALS), flumetsulam and thifensulfuron-methyl, have been used widely, which appeared to overcome the resistance problem. A survey of farmers in Golden Bay indicated that most have herbicide control programmes for giant buttercup based around flumetsulam but some have reported poor control with this herbicide, particularly after several years of use. The research in this thesis was undertaken to determine whether this may be due to evolved resistance.

Seedling progeny from 15 populations of giant buttercup, with known spraying history, were sprayed with a range of doses of flumetsulam, thifensulfuron-methyl and MCPA (Experiment 1) to test for differences in susceptibility. The experiment revealed a large difference in susceptibility between the populations (83-100% and 58-100% mortality at the recommended rate and 2.2 times that rate of flumetsulam applied, respectively). The population with the highest past exposure to flumetsulam showed the lowest mortality and 25% of plants in this population survived a treatment with 5 times the recommended rate. There was a significant declining trend between percent mortality and historical exposure of these populations to flumetsulam. However, most populations with low previous exposure had no or few resistant individuals and only a few populations with high previous exposure had several resistant individuals present, as indicated by their survival above recommended rates. Calculated LD_{50} values did not correlate well to the survival data or to historical exposure to flumetsulam, because the herbicide rates chosen in this experiment were too high.

Measurements of the biomass of giant buttercup, obtained several times after spraying in Experiment 1, showed some evidence of cross resistance to thifensulfuron-methyl but not to MCPA. Biomass yields 3 months after treatment were 1, 2 and 22% of untreated for the recommended rates of flumetsulam, MCPA and thifensulfuron respectively, indicating that flumetsulam and MCPA were equally effective, but that thifensulfuron-methyl was less effective.

In Experiment 2, plants from the most resistant and susceptible populations in Experiment 1, were grown from spare seeds and treated with a wider range of doses (including lower doses) of flumetsulam than in Experiment 1. The LD_{50} values for the two populations in this second experiment differed 5.3-fold and this difference was highly significant. Twenty-nine percent of plants from the population with high past

exposure survived treatment with 25 times the recommended rate of flumetsulam compared to 0% from the population with no past exposure.

In a third experiment the same three herbicides and rates were compared for their damage to perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) sown either in pots (Exp. 3a), or transplanted from the field (Exp. 3b). The total clover yield harvested over 5 months from newly-sown pasture was 80, 59 and 4% that of the untreated control for flumetsulam, thifensulfuron-methyl and MCPA applied at recommended rates, and 95, 40 and 30% respectively for transplanted swards. The total yield of grass was not reduced by any of the herbicides. Overall flumetsulam was the least pasture-damaging herbicide, but rates could not be increased above recommended rates in order to deal with resistance because pasture damage occurred.

The experiments in this study indicate that resistance to flumetsulam may be evolving in giant buttercup in dairy pastures in Golden Bay, but more research is needed in field trials to confirm this. Furthermore, the results indicate that some populations may no longer be resistant to MCPA, but this also needs further study to confirm. Currently-available herbicides may not provide adequate control of giant buttercup in the future if existing management practices continue.

KEYWORDS: *Ranunculus acris*; giant buttercup; MCPA; flumetsulam; thifensulfuron-methyl; efficacy; phenoxy herbicide; ALS inhibitor; resistance; Takaka; New Zealand; pasture tolerance.

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Chapter One

Literature Review

1.1 Giant buttercup *Ranunculus acris* L., subsp. *acris*

1.1.1 Biology and ecology

Ranunculus acris L. is a highly variable species, which has led to it being categorised into several sub-species, the most common and widespread being *R. acris* subsp. *acris* (Coles 1971), (commonly known as giant, meadow or tall buttercup). This is the only sub-species that has naturalised in New Zealand where it has become a serious weed of dairy pastures, and is the subject of this research. Giant buttercup is a polycarpic perennial herb with erect, hairy stems growing up to 1m (Harper 1957). A distinctive feature of this sub-species is that the leaves have three main divisions, with each division being stalkless (Tuckett 1961) (As shown in plate 1.1 at end of chapter, pg 41), although there is a lot of variability in the degree of leaf dissection, and other characteristics such as stem and petiole hairs, petals, and size of seeds (Coles 1971).

Giant buttercup regenerates readily both vegetatively, by shoots from a stout rhizome, and also sexually by seed. Studies have shown most regeneration occurs by seed in Britain (Harper 1957; Sarukhán & Harper 1973) and Russia (Rabotnov & Saurina 1971), but other population studies in Wales indicate both sexual and vegetative reproduction contribute equally (Sarukhán 1974). It is not certain which contributes most to population growth of giant buttercup in New Zealand, but both have been commonly observed in our field work.

Giant buttercup produces bright yellow flowers which are protogynous (the stigma is receptive before the pollen is shed from the anthers) (Clapham et al. 1987) hermaphrodite, self-incompatible, and insect pollinated (Hemborg & Karlsson 1998) (Plate 1.2). In New Zealand, flowering generally begins in October, increases until December where it reaches a peak, then continues into April, and some flowers can be seen all year round (Popay et al. 1989b). Flower stems and leaves die back in winter, and are replaced by small rosettes of leaves which are more frost tolerant, until spring when new larger leaves are produced. Plants produce up to 5000 seeds per year (Tuckett 1961), which are small (generally about 1.5 mm in diameter) and have a rigid hook to aid dispersal (Bourdôt 1983). However, it does not have a well-developed dispersal mechanism as most seeds do not disperse far from the parent plant (Kiviniemi & Eriksson 1999). Seeds are mainly spread by being carried in the hooves, on hair or in the gut of stock, in hay made from infested paddocks, or on machinery, footwear and clothing worn by people, or in irrigation or flood waters (Tuckett 1961; Bourdôt 1983). Seedlings establish readily in dairy pastures in

autumn and spring, and recruitment is increased in bare patches associated with reduced pasture vigor. For example, overstocking, pugging, drought, herbicide damage and poor pasture management create bare micro-sites ideal for germination (Lusk et al. 2009).

Vegetative reproduction occurs in autumn when extension of short stout rhizomes produce new vegetative shoots close to the parent (Sarukhán & Harper 1973). Shoot growth in this way can be promoted by damage to parent plants, for example, by mowing, spraying or heavy grazing and trampling.

1.1.2 Distribution

Giant buttercup is common throughout its native range in central and northern Europe (Coles 1971) and has also spread to other northern hemisphere countries (latitude 74^{o} N to latitude 31^{o} N), most states in the USA, southern territories of Canada, as far north as the Aleutian Islands (latitude 54 N) and as far south as Georgia, USA (latitude 30^{o} N) (Lamoureaux & Bourdôt 2007). In the southern hemisphere it has become naturalised in South Africa, Tasmania, mainland Australia and New Zealand to Latitude 47^{o} S, which is its known southern boundary.

Giant buttercup was first recorded in New Zealand in 1872 (Garnock-Jones 1981), and is now widely distributed throughout the country (Webb et al. 1988). It mainly occurs on damp, well drained areas with a higher rainfall, which do not dry excessively or waterlog (Harper & Sagar 1953). It occurs on roadsides, waste areas, swamp margins and river flats but is also prevalent in dairy pastures where it causes the greatest problem. It is found in the North Island in Northland, Auckland, Waikato, Bay of Plenty, volcanic plateau, Hawke's Bay, Wairarapa, Taranaki and Manawatu, and in the South Island in Tasman, Canterbury, Westland, Otago, Southland and Fiordland (Webb et al. 1988). It is a serious weed in six of the 17 dairy farming regions in New Zealand, which are Tasman district, South Auckland, Hawke's Bay, Taranaki, Wairarapa and Horowhenua (Bourdôt & Saville 2010). As this weed has spread widely beyond its native range in Europe and has a wide climatic tolerance, it is likely it could spread to the remaining uninfested dairy farming regions in New Zealand as well.

1.1.3 Toxicity

Giant buttercup (along with all species in the *Ranunculus* genus) produces an acrid-tasting glycoside, ranunculin, which after being eaten by grazing cattle forms a volatile chemical compound called protoanemonin (Connor 1977). This is known to cause blistering of the lips and tongue, intestinal disorders and in some cases respiratory failure in stock after ingestion (Harper & Sagar 1953), although no cases of poisoning have been reported in New Zealand (Connor 1977). It is believed the concentration of ranunculin varies with season and growth stage. It is for these

reasons that giant buttercup and closely surrounding vegetation is avoided by stock in dairy pastures in New Zealand.

1.1.4 Economic loss from giant buttercup in pastures

Because of its ability to regenerate easily, avoidance by stock and evolved resistance to herbicides, giant buttercup has become a difficult weed to eradicate from dairy pastures. Giant buttercup clumps and surrounding pasture species are avoided by stock (Plate 1.3, pg 42), so consequently infested pastures show reduced productivity, dependent on the amount of the weed present. Utilization of infested pastures may be reduced by up to 50% at peak cover in November (Bourdôt et al. 2003) which is lost grazing opportunity (Plate 1.5, pg 43).

The loss of production caused by giant buttercup was estimated to cost the New Zealand dairy industry $155 million per year, based on 2008/2009 prices (Bourdôt & Saville 2010), and cover estimates of giant buttercup in 2001-02. This was equivalent to 2.1 % of the potential revenue of $7.3 billion that could have been expected had the weed been absent. If this weed was to spread to all remaining uninfested dairy farming regions in New Zealand, a conservative estimate predicted the potential national loss to be as high as $748 million (10.2% of the revenue that could have been expected). In addition, a recent Farmax DairyPro model predicted the economic loss on a typical dairy farm with giant buttercup cover peaking at 12% in November to be $1040 per hectare less than where the weed was absent (King & Rennie 2011).

The costs of four commonly used chemicals that are currently registered for giant buttercup control in pasture are compared in Table 1.1. Excluding the cost of application, this ranges from $28 – $77 per hectare (prices quoted Sept. 2011 from PGG Wrightson, includes GST). The cost of application will vary depending on the situation and many farmers will spray their own pastures. As an example a standard rate for application is $26/ha (excl. GST) (Pangborn 2010).

Table 1.1 Cost of herbicides registered for giant buttercup control in New Zealand (based on prices (incl. GST) from PGG Wrightson, Sept. 2011)

Chemical	Rate/ha	Cost/ha (excl. application)
MCPA 750 g/L (e.g. Agritone 750)	1.5 L (seedlings) - 3 L (large plants)	$21 to $42
MCPB 385g/L (e.g. MCPB 400)	6 L	$90
Preside	65 g + 500 ml/100 L Uptake oil	$60 + $17 = $77
Harmony	20 g	$28

1.2 Chemical control of giant buttercup

1.2.1 Modes of Action

Herbicides can be classified into groups depending on either their chemical structure, or the way in which they act (mode-of-action). Herbicides act by interfering with specific plant processes that are vital for the plant's survival. All current herbicides are based on about 20 target sites, which is low considering the thousands of chemical reactions taking place within plant cells (Moss 2002). There are 14 herbicide mode-of-action groups, some having sub-groups, covering all of the chemical active ingredients available in New Zealand (NZCPR 2011). The rationale for grouping the chemicals in this way is to show users which chemicals have the same biochemical activity and should not be mixed or applied in succession, and what the total number of applications should be (Beresford et al. 2009). It is based on the Herbicide Resistance Action Committee (HRAC) system and is unique to New Zealand's herbicide management requirements. Weeds that become resistant to herbicides in one mode-of-action group should still be controlled with herbicides from a different group. Table 1.2 categorizes the specific mode-of-action groupings for products registered in New Zealand. Those that are registered for selective control of giant buttercup comprise active ingredients in Groups B (ALS inhibitors) and O1 (phenoxy herbicides).

Table 1.2 Herbicide mode-of-action groups based on the New Zealand system (NZCPR 2009).

Group code	Mode-of-action	Chemical family
GROUP A	Inhibitors of AcetylCoA carboxylase (ACCase)	Aryloxyphenoxypropionates, Cyclohexanediones, Phenylpyrazoline
GROUP B	Inhibitors of the enzyme acetolactate synthase- ALS inhibitors	Sulfonylureas, Imidazolinones, Triazdopyrimidines
GROUP C1	Inhibitors of photosynthesis at photosystem II	Triazines, Triazinones, Uracils, Pyridazinones, Phenyl-carbamates
GROUP C2	Inhibitors of photosynthesis at photosystem II	Ureas
GROUP C3	Inhibitors of photosynthesis at photosystem II	Nitriles, Benzothiadiazonone, Phenyl-pyridazines
GROUP D	Photosystem-1-electron diversion	Bipiridyliums
GROUP E	Inhibitors of protoporphyrinogen oxidase	Diphenylethers, Oxidiazoles, Triazolinones
GROUP F1	Inhibitors of PDS	Pyridazinones, Pyridinecarboxamides, others
GROUP F2	Inhibition of 4HPPD	Triketones
GROUP F3	Inhibition of carotenoid biosynthesis (unknown target)	Triazoles, Isoxazolidinones
GROUP G	Inhibitors EPSP synthase	Glycines
GROUP H	Inhibitors of glutamine synthetase	Phosphinic acids
GROUP I	Inhibition if DHP	Carbamates
GROUP K1	Mircotubule assembly inhibition	Dinitroanilines, Benzamides, Benzinedicarboxylic acids
GROUP K2	Inhibition of mitosis/microtubule organization	Carbamates
GROUP K3	Inhibition of cell division	Chloroacetamides, Oxyacetamides
GROUP L	Inhibitors of cell wall (cellulose) synthesis	Nitriles
GROUP N	Inhibitors of lipid synthesis (not ACCase)	Thiocarbamates, Benzofuranes, Chloro-carbonic-acids
GROUP O1	Action like indoleacetic acid (synthetic auxins)	Phenoxy-carboxylic-acids
GROUP O2	"	Benzoic acids
GROUP O3	"	Pyridine, Carboxylic acids
GROUP Z	Unknown mode-of-action	Arylaminopropionic acids, Organoarsenicals

Pasture herbicides that have shown effectiveness against giant buttercup in NZ, Europe, Canada and USA fall into seven different mode-of-action groups (Bourdôt 2011), which are summarised in Table 1.3.

Table 1.3 Herbicides with known activity against giant buttercup (Adapted from Bourdôt 2011).

Group	Mode-of-action	Herbicide	Selective use in pastures	Label claim for giant buttercup in NZ
Group B	ALS inhibitors	flumetsulam	yes	yes
		thifensulfuron-methyl	partial	yes
		metsulfuron	partial	no
		chlorsulfuron	partial	no
Group C3	Photosystem II inhibitors	bentazone	yes	yes
Group E	Protoporphyrinogen oxidase (PPO) inhibitors	oxadiazon	no	no
Group G	EPSP synthase inhibitors	glyphosate	no	no
Group O1	Synthetic auxins-phenoxy carboxylic acids	MCPA	partial	yes
		MCPB	yes	yes
Group O2	Synthetic auxins-benzoic acids	dicamba	Partial, kills legumes	no
Group O3	Pyridine carboxylic acids	aminopyralid	partial, kills legumes	no
		picloram	partial, kills legumes	no

There are only five herbicides registered in New Zealand for selective control of giant buttercup in pastures, MCPA and MCPB (both phenoxys) and flumetsulam and thifensulfuron-methyl (both ALS inhibitors) which are in only two mode-of-action groups (B and O1). The fifth herbicide, bentazone (Basagran), is in a third group (C3) but must be applied in combination with MCPA. This leaves little scope for rotation of herbicides to delay the development of herbicide resistance, a fundamental recommendation for the management of herbicide resistance (Beresford et al. 2009). However it is not known to what extent farmers rotate these herbicides.

1.2.2 Group O1 Synthetic Auxins (MCPA and MCPB)

These herbicides are plant growth regulators (in the chemical family phenoxy carboxylic acids), that mimic natural auxins within a plant resulting in a hormone overdose. Auxins are plant growth hormones that influence plant cell division, differentiation and elongation. Symptoms of herbicide treatment are bending and twisting of stems and leaves, adventitious roots formed at stem nodes, deformed leaves, stems, flowers and abnormal leaves (Moss 2002). Plants normally die quickly within a couple of weeks.

MCPA and 2,4-D were developed during World War II as potential biological-warfare agents and were the first truly selective broadleaf herbicides used in cereal crops. At the time they were considered a major advance in weed technology (Lamoureaux & Bourdôt 2007).

MCPA has been widely used since the 1950s for selective broadleaf weed control in pastures. It is a systemic herbicide absorbed by both the leaves and the roots. MCPA was the chemical of choice by farmers for controlling giant buttercup in pastures because of its low cost. But it is damaging to nitrogen-fixing clovers with effects lasting for several months (Popay et al. 1989a; Hurrell & Bourdôt 1993). Repeated use of this herbicide may lead to reduced pasture vigour and increased recruitment of giant buttercup and other weed seedlings. The reduction in clover content may also lead to reduced animal growth rates (Hartley & Thomson 1982); (Hartley 1983).

Tuckett (1961) found only MCPA, MCPB and 2,4-D gave selective control of giant buttercup in pasture. MCPB at 2.2 kg/ha was found to be as effective as MCPA at 1.5kg/ha, and both were more effective than 2,4-D. However, Tuckett commented that about 20% of established plants re-grew following treatment with MCPB, so this rate is possibly only effective on younger plants. Recent labels for MCPB recommend an application rate of 6L/ha (2.3kg ai/ha), comparable to these early results.

MCPB is a more expensive product than MCPA (Table 1.1) but does not damage clover, which is important when spraying in spring over large areas, as the pasture will remain competitive (Tuckett 1961). This is because giant buttercup converts the non-toxic chemical MCPB to MCPA which is toxic, via the process of β-oxidation, but clover plants cannot carry out this conversion and so are not damaged. However, MCPB is less effective on giant buttercup than MCPA (Popay, 1989), and is generally only used on young plants or in combination with MCPA. Repeat annual applications are often necessary to achieve control which is acknowledged on the label. While these herbicides gave adequate control in the 1950s and 1960s

(Tuckett 1961), 25-30 years later farmers were reporting poor control which was later confirmed to be due to evolved resistance (Bourdôt et al. 1990).

1.2.3 Group B, ALS Inhibitors (flumetsulam and thifensulfuron-methyl)

These herbicides act by inhibiting the enzyme acetolactate synthase (ALS-inhibitor), a precursor for the biosynthesis of the branched-chain amino acids necessary for plant growth and survival; leucine, isoleucine and valine. After treatment these chemicals rapidly inhibit cell division and plant growth, but can take several months to kill plants due to stored reserves of amino acids within the plant. These herbicides are systemic and are absorbed by both the leaves and the roots (Tomlin 1995). Other secondary effects of these herbicides including the buildup of 2-ketobutyrate, disruption of protein synthesis and disruption of photosynthate transport may also contribute to plant death (Shaner, 1991).

Two herbicides within this group have product label claims for giant buttercup in New Zealand. These are thifensulfuron-methyl (Harmony, Chord, Ranger, Backup) and flumetsulam (Preside, Valdo). Thifensulfuron-methyl (sulfonyl-urea chemical group), developed by Du Pont, came onto the market about 1988 and flumetsulam (triazolopyrimidine group), developed by Dow Elanco (now DowAgrosciences) became available on the market about 1992 (Fontaine & Miller 1991; Hare et al. 1993).

Thifensulfuron is damaging to clovers (Sanders & Rahman 1994; Harris & Husband 1997), thus reducing the competitiveness of pasture which may potentially promote population growth of giant buttercup (Tuckett 1961). Flumetsulam has little effect on clovers (Harris & Husband 1997), and can be used in newly established pastures (Young 2010). The efficacy of both these herbicides however may be short lived as their mode of action is one to which many weed species have rapidly evolved resistance (Llewellyn & Powles 2001).

1.2.4 Effectiveness of herbicides used against giant buttercup

Studies show only partial control of giant buttercup has been achieved with herbicides in dairy pastures in New Zealand. Lamoureaux & Bourdôt (2007) reviewed historical experiments with MCPA and MCPB and found that these herbicides gave mean reductions in cover of 59% and 23%, respectively, when applied to dairy pastures in New Zealand in late winter or early spring.

These trials however showed high variability, which was thought to be due to the variation in herbicide resistance between populations and the competitiveness of pastures influencing seedling recruitment after spraying. Annual applications of MCPA over four years were found to result in a steady decline in giant buttercup (Popay et al. 1989b) but it was not completely eradicated and subsequently

returned to pre-spraying levels three to four years later. In another study by the same authors (Popay et al. 1984) they examined the effects of single applications of MCPA at different times of the year and found that the most reliable control resulted from August rather than May applications. Two applications were needed per season for effective control, according to Matthews (1975), and it was suggested the use of MCPA at 1 kg/ha in late winter followed by MCPB at 1.5 kg/ha in late spring or early summer was best.

There is little information in the literature on the efficacy of the two ALS-inhibitor herbicides on giant buttercup in dairy pasture. Lamoureaux & Bourdôt (2007) reviewed the literature and found only two studies (Sanders & Rahman 1994; Harris & Husband 1997) which gave a mean reduction in giant buttercup cover of 53% for thifensulfuron-methyl and only one study which found flumetsulam gave a mean reduction in cover of 67% (Harris & Husband 1997).

The following sections discuss what can be done to control giant buttercup if chemicals can't be used, the possibility of herbicide resistance and what can be done to deal with it, and other reasons for the apparent failure of herbicides to control giant buttercup in the field.

1.3 Non-chemical control

1.3.1 Pasture management through grazing and mowing

Inadequate control of giant buttercup using herbicides has led to the adoption of other weed management practices by farmers. Keeping pastures dense and competitive throughout the year controls population growth and seedling recruitment of giant buttercup to some extent (Bourdôt & Lamoureaux 2002). This can be achieved through good pasture management by preventing over-grazing and pugging in winter. Sowing drought-tolerant pasture species in dry parts of the country can also be an effective way to maintain pasture competitiveness.

Based on observations, giant buttercup is less prevalent in sheep pastures than cattle grazed pastures but it is not known to what extent this is due to grazing by sheep (Brown 1993) or if it is a result of differences in pasture composition or management factors. Sheep (and possibly goats) are known to browse giant buttercup (Popay & Field 1996).

Mowing pastures can reduce flowering and seeding of giant buttercup, and reduce its cover in pastures, but effects are only temporary. Fertiliser addition to deteriorated pastures may promote pasture species but there is little evidence that the abundance of giant buttercup will be reduced (Brown 1993). Although it has not

been tested experimentally, many reports from farmers suggest that cultivating an infested dairy pasture and growing a crop for at least 12 months reduces levels of giant buttercup. However, some plants and seeds survive to re-infest the new pasture. Seeds can be long lived in the soil, for instance James & Rahman (1999) found that 20% of giant buttercup seeds were viable after 16 years in the soil when buried below 4 cm.

1.3.2 Biocontrol

Two plant pathogenic fungi that occur naturally on giant buttercup in New Zealand have been investigated as biocontrol agents. One of these, likely to be a species of *Gnomonia,* was found to be unsuitable (Hardwick et al. 1993) and research was discontinued. The other fungus, *Sclerotinia sclerotiorum,* which causes a watery soft-rot disease that leads to death of infected plants (Bourdôt et al. 1993), showed more potential. There is low risk of the fungus infecting neighbouring crops because some spores may escape from treated pastures (de Jong et al. 2002; Bourdôt et al. 2006) but it does not affect clover species. In a controlled-environment study *S. sclerotiorum* caused 75% mortality of giant buttercup within 21 days (Green et al. 1993). In one field study in dairy pasture a 57% reduction in dry weight of the weed was found after one application of the fungus (Cornwallis et al. 1999) and in another study, a 50% reduction was found (Harvey & Bourdôt 2001). This difference was probably due to field populations being older and more resistant than the 3-month-old plants used in the earlier glasshouse experiments (Lamoureaux & Bourdôt 2007). Further studies found a 60% reduction in the weed could be achieved in the field but results were highly variable between sites (Verkaaik et al. 2004). Although further research is needed on the causes of this variability, before the fungus can be developed as a practical control method for farmers, the high cost of commercialising this fungus as a product has meant no further work has been conducted.

1.4 Herbicide Resistance

The evolution of herbicide-resistant weeds is an increasing problem worldwide, with large areas of productive agricultural land affected in many countries (Heap 2011). Herbicide resistant biotypes become dominant in weed populations when crops or pastures are treated repeatedly with the same chemical year after year (Gressel 1991). The development of selective herbicides was initially seen as a breakthrough in weed control offering large increases in production, but with little restriction on use, overuse of single chemicals/modes of action, and a poor understanding of resistance evolution led to a strong selection pressure for resistant biotypes to evolve (Moss 2002).

Even though selective herbicides have been used widely since the 1950s and herbicide resistance was predicted early on (Blackman 1950), it was not recognised as a serious problem for several decades, until around the mid-1970s. This was much later than the detection of insecticide and fungicide resistance which was seen within several years of widespread pesticide use, and became apparent in the mid-1940s (Georghiou 1986). This is partly because insects and fungi complete many lifecycles in one year allowing resistance genes to build up more quickly, whereas most weeds produce seeds only once per year. Susceptible insects and fungi are normally all killed when sprayed whereas non-resistant weed seeds may be preserved in the seedbank for long periods of time, providing a buffering effect for resistance to develop (Bourdôt & Suckling 1996).

1.4.1 Definitions and methods for determining resistance

The International Survey of Herbicide Resistant Weeds website (Heap 2011) records all new cases of herbicide resistant weeds, and defines resistance as: "the evolved capacity of a previously susceptible weed population to withstand a herbicide and complete its lifecycle when the herbicide is used at its normal rate in an agricultural situation". For a weed to be listed on the site it must fulfill this definition as well as the Weed Science Society of America (WSSA) definition: "the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type". It also must fulfill several other criteria, which are discussed below. These guidelines are accepted by the international weed community as being the desired bases for confirmation of new cases of herbicide resistant biotypes.

For the first detection of a resistant weed biotype the most preferred test is a dose response test under controlled conditions using whole plants grown from seed (Heap 2011). This involves treating plants under controlled conditions (replicating normal field conditions) with herbicides to determine the GR_{50} (dose required to reduce shoot growth by 50%) of the resistant and susceptible populations. A range of doses are used that include sub-lethal and lethal doses for both resistant (R) and susceptible (S) populations. Resistance is confirmed if there is a statistical difference between the resistant and susceptible populations.

Previous studies reporting herbicide resistance have used both the GR_{50} and LD_{50} (dose that kills 50% of individuals) ratios to measure the responses to the herbicides (Bourdôt et al. 1990; Hamouzova et al. 2011). In the case of giant buttercup the GR_{50} test was considered less appropriate than an LD_{50} test because this weed has a large underground root system with large regenerative potential (Lamoureaux & Bourdôt 2007) so a test which only measures the reduced shoot weight would probably not indicate resistance. Past research on resistance in giant buttercup had used LD_{50} values for comparing populations (Bourdôt et al. 1990).

The most preferred method for determining the LD_{50} values is by the probit method (Finney 1971). This method uses the estimates of dose responses, which are usually sigmoidal in shape, and transfers them to a straight line so that regressions can be used to calculate the LD_{50} values.

If the R/S ratio (based on GR_{50} values) is less than 10 fold resistance is difficult to confirm and further clarification is needed. This is determined by using both a scientific and an agricultural field definition of resistance (Heap 2011). The scientific definition only requires there to be a statistical difference between resistant and susceptible populations but does not take into account the field rate of the herbicide. The agricultural definition requires the resistant population to survive the recommended field rate under normal field conditions. It is important when dealing with cases of low-level resistance to use both green-house dose-response studies and field experiments, to show the weed has caused a problem and is difficult to control in the field when treated at the recommended field rate (Heap 2011), as sometimes plants are more susceptible in a glasshouse environment.

Plants tested in a dose-response trial must be grown from seed, not collected from the field as mature plants. This is to avoid using plants which may have had unknown previous herbicide treatments or be of different growth stages. This criterion also distinguishes evolved resistance from the build-up of weeds that were never susceptible to the herbicide in the first place. For example, the build-up of populations of field pansy in cereals with the use of chlorsulfuron was not because this weed evolved resistance, but merely that it was never very susceptible to start with (Bourdôt et al. 1998). Finally the plant must be classified as a weed, at least to species level, and not be a crop plant or volunteer cultivated plant that has been deliberately selected for.

Cross Resistance is where a weed population is resistant to two or more herbicides (same or different chemical class), due to the presence of a single resistance mechanism whereas multiple resistance is where resistant plants have two or more resistance mechanisms or are resistant to herbicides with different modes of action (Moss 2002).

1.4.2 Discovery and occurrence of herbicide resistant weeds

Since the first reported case of *Senecio vulgaris* resistant to simazine in 1968 (Ryan 1970), there has been a steady increase in the number of resistant weed biotypes recorded. The rate of increase in discoveries has been relatively constant since 1980 according to the international herbicide resistance database (Heap 2011) (Fig 1.1). This could however be partially explained by increased awareness and search effort and not be a true indication of the incidences of new cases. To date, 365 herbicide resistant biotypes have been found in 200 species (115 dicots and 85 monocots)

around the world (Heap 2011). Grass weeds represented 40% of all resistant biotypes even though they accounted for only 25% of the world's major weeds (Moss 2002). Other weed families which had disproportionately high numbers of herbicide resistant species compared with their representation as principal weeds were: Amaranthaceae, Brassicaceae, Chenopodiaceae and Scrophulariaceae (Moss 2002).

There are currently 60 countries where herbicide resistant weed biotypes have been reported. The countries with the greatest number of reported resistant biotypes are USA (138), Australia (57), Canada (51), France (33), Spain (32), Israel (27), Germany (26), UK (24), Brazil (24) and Italy, (19) (Heap 2011). Resistance used to be confined to temperate countries with intensive agriculture, especially Europe, North America and Australia, but is now increasing in Asian and South and Central American countries as these countries adopt more intensive agricultural practices.

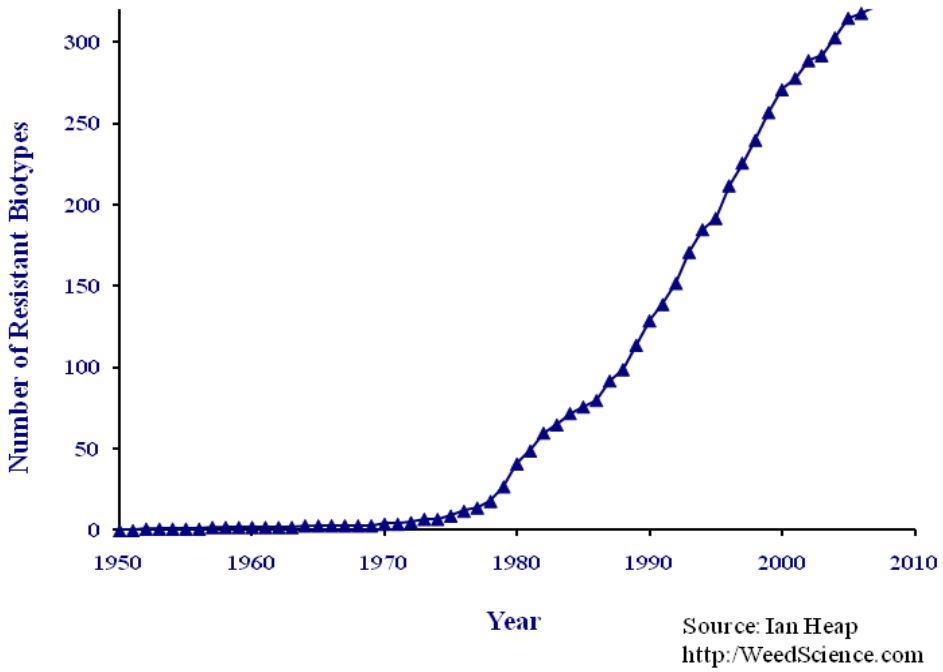

Figure 1.1 Number of resistant weed biotypes recorded worldwide from 1950 – 2010 (from Heap 2011).

1.4.3 Herbicide groups most affected by resistance

Worldwide, herbicide resistant biotypes have been found for all of the 14 mode-of-action groupings available in New Zealand (Heap 2011), although in New Zealand resistant weed biotypes have only been found for four of these 14 groups

(Synthetic auxins, ALS inhibitors, Photosystem II inhibitors and thiocarbamates). Groups are ranked as High-risk (Groups A & B), Medium-risk (Groups C1, C2, C3 & F3) and the rest as Low-risk, based on the number of weed resistant biotypes discovered worldwide (Heap 2011).

Between 1980 and 1990 most cases involved triazine herbicides (Group C1), and examples were found in many countries. It is thought that the evolution of resistance to the triazine herbicides occurred quickly because of high selection pressure and possibly a lack of a buffering effect from the seedbank, as residues remain active in the soil for many months killing any susceptible seedlings that come up within that time (Harrington 2009). Another reason is that these herbicides are often used in crop monocultures where the same crop species is grown, and the same weed control is used, year after year.

Since 1990 resistance to other groups, especially acetolactate synthase (ALS) inhibitors (Group B) and ACCase inhibitors (Group A), has increased dramatically (Fig 1.2). Since 1998, the number of biotypes resistant to ALS-inhibiting herbicides has exceeded the number resistant to triazines (Heap 2011) (Fig 1.2). This was unexpected because triazines have been used widely since the 1960s whereas ALS-inhibitor herbicides were not commercialized until 1982. The first case of resistance to ALS-inhibitor herbicides was reported only 5 years after they became available on the market with chlorsulfuron-resistant prickly lettuce (*Lactuca serriola* L.) (Mallory-Smith et al. 1990). There are now more cases of resistance to ALS inhibitors than to any other chemical class. Currently, 112 of the total 365 worldwide biotypes are related to herbicides that inhibit the enzyme ALS-inhibitor (Heap 2011). Although the number of cases of resistance is increasing steadily, it is due at least in part to the increased awareness, as more people are looking for it (Moss 2002). Other reasons include a high uptake of use for these herbicides because of their popularity (Tranel & Wright 2002). They are popular because they have wide use-ranges, soil residual activity, relatively low toxicity, low dose rates, are relatively inexpensive, and are highly tolerated by pastures and crops (Tranel & Wright 2002).

Although synthetic auxins have been in use for over 50 years, relatively few weeds have evolved resistance to these. In contrast the ALS and ACCase inhibitors have been associated with many more cases of resistance, despite only being available since the early 1980s. Older, broad spectrum pesticides with multi-site action are less likely to select for resistance than modern selective herbicides which target specific single biochemical pathways (Beresford et al. 2009). Therefore new herbicide developments do not always provide an easy solution to overcome resistance (Moss 2002).

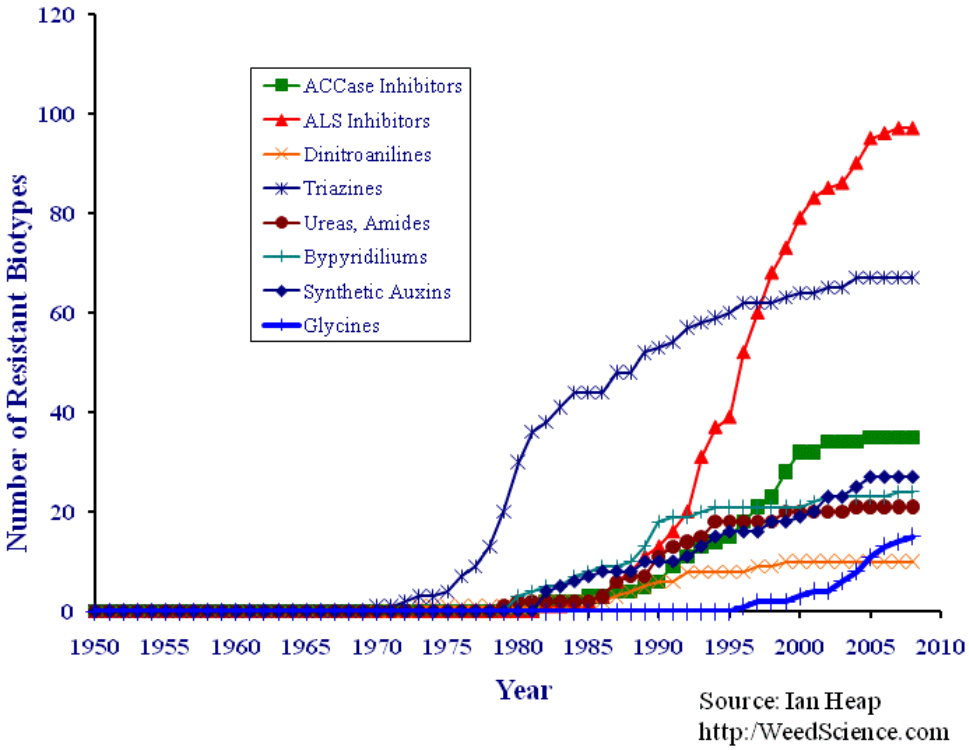

Figure 1.2 Number of resistant weed biotypes recorded worldwide from 1950 – 2010 based on mode-of-action group.

1.4.4 Factors influencing the development of resistance

Both the characteristics of the weed and the herbicide can influence the development of herbicide resistance. Herbicide factors that will increase the likelihood of resistance include a lack of rotation, soil residual activity and a highly specific mode-of-action. Weed characteristics include the initial frequency of the resistant trait to begin with, seed bank longevity and high herbicide efficacy on sensitive weed biotypes (Tranel & Wright 2002; Tharayil-Santhakumar 2004).

Most cases of resistance occur when the same herbicide, or herbicides with the same mode of action, have been used repeatedly over a period of several years (Bourdôt et al. 1990, Rahman et al, 1994; Tranel & Wright, 2002). It is usually associated with intensive agriculture or horticulture involving crop monocultures, or restricted crop rotations, minimum tillage, and systems where herbicides have been relied upon for high levels of weed control. Resistance has occurred in a range of crops but the most widespread problems have been reported in maize, cereal

and rice or horticultural situations such as orchards, nurseries and vineyards (Moss 2002).

The mode of action of the herbicide is an important factor influencing the development of resistance. Some of the newer herbicides that are active at specific sites and have single modes of action tend to be more prone to resistance than older groups with multiple modes of action. Thus herbicides in groups A & B (ALS and ACC'ase inhibitors) tend to have a greater likelihood of developing resistance than other groups, as discussed in the previous section. The high frequency of ALS-inhibitor herbicide resistance cases is partly due to the single-locus-semi-dominant genetics of resistance, the small effects of resistant alleles on plant fitness, and the large number of possible point mutations that can confer resistance to ALS-inhibiting herbicides (Tranel & Wright 2002). Most cases of ALS-inhibitor resistance involve a single nucleotide polymorphism in the ALS gene, resulting in an insensitive target site (Merotto Junior et al. 2010). However, a few cases have involved increased herbicide metabolism resulting in detoxification of the herbicide, and low level resistance to other herbicides with different modes of action (multiple resistance) (Tranel & Wright 2002). The large number of mutations conferring resistance to the ALS-inhibitor herbicides is in contrast to the triazines, where only one mutation has been identified (Tranel & Wright 2002).

Because numerous mutations have been identified it is also difficult to predict how other classes of ALS-inhibitor herbicides will be affected through cross resistance, and different patterns have been observed (McCourt et al. 2006). The mutation site, the specific mutation in the ALS gene and the herbicide binding site are responsible for the different patterns of cross resistance among the five classes of ALS-inhibitor herbicides (McCourt et al. 2006). ALS-inhibitor resistance mechanisms may also differ among biotypes of the same species. For example *Apera spica-venti* (loose silky-bent grass) populations in the Czech Republic showed a wide variation in tolerance to sulfonyl-urea herbicides (Hamouzova et al. 2011), suggesting resistance mechanisms differed between these biotypes.

The biology of the weed can also influence the rate at which resistance can evolve. For example factors which limit herbicide resistance include the length of the weed lifecycle, the seed bank in the soil, lack of mobility of weed seeds and also weed management by non-chemical methods. In theory a longer generation time should slow the rate of herbicide resistance developing. For instance, in many cropping systems some annual weeds only produce seeds once a year but some species, such as giant buttercup, produce seeds continuously over several months. The seed bank provides a buffering effect on selection for resistance, especially in species with persistent seeds, so a weed species that produces large numbers of short-lived seeds will develop resistance faster than one that has a large bank of long-lived

seeds. Mechanical cultivation of soil can aid this process by bringing susceptible seeds to the surface. This may partly explain why herbicide resistance has taken longer to develop than insecticide and fungicide resistance, but did not prevent the rapid development of resistance to some herbicide groups (e.g. ALS and ACCase inhibitors) in many weeds. In contrast to insects and fungal spores which can travel long distances, most weed seeds do not travel far. Transfer of resistant genes over long distances through cross pollination is also unlikely, so farmers have more control over the development and spread of herbicide resistance on their farms than they do of other pesticide resistances (Moss 2002).

1.4.5 Resistance mechanisms

Plant populations can develop resistance from the selection of any phenotypic trait that allows survival after treatment with herbicide (Preston 2002). These can be broadly grouped into two main categories; exclusionary resistance and site-of-action resistance. Exclusionary resistance is where the herbicide molecule is excluded from the site where it causes a toxic response. These can be further grouped into four main categories: a) differential herbicide uptake due to morphological changes, such as waxy or hairy leaves or reduced leaf area, b) differential translocation whereby apoplasmic or symplasmic transport is reduced, c) compartmentation, where herbicides are sequestered in many locations before reaching the site of action and d) metabolic detoxification, where the herbicide is detoxified quickly before it reaches the site of action. Metabolic detoxification has been grouped into four processes (oxidation, reduction, hydrolysis and conjugation).

Site-of-action resistance is where the specific site of herbicide action becomes resistant, which can happen by being altered or overproduced. Firstly the site of action can be altered in such a way that it is no longer susceptible to herbicide. This target based resistance is usually associated with altered binding of the herbicide to the target protein, resulting from a single nucleotide change in the gene encoding the protein to which the herbicide normally binds. For example, in lettuce (*Lactuca sativa*), which is resistant to sulfonyl-urea herbicides, the ALS enzyme (site of herbicide action) is altered so the herbicide can't bind with it (Eberlein et al. 1999). Although this means the herbicide can't interact with the protein it doesn't affect the normal functioning of the enzyme so the plant survives. However, the mutation conferring resistance may also cause other unrelated physiological changes that increase or decrease the fitness of the plant (Tharayil-Santhakumar 2004). For example resistant biotypes have shown reduced photosynthetic ability or poor germination in some cases, or increased germination at lower temperatures in others, e.g. in *Kochia scoparia* (Ragweed). Secondly the site of action can be over produced causing a dilution effect of the herbicide. This means the herbicide can't

inactivate the entire enzyme produced so some of the enzyme produced will carry on normal activity (Tharayil-Santhakumar 2004).

While any biochemical mechanism that improves survival can be selected for, those which confer high levels of resistance tend to be favoured. The two most important mechanisms are target-site modifications and increased rates of herbicide detoxification (Preston 2002). Target site modifications are the most common resistance mechanisms and, for example, have been identified for ALS, ACCase and triazine herbicide resistance cases. Differences in plant metabolism are also common and are sometimes used to confer selectivity to herbicides, as the crop is able to metabolise the chemical but the target weed is not (Moss 2002). The reason these two resistance mechanisms are common in weeds is thought to be partly due to their greater fitness under selection pressure and small fitness costs in the absence of herbicide selection pressure. Other mechanisms, such as reduced translocation of herbicide, e.g. paraquat resistance in *Hordeum* spp and glyphosate resistance in *Lolium rigidum*, tend to occur only where target site and metabolism of the herbicide are impossible (Preston 2002). Other mechanisms involved in resistance have been identified but are less well documented and in many cases the actual mechanisms of resistance remain unknown.

1.4.6 Evolution of resistance

There are two ways resistance may arise in a weed population, as described by Moss (2002). Firstly, a major gene or genes causing resistance may be present at very low levels because of random mutations, which may have occurred before or after the introduction of the herbicide. The herbicide kills the majority of susceptible plants but allows resistant individuals to survive and reproduce, until their progeny dominate the population. The proportion of resistant individuals in the population gradually increases until it becomes obvious that the weed is not being controlled adequately (typically when 10-20% of plants fail to be controlled). The proportion of resistant genes within the whole population is likely to have been increasing for many years before the problem is recognised in the field. Target site resistance is considered monogenic and absolute, meaning resistant plants are usually unaffected by the herbicide so the level of resistance in the population depends on the number of resistant and susceptible plants (Moss 2002).

Secondly, selection may act on the variation in a weed population to achieve a progressive increase in resistance over several generations. This variation may be conferred by several genes, each producing a small effect but together produce a polygenic phenotypic trait. Selection may be acting on resistance genes which are common in the population, but which individually cause a small advantage. Quantitative variation usually means there will be a range of responses to the herbicide, from susceptible to partially resistant to highly resistant plants in one

population. In this case, resistance is due to a progressive shift or increase in the level of resistance in the whole population, rather than the increase in the proportion of very resistant individuals. Enhanced metabolism resistance is generally considered to be conferred by polygenic inheritance. Although it was assumed polygenic resistance was rare in the field, there is now some evidence that non-target site resistance e.g. increased metabolism or reduced translocation (probably polygenic) are more common than previously thought (Doyle & Stypa 2004).

Herbicides are generally strong selecting agents for resistance evolution in weed populations because they cause high mortality and leave few survivors (Preston 2002). Selection for resistance is dependent on many factors but is largely influenced by the level of resistance present in the population to begin with and the intensity of selection pressure (Preston 2002). Firstly the initial frequency of the resistance trait in a weed population will affect the rate at which resistance evolves. The genetic basis of resistance such as the number of genes/alleles involved, the degree of dominance of resistant alleles and mode of inheritance are also important. Secondly, the stronger the selection pressure is, the more rapidly resistance will evolve. This will depend on such things as the frequency of herbicide use, herbicide persistence, pattern of weed emergence, intrinsic activity and the specificity of the herbicide. Fitness differences (advantage of phenotype in terms of survival and reproductive success) will determine how long the resistant biotype persists in a population after the herbicide selection pressure is removed (Maxwell et al. 1990).

Many factors affect the evolution of resistance under herbicide selection, one important factor being the intensity of selection, resulting from the herbicide use rate (Manalil et al. 2011). Although herbicides normally cause high mortality, use rates vary from country to country, and rate cutting occurs commonly for economic and environmental reasons resulting in a lower than normal kill rate. Environmental variability, decay kinetics for soil residual herbicides, and growth stage of the weed can also effectively result in lower than normal rates being applied (Zhang et al. 2000).

Recent studies have shown that low rates of herbicides can lead to the evolution of polygenic resistance through a build-up of resistance alleles, as the intermediate resistant forms are not all killed (Renton et al. 2011). This is especially true for cross-pollinated species. It is thought that low rates increase mutation frequencies creating resistant traits for selection to work on. For example, using lower than recommended rates of diclofop-methyl can lead to rapid evolution of resistance to that herbicide in annual ryegrass (*Lolium rigidum*) and cross resistance to other herbicides (Manalil et al. 2011). While low rates rapidly select for multi-factorial

resistance, high rates encourage the evolution of major single gene (target site) resistance (Gressel 2011). Herbicides should be used at rates which give a high level of kill, to stop the build-up of weak resistance traits, because non-target site resistance mechanisms often confer resistance to multiple herbicide groups and are therefore more difficult to manage (Powles & Yu 2010).

The biological processes that influence resistance are complicated and involve an interaction of life history processes and population genetics. Gene flow and fitness are important processes influencing resistance dynamics (Maxwell et al. 1995).

1.5 Herbicide resistance in New Zealand weeds

Herbicide resistant biotypes have been officially documented in ten weed species in New Zealand (Heap 2011). The first New Zealand case was reported in fathen (*Chenopodium album*) to the herbicide atrazine in maize crops (Rahman 1990). Reports in the late 1970s indicated that fathen was becoming difficult to control in the Waikato region and later near Gisborne, and uncontrolled patches were increasing in size over time. Seedling progeny were grown from some of these populations and treated with a range of doses of atrazine in glasshouse trials and found to tolerate 60 kg/ha (35 to 60 times the normal field dose) (Rahman 1982).

In 1980, willow weed (*Polygonum persicaria)* was also found in a maize crop near Hamilton that was not controlled by atrazine after having been treated annually since 1970 (Rahman & Patterson 1987). Glasshouse trials found seedling progeny from surviving plants in this population were unaffected by 20 kg/ha atrazine whereas populations with no past exposure were completely controlled (Rahman & Patterson 1987). The resistant plants were cross-resistant to eleven other triazines (Rahman & Patterson 1987). It is not surprising that the first cases of resistance were found in maize crops because of the way they are treated regularly with the same herbicides year after year.

In the late 1970s a Hawkes Bay farmer reported large patches of nodding thistle (*Carduus nutans*) on his sheep farm in Argyll not being controlled by 2,4-D anymore. The response of this population, and another nearby population at Matapiro that was controlled by the herbicide, were compared by treating both populations with 20 mg/plant of MCPA or 2,4-D. The tolerance varied greatly between the two populations indicating a genetic difference (Harrington & Popay 1987). Seedling progeny from these two populations were then compared in a series of dose response tests which showed the Argyll population required between five and 30 times more MCPA to give the same level of control. When comparing thistles from both populations growing in pastures, the Argyll biotype had an LD_{50} 6.7 times

greater than that of the Matapiro population (Harrington et al. 1988). Seeds from 28 different populations of nodding thistle were later collected from around Hawke's Bay, Waikato and the Manawatu and the seedling progeny tested for susceptibility to MCPA. Fourteen of these populations were more resistant than the Matapiro plants indicating that resistance had arisen independently in many different areas. After gathering information on spraying history from seven of the most resistant and seven of the most susceptible populations, it was found that resistance had only occurred when plants had been treated year after year with 2,4-D. Further research showed that the Argyll thistles were also cross resistant to MCPA and MCPB but not resistant to picloram, clopyralid, dicamba, mecoprop or glyphosate or chemicals from other groups (Harrington 1989). Resistance is suspected as the cause of herbicide treatment failure in populations in the South Island also, but thorough tests have not been made to confirm this.

In the late 1980s giant buttercup was found to be resistant to the phenoxy herbicides MCPA and MCPB which had been relied upon by farmers since their introduction in the 1950s (Bourdôt et al. 1990). While giving adequate control in the 1950s and 1960s (Tuckett 1961), some farmers were finding that 25-30 years later, these herbicides were not effective anymore. Research by Bourdôt & Hurrell (1988) found that seedling progeny of giant buttercup from different dairy farms in Golden Bay varied widely in their tolerance of MCPA. The same level of variability between farms was also found in the North Island in Taranaki, Hawke's Bay and Wairarapa (Bourdôt et al. 1990). This variable control reflected the level of control achieved in the field by farmers, supporting the idea of genetically based herbicide resistance. The level of resistance was also correlated with the past exposure to MCPA and MCPB (Bourdôt & Hurrell 1990; Bourdôt et al. 1990). Later studies confirmed MCPA resistance is a heritable trait in giant buttercup and that populations can evolve resistance given sufficient selection pressure (Bourdôt & Hurrell 1991), provided by regular spraying of pastures.

Another case of resistance to phenoxy herbicides was found in slender winged thistle (*Carduus pycnocephalus*). Seedling progeny from a population in Hawkes Bay were tested for resistance to MCPA after reports that phenoxy herbicides were failing to control it in this field population (Harrington 1989). Only 13% of 3-month old seedlings were killed in this population with a dose usually lethal to susceptible thistle populations.

Pot trials at Massey University found a resistant biotype of Onehunga weed (*Soliva sessilis*), from turf at Helensville. This biotype was resistant to clopyralid, triclopyr, picloram/2,4-D and picloram/triclopyr mixtures (Harrington et al. 2001).

In 1992/93 chlorsulfuron failed to control chickweed (*Stellaria media*) in two fields rotationally cropped with wheat, barley and oats, at Tussock Creek near Winton and at Riversdale in Southland (Seefeldt et al. 2001). Herbicides had been used annually for ten years in these fields, giving effective control in the past, so resistance was suspected, but it may have been possible that the application equipment was faulty or methods were not followed correctly. Trials by DuPont found that applications at four times the normal rate (80 g/ha) had no effect on chickweed at Riversdale and treatment by the farmer at Tussock Creek had no effect (Bourdôt 1996). In 1993/4, seeds were collected from the suspected resistant population and nearby populations that had never been treated with herbicide. ALS extracted from the suspected resistant population had decreased sensitivity to both chlorsulfuron and tribenuron, compared to that of the control plants, supporting the idea that the supposed resistant population was genetically different (Bourdôt & Suckling 1996).

Chilean needle grass (*Nassella neesiana*) occurs on several farms in Marlborough and in Hawkes Bay. In 1990, aerial applications of dalapon at 2.2 kg/ha were made to stop plants flowering and seeding. In 1992, patches of Chilean needle grass were found still flowering after that season's application of dalapon, and the same thing occurred the following year. Clonally produced plants and seedlings grown from two populations suspected to be resistant, and two populations of plants never exposed to dalapon, were treated with 1, 2, 3 and 6 kg/ha dalapon. Panicle production was unaffected in the suspected resistant population at all rates but was reduced in previously untreated populations above 1-2 kg/ha. This confirmed that plants making up the two patches in the field were resistant to dalapon and able to flower at very high dose rates (Hartley 1994).

Black nightshade (*Solanum nigrum*) plants were found to be poorly controlled by cyanazine in a pea crop in the Manawatu (Harrington et al. 2001). Seedling progeny were subsequently tested and found to be resistant to cyanazine, turbuthylazine, atrazine and prometryn. Plants were unaffected by a dose of terbuthylazine 30 times that of the recommended rate.

Chenopodium album (fathen) was found to be resistant to dicamba in maize fields (James et al. 2005), and survived treatment with four times the recommended rate.

Resistance to paraquat was also discovered in black nightshade species (*S. nigrum* L. and *S. americanum* Mill.) in kumara fields in Northland (Lewthwaite & Triggs 2009), but this has not, to date, been officially recorded with the International Survey of herbicide resistant weeds (Heap 2011).

1.6 Management of herbicide resistance

1.6.1 Herbicide resistance in giant buttercup

Resistance to the phenoxy herbicides MCPA, MCPB, 2,4-D and 2,4-DB has evolved in populations of giant buttercup, slender winged thistle and nodding thistle in several areas of New Zealand. The resistant biotypes cannot be controlled by any of these herbicides because of cross resistance (Harrington 1989; Bourdôt et al. 1994). This makes selective control of these weeds difficult in pastures because there are limited alternative chemicals available to use. Other herbicides that have some selectivity on broadleaved weeds in pastures are clopyralid, asulam, and bentazone but they have little effect on giant buttercup (Bourdôt et al. 1994).

The mechanism responsible for phenoxy resistance in nodding thistle is thought to be due to enhanced degradation of the 2,4-D molecules and less movement of metabolites within the plant rather than reduced absorption, although the exact pathways of degradation could not be determined (Harrington & Woolley 2006). Nodding thistle is still susceptible to chemicals such as clopyralid, dicamba and products containing picloram, but these are all damaging to clover so cannot be used selectively (Harrington 1989; Rahman et al. 1994). One option is to treat pre-flowering thistles with a weed wiper, in which case there are several other herbicide options because the pasture does not come into contact with the herbicide.

It is not known what mechanism is involved with MCPA resistance in giant buttercup but McNaughton (1991) concluded that it was most likely due to less translocation of the herbicide to the rhizome and greater decarboxylation within leaves of resistant plants. The advent of newer herbicides (ALS inhibitors), based on different chemistry, appeared to overcome the problem by targeting different sites of action. Flumetsulam, and to a lesser extent, thifensulfuron-methyl replaced MCPA and MCPB in areas with resistance to phenoxy herbicides in giant buttercup.

Bourdôt et al. (1994) found resistant giant buttercup plants showed cross resistance to 2,4-D and MCPB, but that glyphosate and the sulfonylureas, tribenuron-methyl, chlorsulfuron and thifensulfuron still gave a high level of kill. There was a lower, temporary cross resistance to chlorsulfuron and thifensulfuron, as indicated by slower apical mortality in resistant plants. So even then there was indication that resistance could occur with ALS-inhibitor herbicides despite plants never having been exposed to them. This cross resistance was, therefore, a result of past exposure to phenoxy herbicides alone.

As there are only four herbicides available in New Zealand for selective control of giant buttercup, and these fall within only two mode-of-action groups, there is little flexibility for farmers to rotate herbicides from different groups, as is required for effective herbicide resistance management (Martin et al. 2005). However, there is more scope than for control of nodding thistle in pastures, where only phenoxy herbicides can be used selectively. Also it is not known to what extent farmers rotate these herbicides, or whether they use the same one repeatedly, until they notice control is not as effective and then change to using a different one. Alternatives to these four herbicides may need to be found. For instance, there are some herbicides that have not yet been tested in New Zealand which may be effective, and some which may be effective but are not registered for giant buttercup, and there is potential for biocontrol options to be developed further (Bourdôt 2011). However, as discussed earlier the discovery of newer herbicides may not offer an easy answer. Modern herbicides which target single specific sites in biochemical pathways are more likely to select for resistance than older broad spectrum herbicides which target multiple sites (Beresford et al. 2009).

Weed wiper application of herbicides may offer more options for using the herbicides which have shown efficacy against giant buttercup overseas (Bourdôt 2011) or in New Zealand, but these methods have not been investigated on giant buttercup in New Zealand. Giant buttercup may be a good candidate for this control method because of its size (up to 1 m in height) (Harper 1957) and because it is avoided by dairy cattle, leaving it standing well above the other pasture species.

Herbicide resistance has evolved because too much reliance has been placed on herbicides to control weeds. A more broadly based approach to weed management is needed in which herbicide use is integrated with non-chemical methods of weed management.

1.6.2 Methods for delaying resistance

There are two broad objectives for managing herbicide resistance which are, preventing/delaying evolution, and reducing it once it has developed. Because control of resistant weeds in pastures is difficult, effort should concentrate on prevention of resistance occurring in the first place, and farmers should follow “herbicide resistance management practices” as described by Martin et al. (2005). These include practices such as alternating herbicides from different mode-of-action groups where possible, limiting the number of herbicide applications, restricting use to certain crop growth stages, weed wiper technology using other non-selective herbicides, and incorporating non-chemical control techniques such as pasture renewal and inter-cropping into the farm system.

Leathwick & Bourdôt (1991) developed a phenological model used to evaluate the effect of phenoxy herbicide application date on the rate of increase of resistance in a susceptible population. They found delaying spraying until late summer/autumn rather than the usual spring spraying time reduced selection pressure for resistant phenotypes and delayed the onset of resistance. However, there was a short term cost of lost pasture persistence and a long term cost of seed entering the seed bank.

1.6.3 Decline in resistance over time

The rate of decline of resistance in a weed population after herbicide treatment has stopped depends on many factors including life-history and immigration processes, inheritance and reproduction mechanisms and the relative fitness of the resistant and susceptible phenotypes (Maxwell et al. 1990). According to a model proposed by these authors, resistance should decline in a weed population soon after herbicide application has stopped, provided the susceptible phenotype is more ecologically fit. In some cases the herbicide resistant individuals are less fit than the wild types (Gressel & Segel 1982), but there is conflicting evidence for this case in other studies.

In glasshouse studies with potted plants, atrazine-resistant phenotypes of *Senecio vulgaris* and *Amaranthus retroflexus* (Conard & Radosevich 1979) and a dinitroanaline-resistant phenotype of *Eleusine indica* were less competitive than the susceptible phenotype (Valverde et al. 1988). A triazine-resistant phenotype of *Phalaris paradoxa* was competitively equal to its susceptible phenotype and superior in other ecological attributes (Rubin et al. 1985). In the field there is some anecdotal evidence that if the herbicide selection pressure is removed, resistance declines (Maxwell et al. 1990).

Harrington (1990) found that resistant and susceptible populations of nodding thistle are likely to be equally ecologically fit, and thus if selection pressure was reduced in resistant populations, the proportion of resistant individuals may not decline.

Bourdôt et al. (1996) found MCPA-resistant biotypes of giant buttercup were only slightly less fit than their susceptible counterparts and he estimated treatment would need to be withheld for 28 years for it to return to a level of susceptibility enabling MCPA to give effective control. The LD_{50} of susceptible plants with herbicide treatment stopped for 5 years was two thirds that of continued herbicide treatment plants. The susceptible plants yielded higher dry weights than the resistant plants at low densities, but not at high densities.

Tranel & Wright (2002) concluded that for ALS-resistant weed biotypes, generally the effects of the resistant alleles on plant fitness in the absence of herbicide selection are minimal, so resistance may not decline if herbicide usage is stopped.

According to the model by Maxwell et al. (1990) the most significant influence of relative competitive ability of resistant and susceptible plants occurs soon after the herbicide has been stopped. Within the first 3 years after herbicide suspension 30% resistance remained when the competitive abilities were assumed equal.

Having an adjacent population of susceptible plants can reduce the maximum level of resistance obtained in a population, or reduce time for recovery through providing a source of immigrant pollen. Leaving unsprayed strips in herbicide application was also suggested as a way of reducing efficacy to discourage the evolution of resistance, based on the results of their model.

1.7 Other causes of herbicide failure

Apparent failure of a herbicide to control a weed in the field may be due to many reasons besides the increase and spread of a herbicide resistant biotype (Moss 2002). Environmental variability (weather, soil type, soil decay kinetics, growing conditions, drought, stress, etc.) all play a big role in the effect herbicides have on weeds (Heap 2011; Manalil et al. 2011). Because herbicides disrupt the processes which allow plants to grow and survive, they are all affected by light intensity and temperature. Some herbicides work more quickly in bright sunlight, for example paraquat, while others work more quickly when it is warm and the plants are growing quickly (Young 2010). Proper application of herbicides can make the difference between achieving good control and the apparent failure to control weeds with a product. Some of these reasons are use of an inappropriate product, incorrect application rate, inappropriate application method, blocked spray nozzles, application at wrong time of year, application to weeds at incorrect phenological stage, application under unsuitable weather conditions, and faulty product (Bourdôt & Suckling 1996).

In the case of giant buttercup, the activity of flumetsulam herbicide is known to increase with increasing pH and decreasing organic matter content in the soil (Young 2010). This is a possible explanation as to why farmers have reported control to be variable in the field, and have found the herbicide works better in some paddocks than in others. However, as flumetsulam is mainly absorbed through the foliage, other factors such as plant age, how actively it is growing and timing of application may also be important.

Although flumetsulam, MCPA and thifensulfuron can be applied in spring or autumn, Popay et al. (1984) found the most effective spraying time for MCPA was in August and September. It was thought the herbicide was more effective at this time because the plants were growing vegetatively.

1.8 Reasons for undertaking the study

This study was undertaken because Golden Bay dairy farmers, with giant buttercup infested pastures, have commented that the ALS-inhibitor herbicides, such as flumetsulam and thifensulfuron-methyl, are failing to give adequate control of this weed. These chemicals have been widely used on giant buttercup after this weed became resistant to MCPA in the 1980s. This research was undertaken to investigate these claims further and in particular to determine if evolved resistance to these newer herbicides could partly explain these observations.

The specific objectives of this thesis were:

1. To survey dairy farmers in Golden Bay to find out how widespread their concerns were with regard to control of giant buttercup with herbicides
2. To compare the relative efficacy of flumetsulam, thifensulfuron-methyl and MCPA on giant buttercup populations
3. To determine whether giant buttercup from populations with a high history of use of flumetsulam are more difficult to control than populations with no history of spraying
4. If a difference was found in flumetsulam efficacy, to further investigate the magnitude of the difference in susceptibility between resistant and susceptible populations
5. To compare the effects of the three herbicides on new and established pasture swards to determine whether there was any scope for increasing the rates of herbicides without damaging the pasture in order to deal with any resistance that was found.

Plate 1.1 Close up of giant buttercup leaf showing deeply dissected leaf morphology (Photo by G.W. Bourdôt).

Plate 1.2 Close up of giant buttercup flower (Photo by G.W. Bourdôt).

Plate 1.3 Clumps of giant buttercup plants in dairy pasture showing how grazing cattle avoid it and closely surrounding vegetation (Photo by G.A. Hurrell).

Plate 1.4 A typical dairy pasture in Takaka, Golden Bay, infested with giant buttercup (Photo by G.W. Bourdôt).

Plate 1.5 A dairy pasture badly infested by giant buttercup in Takaka, Golden Bay, New Zealand (Photo by G. A. Hurrell).

Chapter Two
Comparisons of the responses of giant buttercup populations to three herbicides

2.1 Introduction

The control of giant buttercup in pastures has been problematic for farmers since the late 1970s. This is because the extensive use of the 'phenoxy' herbicides (MCPA and MCPB) since the 1950s, which previously gave adequate control (Tuckett 1961), had led to resistance evolving to them, leaving them virtually ineffective on many farms (Bourdôt & Hurrell 1988). Following the decline in the use of phenoxy herbicides, two newer herbicides, thifensulfuron-methyl and flumetsulam, based on a different mode-of-action (both acetolactate synthase (ALS) inhibitors) have been introduced and widely used, especially flumetsulam. These have been on the market for nearly two decades and farmers are now reporting that their effectiveness has been declining, particularly with repeated use over several years. This scenario is very similar to what occurred prior to the discovery of phenoxy resistance in giant buttercup, slender winged thistle and nodding thistle in New Zealand (Harrington et al. 1988; Harrington 1989; Bourdôt et al. 1990) and in other cases of resistance both in NZ and overseas. This leads to the hypothesis that giant buttercup may now be evolving resistance to these newer herbicides as well.

Currently these two newer herbicides, flumetsulam, and to a lesser extent thifensulfuron-methyl, are the herbicides of choice by farmers for broad-acre control, because they are the only herbicides with label claims for giant buttercup in pastures besides MCPA and MCPB. Since they are both ALS inhibitors, their mode of action makes them quite susceptible to resistance developing through repeated use (Llewellyn & Powles 2001). Since ALS inhibitors first came on the market in the early 1980s, the number of weed biotypes resistant to them has increased rapidly, with the first case, prickly lettuce (*Lactuca serriola* L.), being reported only 5 years after the introduction of chlorsulfuron onto the market (Mallory-Smith et al. 1990). There are now more cases of resistance to ALS inhibitors than for any other mode-of-action group (Heap 2011), and these make up nearly a third of all known cases worldwide.

Resistance to herbicides in pasture weeds can be of more concern than in crops because often the only alternative chemicals available for control are damaging to valuable pasture species (Bourdôt & Suckling 1996). There are only four selective herbicides currently registered for giant buttercup control in New Zealand, as mentioned earlier (Section 1.2.1 of this thesis), which are MCPA and MCPB (phenoxy group) and flumetsulam and thifensulfuron-methyl (ALS inhibitors). With

only two mode-of-action groups this leaves little scope for rotating herbicides from year to year which is recommended for the management of herbicide resistance (Martin et al. 2005), although there is more scope than for some other pasture weeds, for example nodding thistle, where only phenoxys can be used (Harrington 1989). It is not known however, whether farmers do rotate these herbicides or repeatedly apply their preferred herbicide year after year.

Although resistance is a likely reason for the apparent failure of flumetsulam to control giant buttercup in the field in some cases, there may be many other reasons. Some of these include inappropriate application methods, faulty product, wrong plant growth stage, and unfavourable environmental conditions (Bourdôt & Suckling 1996). For instance it says on the label for flumetsulam that activity is known to increase with increasing pH and decreasing organic matter content in the soil, as it is taken up by both roots and foliage. Herbicide residues are known to disappear faster for some chemicals in soils with low pH and high organic matter (Rahman et al. 2011). This may partly explain the variability in level of control between paddocks that farmers have sometimes observed, but does not explain their reports of decreasing efficacy over the last decade.

Past research shows only partial control of giant buttercup is achieved with the phenoxy herbicides MCPA and MCPB, and that results are highly variable due to resistance and other factors (Lamoureaux & Bourdôt 2007). However there is very little published empirical data that objectively compares the efficacy and variability of the two newer herbicides flumetsulam and thifensulfuron-methyl with the phenoxys on giant buttercup.

The aim of this experiment was to test the hypothesis that giant buttercup may now be evolving resistance to the newer ALS herbicides, by three objectives:

1) To survey farmers on their past experience with using flumetsulam, thifensulfuron-methyl and MCPA for giant buttercup control, including their history of use and how effective they have found these herbicides to be. This would enable farms to be ranked according to their historical exposure to these herbicides.
2) To test whether variability in efficacy of the three herbicides on giant buttercup populations correlates with their historical exposure to those herbicides.
3) To compare the overall efficacy of flumetsulam, thifensulfuron-methyl and MCPA on a wide range of giant buttercup populations.

2.2 Survey

2.2.1 Methods

A list of farmers in Golden Bay, known to have giant buttercup on their properties was obtained from a local dairy farmer Graeme Ball and Dairy New Zealand board member Sue Brown. Of the 47 property owners on the register, 30 names were randomly selected (for logistical reasons). They were sent either a letter or email explaining the project and asked to fill in a questionnaire about past herbicide use on their property and control outcomes for giant buttercup (Appendix 1). Each person was later phoned and their answers to the questions were recorded. Twenty-one out of the thirty farmers were able to be contacted and could answer the questions in the survey. Information was sought on their experiences with using flumetsulam and thifensulfuron-methyl including the history of use, application rates, number of applications, time of year, how effective the applications were initially and longer term. Also the history of use of MCPA or other herbicides and how well these have worked in the past was recorded.

Each farm's history of herbicide use was then ranked according to the intensity of historical spraying of flumetsulam, by the number of times the herbicide had been applied in the last 12 years.

2.2.2 Results of Survey

Seventeen out of the 21 farmers had used flumetsulam within the past 12 years, their responses ranging from once, to every year since the herbicide came on the market (Table 2.1). Farmers reported mixed success with using flumetsulam; some said it worked well, others not so well, and six (29%) made the comment that they found it to be less effective now than when they first started using it several years ago. One farmer said it worked better now than when he first started using it, probably because the density of buttercup was now lower and the pasture sward more competitive. Other farmers commented that when spraying flumetsulam on the same day with the same tank mix it would work well in one paddock and not in another and control was often variable. They thought this may have been because it is sensitive to cloud cover and/or other environmental effects. Others commented that it seems to work better in higher drier paddocks than lower areas that are prone to bogging and flooding or on river flats. One farmer commented that when he tried using MCPA recently after not using it for 10 years it worked quite well, even though this buttercup had been very resistant to this herbicide in the past. This may indicate a decline in the frequency of the resistant biotype over time and replacement with a more susceptible biotype, which was implicated by previous research (Bourdôt et al. 1996) or maybe due to an improved formulation of the herbicide. Only one farmer out of the 21 used the phenoxy herbicides

(MCPA) as the primary control method, while the others used phenoxy herbicides to varying degrees ranging from no use to regular use combined alternately with other herbicides.

Of the 21 farmers contacted, six had used thifensulfuron-methyl but only once or twice as they said it was too damaging on the pasture to ever use again. Since no farmer had used this chemical regularly on the same paddocks it was unlikely that sufficient selection pressure had resulted in resistance from its use but it was still included in the study to test for cross resistance between the two ALS herbicides.

One farmer said he suspected resistance to flumetsulam was occurring, because it had worked better in the past than it does now, but he was unaware that most of the buttercup on his property was celery-leaved buttercup (*R. sceleratus*), rather than giant buttercup. This may be because the herbicide had been more effective on giant buttercup and the celery-leaved buttercup was selected for over time. Equally, it may be because celery-leaved buttercup is an annual plant that may well have been controlled by the herbicide but due its large seed bank, it re-grew every year. However the farmer reported spraying with flumetsulam the previous spring (3 months before our visit), and the celery-leaved buttercup seemed unaffected at this time, which may indicate some level of tolerance to flumetsulam. In order to test this, seed would need to have been collected from this and a non-treated population and the tolerance of seedling progeny compared.

Table 2.1 Results from survey of farmers about their herbicide use on giant buttercup.

Question	Number of farmers
Number of farmers who responded	21 (out of 30 contacted)
Have used flumetsulam?	17
Have used thifensulfuron-methyl?	6
Have used phenoxy herbicides in last 12 yrs?	11
Noticed decline in efficacy with flumetsulam?	6

2.3 Experiment 1 Comparing the dose responses of giant buttercup populations to flumetsulam, thifensulfuron-methyl and MCPA

2.3.1 Methods for seed collection

Seeds of giant buttercup were collected from paddocks where control was reported satisfactory and those where herbicide resistance may be occurring, based on the farmer's information supplied in the previous survey. Twelve of the Golden Bay farms included in the survey were visited between January 16-20th 2010 and seeds

were collected from them, and also from a roadside patch at Leithfield, North Canterbury (Fig 2.1). It was presumed the roadside patch from North Canterbury had never been treated with any of these herbicides, and the previous studies on giant buttercup had used seeds collected from Saltwater Creek nearby (Bourdôt et al. 1990).

For each paddock from which seed was collected, the farmer was again questioned about how many times the site had been treated with the different herbicides. Seed was collected from 15 different buttercup populations in total (Table 2.2), with a range of past treatments from no herbicide use in the last 35 years to flumetsulam every year since it came on the market, and from one farm with no past use of flumetsulam but high phenoxy usage (Table 2.3). This latter site was included in the study to test for multiple/cross resistance between and within the two mode-of-action groups. Locations of the 15 sites where seed was collected from are shown in Figure 2.2 and Table 2.2. Seeds were collected from as many plants as were available and from right across each entire paddock.

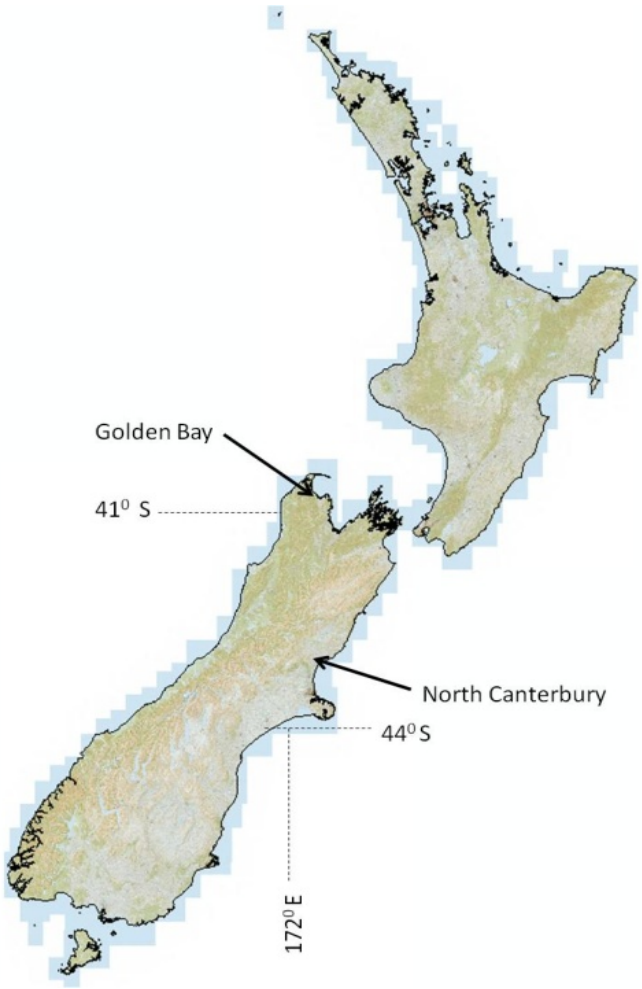

Figure 2.1 Map of NZ showing location of sampling regions

Table 2.2 Farm code, district and map coordinates (Latitude/Longitude, decimal degrees World Geodetic System 84) of the sites where seeds were collected for the study**.**

Farm	District	Latitude	Longitude
B	Golden Bay	-40.8544	172.7518
Bh	Golden Bay	-40.8457	172.7648
Br	Golden Bay	-40.6614	172.6459
C	North Canterbury	-43.1990	172.7351
Dp	Golden Bay	-40.7360	172.6854
Dt	Golden Bay	-40.8146	172.7579
Fb	Golden Bay	-40.8283	172.7944
Fm	Golden Bay	-40.8327	172.7930
G	Golden Bay	-40.8718	172.7813
J	Golden Bay	-40.8399	172.8179
Lm	Golden Bay	-40.8848	172.7887
Ls	Golden Bay	-40.8888	172.7896
P	Golden Bay	-40.8665	172.8554
Pg	Golden Bay	-40.8649	172.8027
R	Golden Bay	-40.8346	172.8023

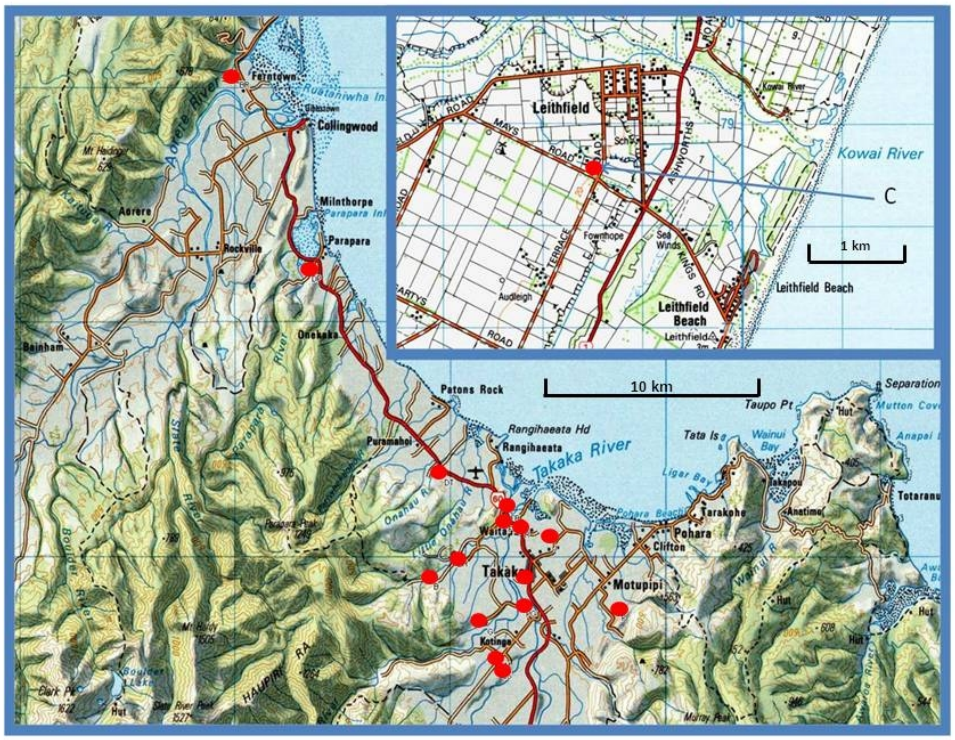

Figure 2.2 Location of 14 sites in Golden Bay where seed was collected from and the one North Canterbury site (inset). Sites are shown by red dots.

Table 2.3 List of the 15 populations of giant buttercup used in Experiemnt 1 and their approximate number of treatments of flumetsulam, thifensulfuron and MCPA/B over the last 12 years (1997 – 2010). Populations are ordered according to increasing intensity of historical exposure to flumetsulam. Population codes identify farmer name and locality.

Population code	flumetsulam	thifensulfuron-methyl	MCPA/B
J	nil	nil	unknown
Dp	nil	nil	nil
Dt	nil	nil	nil
C	nil	nil	nil
R	nil	2	high
P	3	nil	occasional
Pg	6	nil	occasional
Lm	6	nil	high in past, occasional last 12 yrs
Br	7	nil	occasional
Ls	8	nil	occasional
Fm	8	1	occasional
Fb	8	1	occasional
B	10	nil	high in past, occasional last 12 yrs
Bh	10	nil	high in past, occasional last 12 yrs
G	12	nil	regular use

2.3.2 Seed germination, seedling planting and experimental design

On 29 March 2010, half of all seeds collected were soaked overnight in 0.02% solution of KNO_3 (about 300 from each population). On 30 March seeds were dried on paper towels and dusted with a 50:50 w/w mix of captan (Captan 80W, 800 g/kg) and benomyl (Benlate, 500 g/kg) wettable-powder fungicides, using approximately 0.5 g powder per 5 g seed. The seeds were then sown into trays of damp vermiculite (one tray per population), lightly covered with vermiculite, then trays placed in clear plastic bags into a Contherm growth cabinet set to 20^{o}C and 10^{o}C, light and dark on a 12 h cycle. The first seedlings emerged on the 12 April, 14 days after sowing (Plate 2.1, end of chapter, pg 75). Trays were moved into an un-heated glasshouse two days later, with its temperature limited to 20^{o}C to prevent seedlings becoming too etiolated. Watering was by hand as required.

On 20 April the buttercup seedlings were pricked out and planted into 90 mm (500 ml) square pots (2 seedlings per pot) filled with potting mix, watered and left in the glasshouse. The potting mix was a mixture of 60% bark wood chips, 40% crusher dust, with agricultural and dolomite lime, Osmocote® slow release fertiliser and other minerals added (Appendix 2). Seedlings were still at the cotyledon stage (Plate 2.2). The next day all pots were randomly assigned to the treatments (Table 2.4), labeled, and organised into a split block design (as shown in Figure 2.3).

The treatments were a logarithmic series (2.236 multiplier) of five doses of thifensulfuron-methyl (Harmony, DuPont NZ Ltd), the dimethylamine salt of MCPA (Maestro 750) and flumetsulam (Preside) (both Dow Agrosciences NZ Ltd), each with two rates above and below the recommended rates plus an untreated control, making 16 treatments in total (Table 2.4). The middle rate chosen for thifensulfuron-methyl, however, was slightly lower (33%) than the field recommended rate for giant buttercup because this product had undergone a change to its formulation strength (750 g/kg to 500 g/kg) and the lower strength product (500 g/kg) was used for the experiment, but unfortunately using the outdated field rate of use (20 g instead of 30 g/ha).

Table 2.4 Dose-rate treatments for the herbicides used in Experiment 1, (five rates of each herbicide plus an untreated control).

Proportion of recommended rate	flumetsulam (g ai/ha)	thifensulfuron-methyl (g ai/ha)	MCPA (kg ai/ha)
0.0	0.0	0.0	0.0
0.20	10.4	2.0	0.25
0.45	23.2	4.5	0.56
1 (label rate)	52.0	10.0	1.25
2.24	116.3	22.4	2.79
5.00	260.0	50.0	6.25

The number of seedlings that germinated only allowed for six replicates of 11 populations plus four replicates of four populations. This meant arranging the blocks in the glasshouse as follows:

Four blocks of 16 treatments * 15 populations = 240
Two blocks of 16 treatments * 11 populations = 176
4(240) + 2(176) = 1312 pots in total (2624 seedlings in total as 2 seedlings per pot).

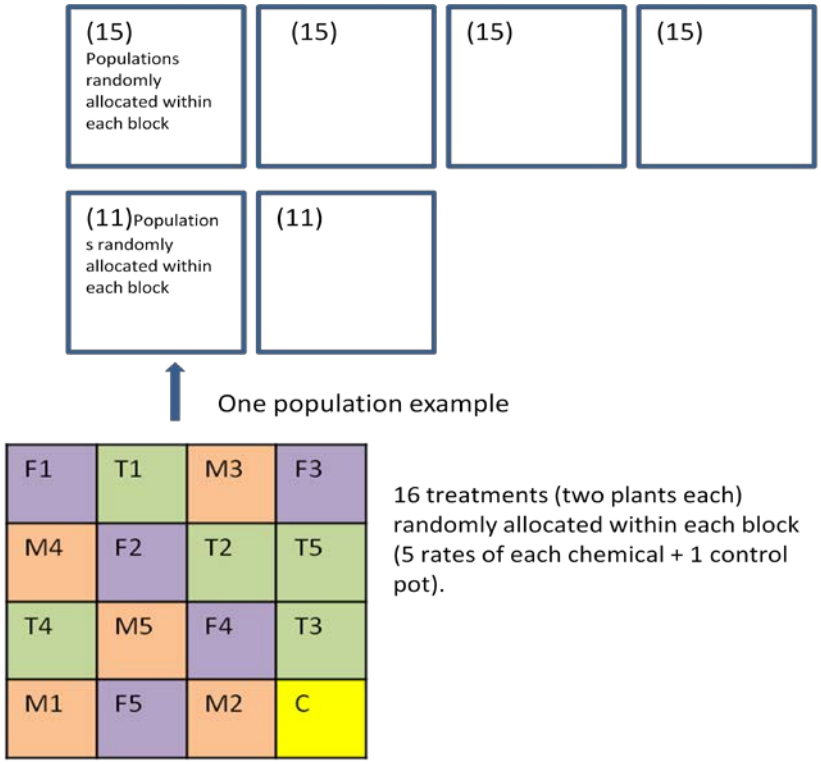

Figure 2.3 Diagrammatic layout of experiment 1**:** Split plot design, 4 blocks of 15 main plots (populations) plus 2 blocks of 11 main plots, 16 sub-plots (treatments) for each main plot. F = flumetsulam, T = thifensulfuron-methyl, M = MCPA, C = control.

After 10 days some unthrifty seedlings that had not transplanted well were replaced with spare giant buttercup seedlings from the appropriate populations.

On 24th June, about halfway through the growing phase, all blocks were taken out of glasshouse and replaced in reverse order to prevent the plants at the back of the glasshouse becoming etiolated because of lower daytime light level. This was because plants at the back were partially shaded due to a high end wall in the glasshouse (Plate 2.3).

On 6th August the buttercup plants were moved out of the glasshouse into a nearby shadehouse to allow more room for ease of assessments, and because the leaves and stems of the buttercup plants had become tangled and etiolated from growing too closely together (Plate 2.4). Plants were arranged into groups of the same treatment and spaced further apart, to allow better coverage of herbicide to all leaves during belt sprayer application. As plants were quite etiolated some damage occurred to leaves and petioles when being separated, and became droopy as some couldn't support their own weight. Although the plants had long petioles they had large normal leaves, more typical of giant buttercup in the field when growing amongst long dairy pasture (personal observation).

2.3.3 Spray application

On the 20th August (14 days after being transferred to the shadehouse) the now 4-month old buttercup plants were moved to a sheltered location nearby for spraying. Spray treatments were then applied between 8am-11am under calm cloudy conditions (temperature slowly increased from 5-10˚ C). Plants were mostly dry but there were some water drops on the undersides of some leaves. It had been cold and rainy the day prior to spraying and was fine, cool and cloudy for two days after spraying. The chemicals were mixed up with water immediately prior to spraying and the calibration of the sprayer checked. Mixing rates are shown in Tables 2.5, 2.6 and 2.7.

Table 2.5 Rates for flumetsulam* (Preside™, Dow Agrosciences NZ Ltd) used in Experiment 1.

flumetsulam	g ai/ha	g product/ha	mixing rate (g/L)
	10.40	13.00	0.065
	23.25	29.07	0.145
Label rate	52.00	65.00	0.325
	116.26	145.33	0.727
	260.00	325.00	1.625

*field rate 65 g/ha in 200 litres water plus Uptake oil 500 ml/100L. Preside™ contains 800 g/kg flumetsulam = 52 g ai/ha @ 65 g Preside/ha. Adjuvant rate 1 L/ha = 5 ml/L.

Table 2.6 Rates for MCPA* (Maestro™ 750, Dow Agrosciences NZ Ltd) used in Experiment 1.

MCPA	kg ai/ha	L product/ha	mixing rate mls/L
	0.25	0.33	1.667
	0.56	0.75	3.727
Label rate	1.25	1.67	8.333
	2.79	3.73	18.632
	6.25	8.33	41.662

*1.5-3 L/ha in 120-280 litres water. The MCPA product to be used contains 750 g /kg MCPA = 1.125-2.25 kg ai/ha

Table 2.7 Rates for thifensulfuron-methyl* (Harmony®, Du Pont NZ Ltd) used in Experiment 1.

thifensulfuron-methyl	g ai/ha	g product/ha	mixing rate (g/L)
	2.00	4.00	0.020
	4.47	8.94	0.045
Label rate	10.00	20.00	0.100
	22.36	44.72	0.224
	50.00	100.00	0.500

*20 g/ha in 200 L water. Harmony contains 500 g/kg thifensulfuron-methyl = 10 g ai/ha

The herbicides were applied by a moving conveyer belt sprayer with the sprayer delivering 200 L/ha at 210 kPa, at a flow rate of 0.64 L/min through a single 8002EVS flat-fan Teejet® (Spraying System Ltd) hydraulic nozzle mounted 30cm above the plants (swath width 50cm). The conveyor belt speed was 1.06 m/s.

The plants were sprayed in the order of the low rate to high rate of flumetsulam, thifensulfuron-methyl then MCPA. Plants were put through in treatment groups, made up of the replicates and populations, which were randomised within each treatment. Each treatment consisted of 82 pots in trays of up to 10 per tray which were put through individually, taking about one minute to spray each treatment. The spraying equipment was flushed with clean water between treatments and all equipment double-rinsed with water between chemicals. After spraying, trays were replaced on tables in the shadehouse, in treatment groups to prevent cross contamination of wet plants.

A few days later, plants were rearranged back into the split plot design and left for the remainder of the experiment. Plants were watered regularly by hand from above throughout the experiment to prevent drought stress and treated with Phostrogen (PBI Home and Garden Ltd, Hertforshire, UK) liquid fertiliser on 25

August and again with Nitrophoska Blue (Ravensdown fertilizer, NZ) granular fertiliser on 21 October (5-6 granules per pot).

Temperature was recorded at hourly intervals throughout the experiment with a TinyTag temperature datalogger (Gemini Dataloggers UK). Mean daily temperatures, as measured in the shadehouse, from the time of spraying onwards, steadily increased throughout the 3 month period of the experiment (Figure 2.4) which was typical of growing conditions for Canterbury at that time of year. The mean, minimum and average temperatures were similar to those experienced in the preceding 5 years (source NIWA Virtual Climate Weather Station http://cliflo.niwa.co.nz/ accessed 22nd Sept. 2011).

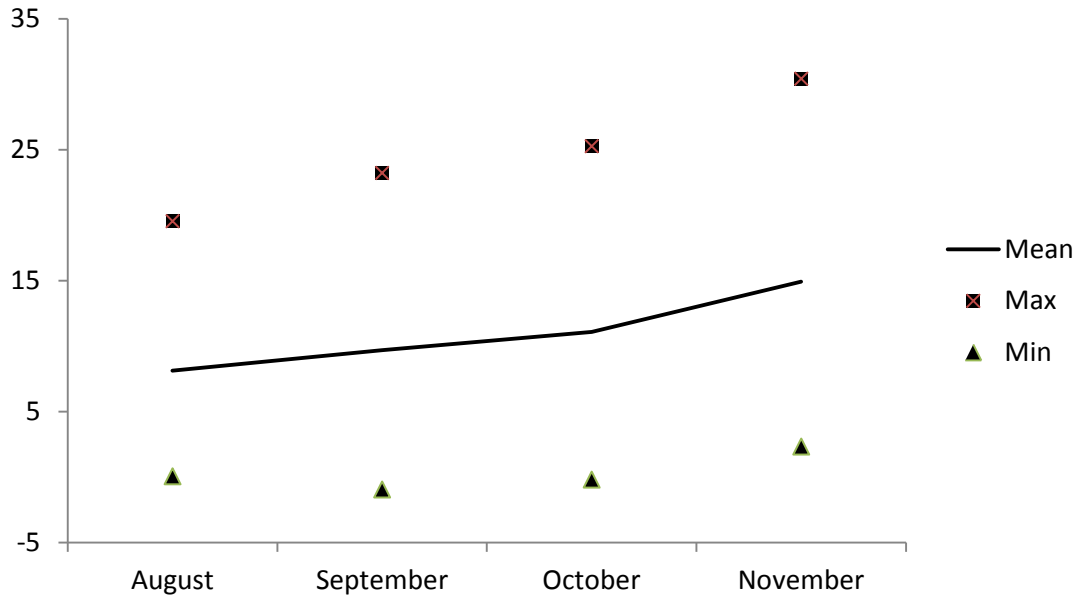

Figure 2.4 Min, max and mean monthly temperatures for 20th August, to late November, recorded in the shadehouse throughout Experiment 1.

2.3.4 Assessment

On 17 August each of the two plants per pot were given a vigour score prior to spraying based on their size and robustness which ranged from 0-5, with 0= dead and 5=very big and healthy, and then the sum of the two scores was calculated, giving a maximum score of 10 for each pot.

Plants were assessed at 27, 59, 81 and 101 days after treatment. This was done by estimating the amount of green tissue (biomass) remaining in each pot relative to the untreated control for that population and replicate. About 3 months after treatment (101 days) the number of surviving plants in each pot was also recorded. A plant was considered dead if there was no green plant material left on the surface of the pot and the remaining dead material pulled away from the soil surface easily.

2.3.5 Data Analysis

The final mortality data were analysed using GenStat's (Ver 13) ANOVA procedure. Means of percent mortality were calculated for each population (averaged over all five rates) and for each rate separately. Separate LSDs (Least Significant Differences) were calculated for comparing between and within populations of four and six replicates.

The GenStat output gave LSDs for comparing two populations both with six replicates. To compare two populations both with four replicates, an LSD was calculated by multiplying the LSD by $\sqrt{}$ (6/4) and to compare between two populations with six and four replicates, the multiplier was $\sqrt{}(1/6 + 1/4) \div \sqrt{}(2/6)$.

The percent mortality for each population (averaged over all five herbicide rates) for each herbicide was plotted against number of past treatments of flumetsulam and regression slopes fitted to the data using Microsoft Excel, then graphs reproduced in CoPlot. A Student's t-test (slope/[se(slope)]) was used to determine whether there was a significant increasing trend.

For each population and herbicide, LD_{50} values (dose required to kill 50% of population (g or kg ai/ha)) were calculated. This was done by fitting a probit curve (using block contrasts for the six blocks) relating the percent mortality to the $\log_{10}$dose rate using a generalised linear model assuming a binomial distribution and a "probit link function" using Genstat (VSN International, Version 13). The parameters (constant and slope) from the Genstat printout were then used to calculate LD_{50} values for each population and herbicide in Microsoft Excel using the following formula:

(equation 1) $Log_{10}(LD_{50})$ = -(constant/slope), then

(equation 2) $LD_{50} = 10^{(Log10(LD50))}$

The parameter estimates from Genstat (constant and slope) were also used to plot the fitted probit curves for each herbicide and population, onto graphs in Microsoft Excel. The formula used to calculate the curves was:

Percent mortality = cumulative normal distribution function
(constant + slope*X) * 100, where X is $\log_{10}$ herbicide dose

Actual data points of percent mortality at each rate for each population were also plotted onto the graphs along with the probit curves. The $\log_{10}(LD_{50})$ and LD_{50} values could then also be determined by reading from off these graphs. For some populations the curve showed a very poor fit to the data because mortality was

close to 100% for the higher rates, so the curve was re-calculated using mortality data from only the bottom three rates instead of all five. For these graphs the curves were only plotted between data values for the lower three rates (as indicated in Figs 2.8, 2.9 and 2.10). The reason for omitting the top two rates from the Genstat analysis was that when the fitted probit curve gets close to 100% (or 0%), the data does not conform to the statistical algorithm, (akin to trying to divide something by 0 for which the answer is ∞, infinity). One rate with approximately 100% is generally acceptable, but with two or more rates with values about 100%, the fitted curve for the highest rate is very close to 100%, and unrealistic results occur.

The $\log_{10}(LD_{50})$ values generated for each population and herbicide were also plotted on graphs against past number of treatments of flumetsulam (exposure history) and a regression line fitted to the data. A Student t-test (slope/[se(slope)]) was used to determine whether there was a significant trend of increasing LD_{50} with increasing historical exposure to flumetsulam.

Biomass data collected in the trial were summarised in CoPlot in various ways.
The reductions in biomass of giant buttercup plants over time and herbicide rate were summarised graphically in CoPlot.

2.4 Results

2.4.1 Biomass data

At 27 days after spraying, MCPA-treated giant buttercups showed a much greater reduction in biomass than those treated with either flumetsulam or thifensulfuron-methyl treated, and there was a strong rate effect (Figure 2.5). At 59 days all three herbicides had reduced the biomass of buttercups to a similar low level. At 81 days, there was some evidence that survivors of thifensulfuron and MCPA treated buttercups were re-growing at recommended rates and below, but the biomass of flumetsulam treated plants continued to decline.

At 101 days surviving plants treated with either MCPA or thifensulfuron-methyl showed large increases in biomass compared to that at 81 days at 0.2 and 0.45 times recommended rates (70-80% that of untreated at 0.2 times recommended rate for MCPA and thifensulfuron respectively). At the recommended rates and above MCPA treated plants had no regrowth, but thifensulfuron-methyl plants showed some regrowth. Flumetsulam treated plants showed some regrowth of survivors at 0.2, 0.45, 2.2 and 5 times recommended rates, when averaged over all populations, while at the recommended rate biomass was only about 1% that of untreated.

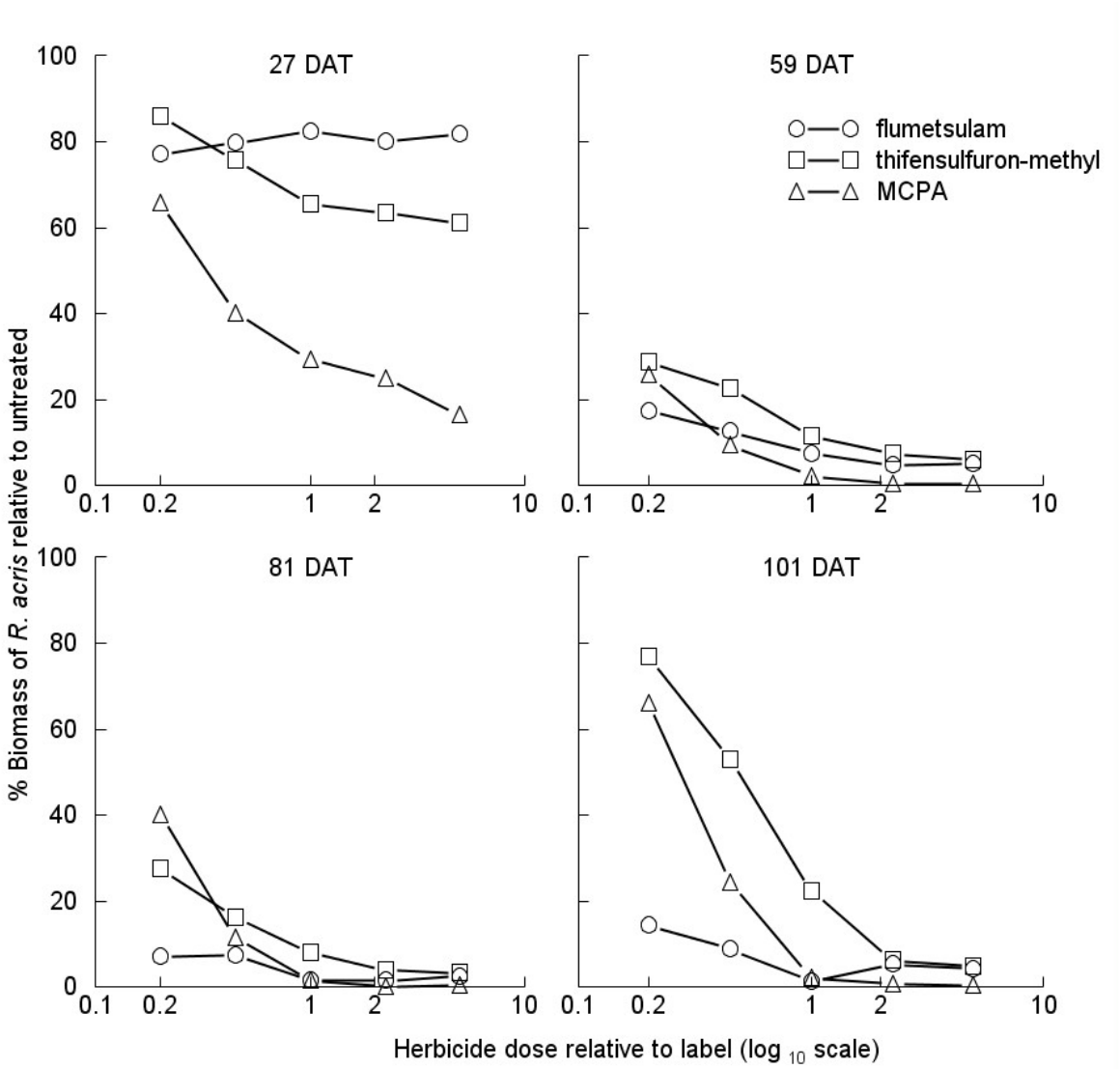

Figure 2.5 Reduction in biomass (relative to untreated plants) of 4-month old giant buttercup plants at 27, 59, 81 and 101 days after treatment with flumetsulam, thifensulfuron-methyl and MCPA applied at 5 rates in Experiment 1. Data are the mean of 15 populations (82 pots per treatment).

There was a correlation between tolerance to flumetsulam and past exposure to flumetsulam in the seedling progeny that became more pronounced over the 3-month period (o symbol, Figure 2.6), as was indicated by the R^2 values that increased at each assessment time. At 101 days after treatment the biomass of flumetsulam treated buttercups was generally higher for populations that had had a higher number of past treatments of ALS herbicide.

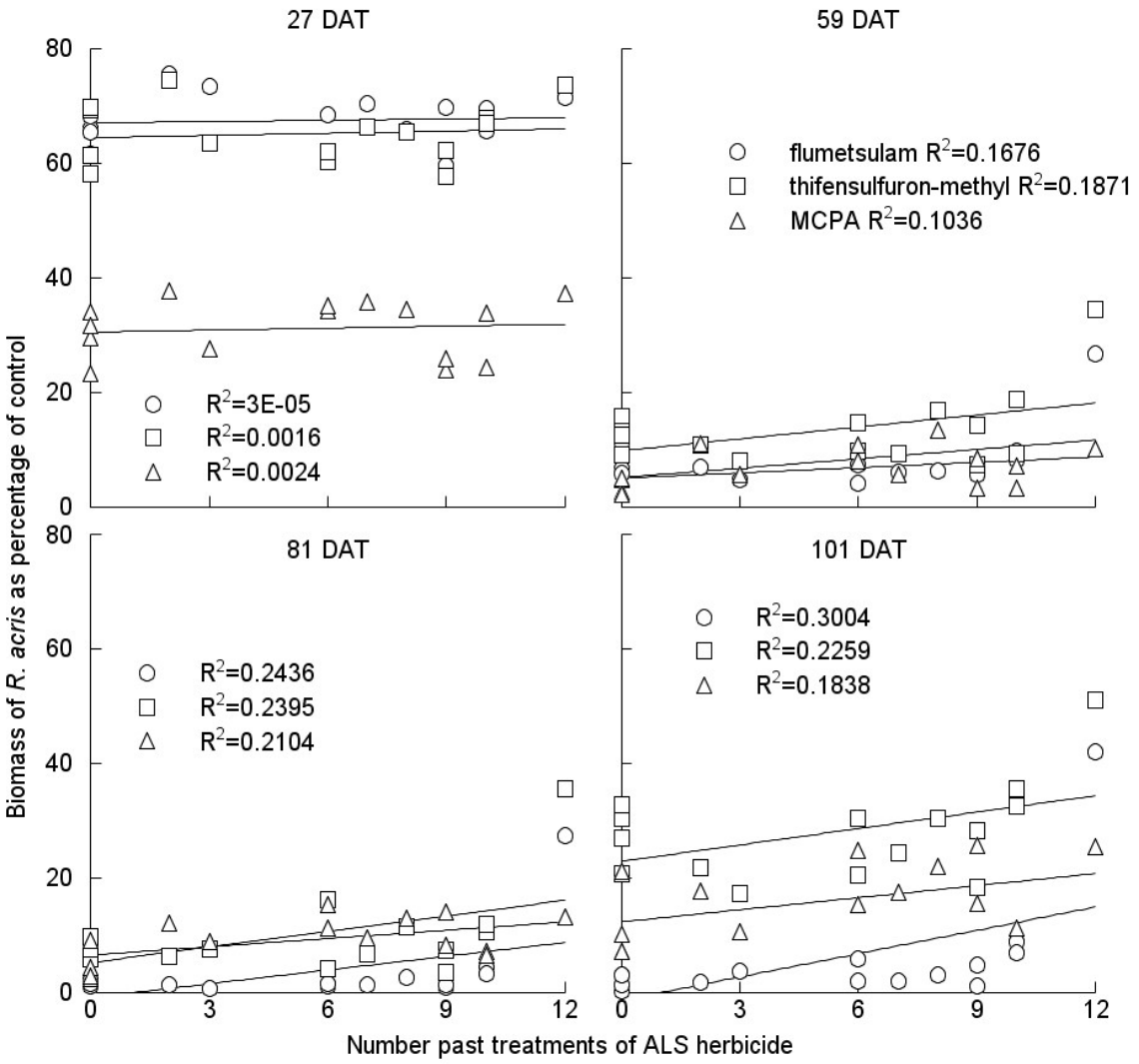

Figure 2.6 Reduction in biomass of giant buttercup plants (4 months old at time of treatment) at 27, 59, 81 and 101 days after treatment with flumetsulam, thifensulfuron-methyl and MCPA applied at five rates, correlated with past exposure to flumetsulam in Experiment 1. Data is the mean over all 5 application rates for each herbicide.

There were differences in the responses to the three herbicides when comparing three populations that differed in their past exposure to flumetsulam: 'C' with no previous exposure, 'R' with no ALS exposure but frequent phenoxy exposure, and 'G' with frequent phenoxy and ALS exposure, (Figure 2.7).

At 27 days after treatment with flumetsulam, the biomass of population 'C' was similar, but slightly lower than the biomass of populations 'R' and 'G' at all rates. At 59, 81 and 101 days the biomass of populations 'C' and 'R' were much lower than population 'G' at all rates, although less so for the treatment at the recommended rate (Figure 2.7).

At 27 days after treatment with thifensulfuron the biomass of population ‘C’ was again reduced slightly more than populations ‘R’ and ‘G’ at all rates. At 59 and 81 days the biomass of populations ‘C’ and ‘R’ were again much lower than for population ‘G’, however at 101 days, although the biomass of populations ‘C’ and ‘R’ tended to be much lower than ‘G’ at all rates, results for 0.45 times recommended rate were unexpectedly higher for population ‘C’ (Figure 2.7).

At 27 days after treatment with MCPA, the biomass of population ‘C’ was consistently lower than populations ‘R’ and ‘G’ at all rates. At 59, 81 and 101 days the biomass of ‘C’ was reduced more than ‘R’ and ‘G’ only at 0.2 and 0.45 times recommended rates, while at the recommended rate and above biomass for all populations was virtually zero (Figure 2.7).

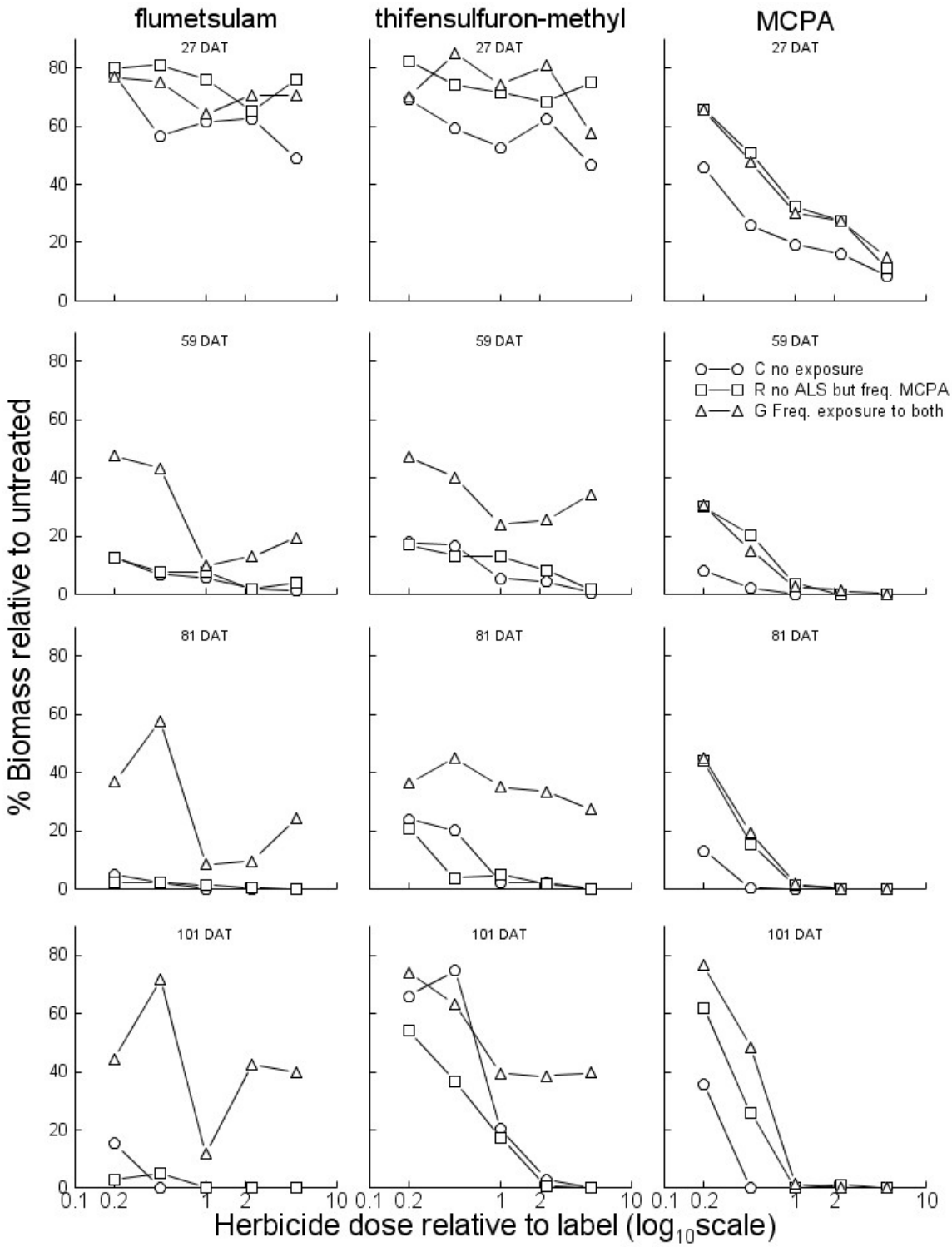

Figure 2.7 Dose responses (biomass) of giant buttercup plants (4 months old at time of treatment) to flumetsulam, thifensulfuron-methyl and MCPA at 27, 59, 81 and 101 days after treatment in Experiment 1. Data is for three populations only with differing past exposures to flumetsulam: 'C' with no previous exposure, 'R' with no ALS exposure but frequent phenoxy exposure, and 'G' with frequent phenoxy and ALS exposure.

2.4.2 Mortality Data

The giant buttercup populations varied in their responses to the five rates of flumetsulam. At the recommended field application rate (1 in Table 2.8) this was the most effective of the three herbicides compared in this experiment with an average mortality of 97% across all populations. Population 'G' had the lowest mortality of all the populations for rates of 0.45 to 5.0 times recommended rate. This difference was most pronounced at 2.2 times the recommended rate where Population 'G' had lower mortality than all other populations (by at least 25%) and significantly lower mortality than 12 of the other 14 populations. Flumetsulam caused 50% or higher mortality in nine of the 15 populations at 0.2 times the recommended rate.

Table 2.8 Percent mortality for the 15 populations of giant buttercup after treatment with each of five rates of flumetsulam in Experiment 1. LSDs (in footnote) are for comparing populations with 4 or 6 replicates with other populations with 4 or 6 reps. All populations have six replicates apart from the last four (shown in brackets) which have four. Stars indicate populations with significantly higher mortality than the population with the lowest mortality (underlined) at each rate.

	Proportion of recommended rate				
Population	0.2	0.45	1	2.2	5
J	58.3	100*	100	100*	100
Fb	58.3	100*	100	100*	100
B	50	83.3*	100	83.3	91.7
Bh	50	66.7	91.7	91.7*	91.7
Br	58.3	75	100	100*	100
Ls	50	75	100	100*	100
Dp	50	91.7*	91.7	100*	100
Dt	33.3	100*	100	100*	100
C	33.3	91.7*	100	100*	100
R	75.0*	83.3*	91.7	100*	100
G	50	50	83.3	58.3	75
(Pg)	37.5	75	100	100*	100
(P)	50	100*	100	100*	100
(Fm)	62.5	100*	100	100*	100
(Lm)	37.5	87.5*	100	87.5	100
Mean	**50.3**	**85.3**	**97.2**	**94.7**	**97.2**

P<0.001; LSD (5%) for comparing 6 vs 6 reps = 29.4, 4 vs 4 reps = 36.0 and 4 vs 6 reps = 32.8

Thifensulfuron-methyl, by comparison, was the least effective of the three herbicides compared here with the overall lowest mortality percentages across populations at each rate (Table 2.9). Although the middle rate used was slightly lower than the recommended application rate (10 g instead of 15 g/ha), mortality

was still lower than the other two chemicals overall. At Rate 4, (22.4 g ai/ha) which is equivalent to 1.5 times the recommended application rate, this chemical gave only 75% mortality across all populations. This compares to flumetsulam and MCPA which gave 97 and 95% mortality at the recommended rate, respectively. No one population stood out with consistently lower mortality across all rates, although population 'G' had the lowest mortality of all populations at 5 times the recommended rate by at least 17% but only significantly lower than 10 of the other 14 populations.

Table 2.9 Percent mortality for the 15 populations of giant buttercup after treatment with each of five rates of thifensulfuron-methyl in Experiemnt 1. LSDs are for comparing populations with 4 or 6 replicates with other populations with 4 or 6 replicates. All populations have 6 reps apart from the last four which have four, shown in brackets. Stars indicate populations with significantly higher mortality than the population with the lowest mortality (underlined).

	Proportion of recommended rate				
Population	0.2	0.45	1	2.2	5
J	16.7	0	50	58.3	91.7*
Fb	8.3	25	58.3	83.3	91.7*
B	25	16.7	50	58.3	83.3
Bh	16.7	16.7	33.3	66.7	100*
Br	8.3	8.3	50	100	91.7*
Ls	8.3	41.7	41.7	75	75
Dp	0	0	58.3	75	100*
Dt	16.7	33.3	66.7	58.3	100*
C	0	8.3	58.3	66.7	100*
R	8.3	33.3	41.7	91.7	100*
G	16.7	41.7	58.3	75	<u>58.3</u>
(Pg)	25	0	62.5	87.5	100*
(P)	0	50	50	100	100*
(Fm)	12.5	25	50	75	75
(Lm)	12.5	12.5	50	50	87.5
Mean	**11.7**	**20.8**	**51.9**	**74.7**	**90.3**

P<0.001; LSD (5%) for comparing 6 vs 6 reps = 29.4, 4 vs 4 reps = 36.0 and 4 vs 6 reps = 32.8

As with the two ALS herbicides, the populations also varied in their responses to treatment with the five rates of MCPA but overall no one population stood out with the lowest mortality across all rates (Table 2.10). This chemical was the second most effective herbicide of the three tested here when comparing mortality over all populations at the recommended application rate (95% mortality) (Table 2.10), but had the lowest number of plants surviving above recommended rates. Population Pg had the lowest percent mortality (highest tolerance) at 0.45, 1.0, and 5.0 times recommended rates, while populations 'Dp', 'Dt' and 'C' appeared to have highest

percent mortality (lowest tolerance) across several rates. This may correlate to historical exposure to MCPA as population 'Pg' had high previous exposure, while 'Dp', 'Dt' and 'C' had no past exposure to MCPA (Table 2.3).

Table 2.10 Percent mortality for the 15 populations of giant buttercup after treatment with each of five rates of MCPA in Experiment 1. LSDs are for comparing populations with 4 or 6 reps with other populations with 4 or 6 reps. All populations have 6 reps apart from the last four which have four, shown in brackets. Stars indicate populations with significantly higher mortality than the population with the lowest mortality (highlighted).

	Proportion of recommended rate				
Population	0.2	0.45	1	2.2	5
J	16.7	66.7	91.7	100	100
Fb	8.3	25	91.7	100	100
B	50	75	100	100	100
Bh	41.7	58.3	100	100	100
Br	33.3	58.3	91.7	100	100
Ls	25	33.3	100	100	100
Dp	58.3	100	100	91.7	100
Dt	41.7	91.7	91.7	100	100
C	50	100	100	100	100
R	16.7	58.3	100	91.7	100
G	8.3	50	91.7	100	100
(Pg)	12.5	25	87.5	100	87.5
(P)	25	87.5	100	100	100
(Fm)	37.5*	75	87.5	100	100
(Lm)	0	75	87.5	100	100
Mean	**28.3**	**65.3**	**94.7**	**98.9**	**99.2**

P<0.001; LSD (5%) for comparing 6 vs 6 reps = 29.4, 4 vs 4 reps = 36.0 and 4 vs 6 reps = 32.8

When averaged over all five rates, plants treated with flumetsulam differed in their tolerances (Table 2.11), but overall showed high mortality (63-91%). Population 'G' had significantly lower mortality than all other populations by 15% or more, (63% mortality compared with 78-91% mortality for the other 14 populations).

Thifensulfuron-methyl was least effective overall resulting in 39-57% mortality for the 15 populations (Table 2.11) when averaged over all five rates. However, as stated earlier, a slightly lower than recommended application rate was used for the middle rate. Data showed thifensulfuron-methyl had the lowest range of responses across the populations of the three herbicides (18%) compared with flumetsulam and MCPA (28% and 31% respectively).

MCPA resulted in a high level of mortality similar to that from flumetsulam (59-90%) for the 15 populations when averaged over all five rates (Table 2.11).

Population 'Pg' had lower mortality than all other populations by at least 6% (59% mortality) and significantly lower mortality than nine of the other 14 populations. Populations 'Lm', 'Fb', 'Ls', 'R' and 'G' also showed overall lower mortality (higher tolerance) than most other populations (65-73%), while 'C', 'B', 'Dt' and 'Dp' showed the highest overall mortality (lowest tolerance)(85-90%).

Table 2.11 Percent mortality for the 15 populations of giant buttercup treated with flumetsulam, thifensulfuron-methyl or MCPA in Experiment 1. Data is averaged over all five rates. LSDs are for comparing populations with four or six reps with other populations with 4 or 6 reps. Populations with four reps are indicated in the last four rows of the table (in brackets) all other populations have six reps. Stars indicate values significantly higher than the population with the lowest mortality (underlined).

Population	flumetsulam	thifensulfuron-methyl	MCPA
J	91.7*	43.3	75.0*
Fb	91.7*	53.3	65.0
B	81.7*	46.7	85.0*
Bh	78.3*	46.7	80.0*
Br	86.7*	51.7	76.7*
Ls	85.0*	48.3	71.7
Dp	86.7*	46.7	90.0*
Dt	86.7*	55.0*	85.0*
C	85.0*	46.7	90.0*
R	90.0*	55.0	73.3
G	<u>63.3</u>	50.0*	70.0
(Pg)	79.3*	51.8	<u>59.3</u>
(P)	86.8*	56.8*	79.3*
(Fm)	89.3*	44.3	76.8*
(Lm)	79.3*	<u>39.3</u>	69.3
Mean	**84.1**	**49.0**	**76.4**

LSD (5%) for comparing 6 vs 6 reps = 13.1, 4 vs 4 reps = 16.1 and 4 vs 6 reps = 14.7

Probit curves fitted to the mortality data are given for all the populations for flumetsulam, thifensulfuron-methyl and MCPA (Figures 2.8, 2.9 & 2.10, respectively). The fitted curves were a poor fit for both flumetsulam and MCPA. This is because the mortality was high at the median rate and above and the LD_{50} values were in some cases off the scale of the graphs (below the range of rates chosen for this experiment). The mortality for the lowest rates was, in some cases, above 50% so an LD_{50} could not be calculated. This was the case for 11 of the 15 populations for flumetsulam and four for MCPA. For some of these populations with poorer fitted curves the data for only the lowest three rates was used to enable a curve to be fitted. However, data for thifensulfuron-methyl was a better fit because mortality was lower across the range of rates (Figure 2.9).

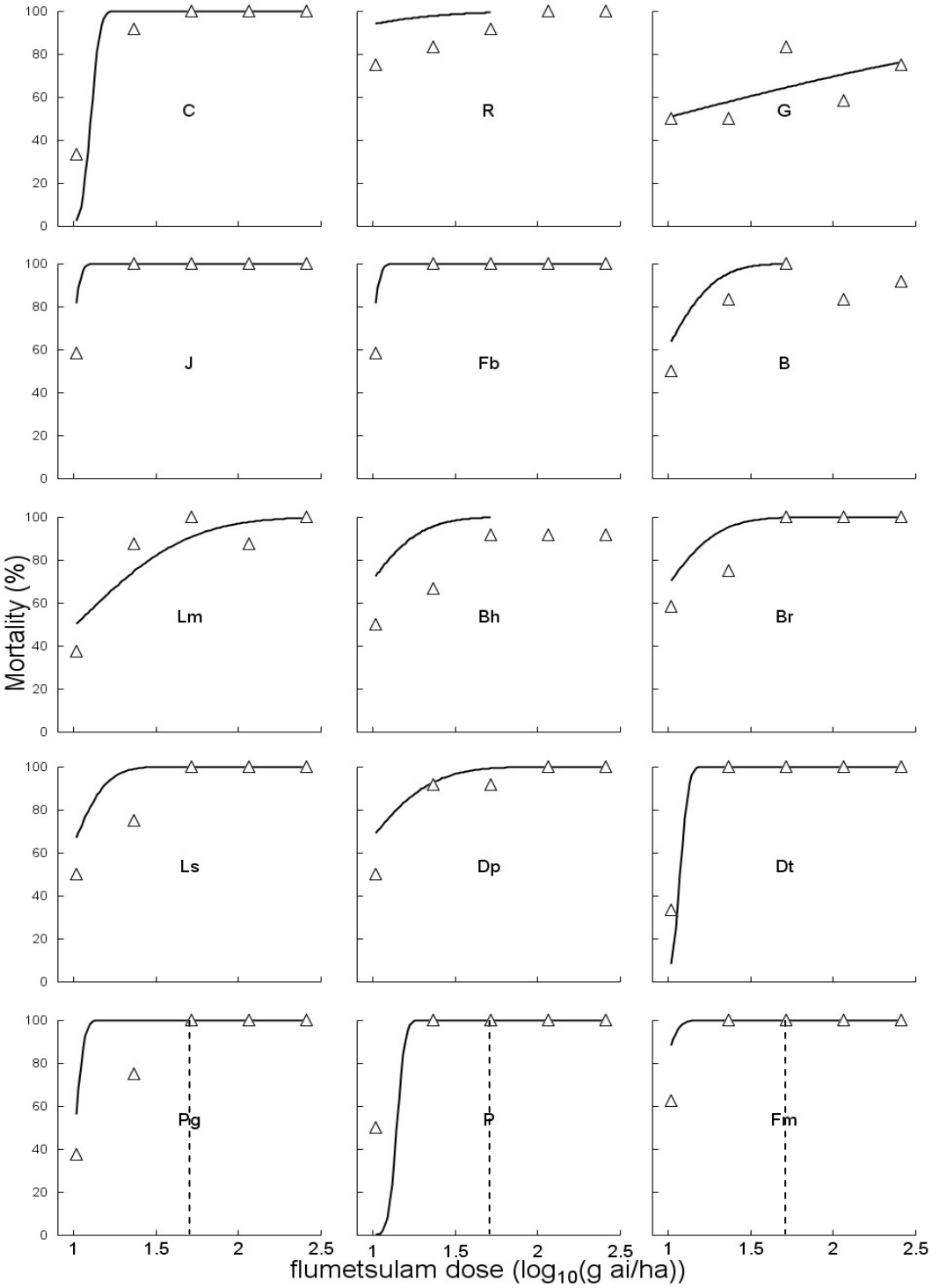

Figure 2.8 Percent mortality for the 15 populations of giant buttercup treated with 5 rates of flumetsulam in Experiment 1, with fitted Probit curves. Populations 'B', 'Bh' and 'R' use only the bottom 3 rates to plot curve. Vertical dotted line is the middle rate.

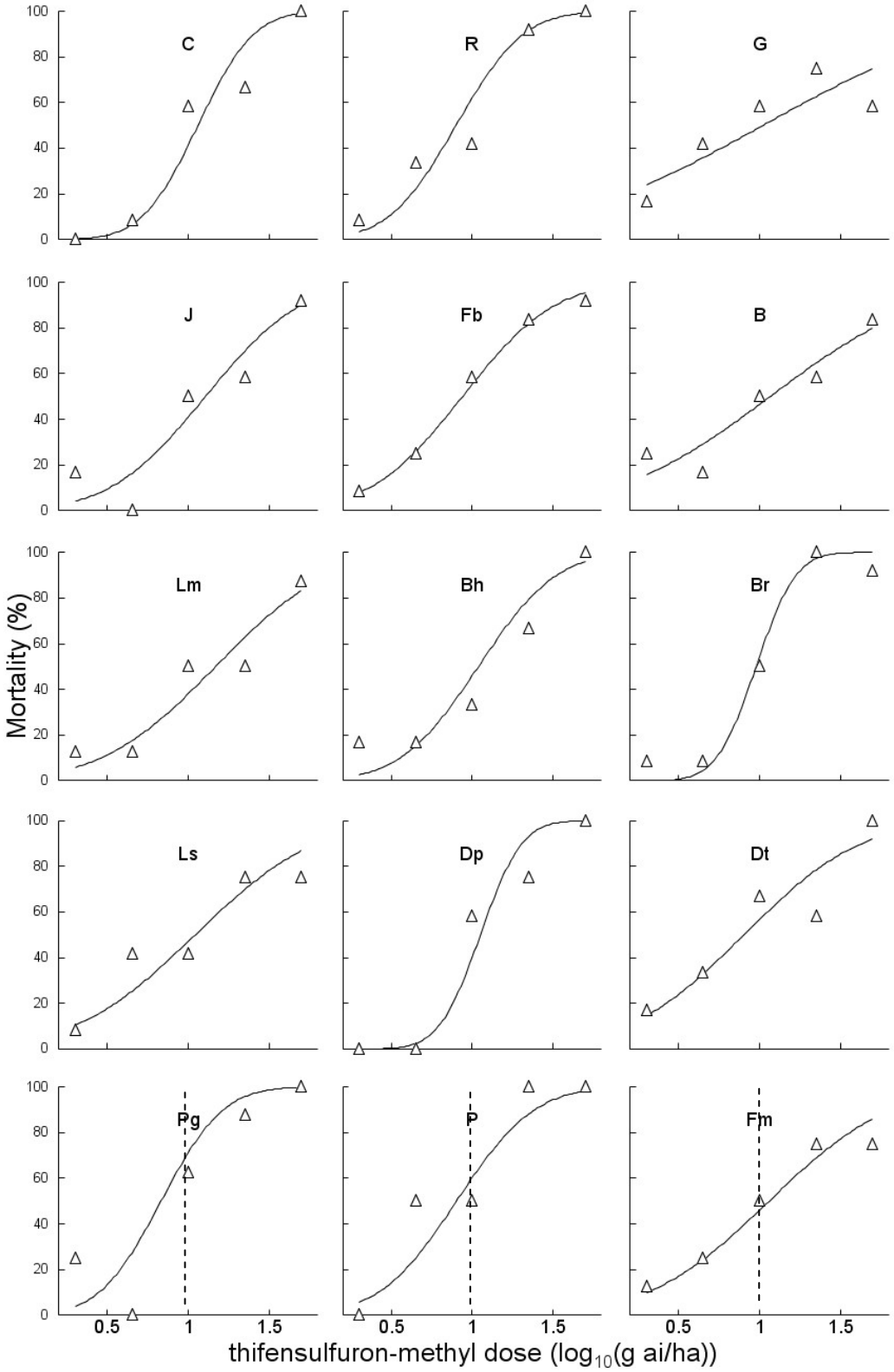

Figure 2.9 Percent mortality for the 15 populations of giant buttercup treated with 5 rates of thifensulfuron-methyl in Experiment 1, with fitted Probit curves. Vertical dotted line is the middle rate used.

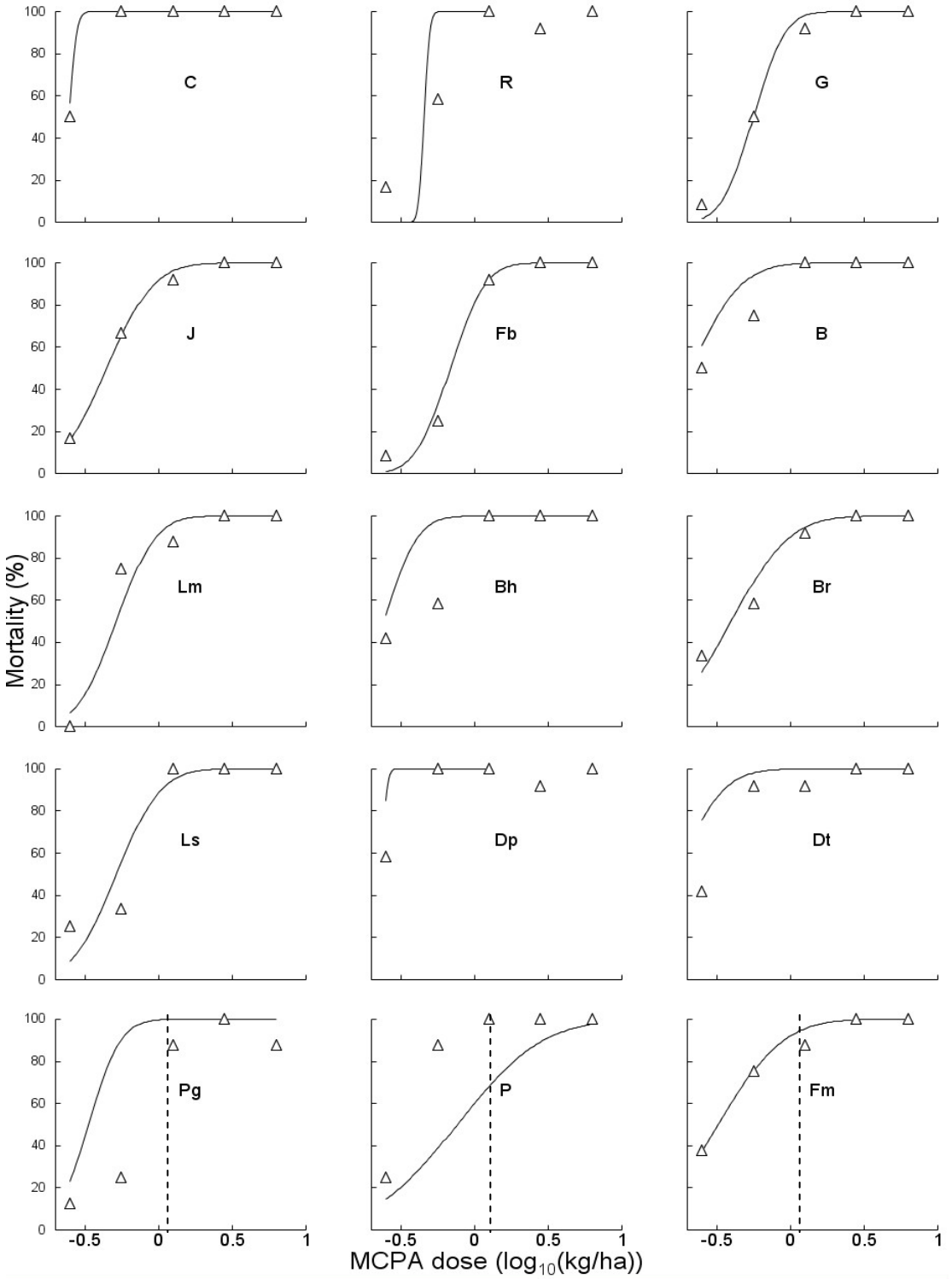

Figure 2.10 Percent mortality for the 15 populations of giant buttercup treated with 5 rates of MCPA in Experiment 1, with fitted Probit curves. Populations 'R' and 'Dp' use only bottom 3 rates to calculate curve. Vertical dotted line is the middle rate used.

$Log_{10}LD_{50}$ values (calculated by Genstat and back-transformed to derive LD_{50} values) for flumetsulam varied considerably between populations (Table 2.12) but did not correlate well with increasing past exposure to flumetsulam (Figure 2.11, R^2=0.03), although the trend upward with increasing historical exposure to flumetsulam is in support of the hypothesis of evolved resistance. However there was a better correlation (P<0.05) when the percent mortality, averaged over all five rates (data from Table 2.11) for flumetsulam, was plotted against historical exposure to flumetsulam (Figure 2.12, R^2= 0.34) at 101 days after spraying, again in support of the hypothesis that resistance to flumetsulam is increasing. The correlation was not so good for thifensulfuron-methyl or MCPA (R^2 = 0.023 and 0.166 for thifensulfuron and MCPA respectively).

Table 2.12 $Log_{10}LD_{50}$ and back-transformed LD_{50} values (g ai/ha for thifensulfuron and flumetsulam, kg ai/ha for MCPA) produced using GenStat parameter estimates for each population and herbicide in Experiment 1 as per equation 1 and 2. For all populations five rates were used to calculate the values except those underlined which were based on only the bottom three rates.

	thifensulfuron-methyl		flumetsulam		MCPA	
Population	$Log_{10}LD_{50}$	LD_{50}	$Log_{10}LD_{50}$	LD_{50}	$Log_{10}LD_{50}$	LD_{50}
B	1.07	11.64	0.93	8.45	-0.67	0.21
Bh	1.04	10.95	0.83	6.76	-0.62	0.24
Br	0.98	9.52	0.85	7.14	-0.40	0.40
C	1.06	11.45	1.10	12.72	-0.61	0.25
Dp	1.05	11.25	0.84	6.89	-0.64	0.23
Dt	0.90	8.00	1.07	11.82	-0.78	0.17
Fb	0.94	8.73	0.98	9.48	-0.17	0.68
G	1.02	10.50	0.97	9.33	-0.25	0.56
J	1.11	12.76	0.98	9.48	-0.35	0.45
Ls	1.04	11.04	0.94	8.65	-0.28	0.52
R	0.90	7.96	-0.22	0.60	-0.34	0.46
Fm	1.06	11.47	0.92	8.39	-0.49	0.32
Lm	1.17	14.70	1.02	10.37	-0.29	0.52
P	0.83	6.78	1.01	10.25	-0.47	0.34
Pg	0.90	8.01	1.14	13.95	-0.12	0.76

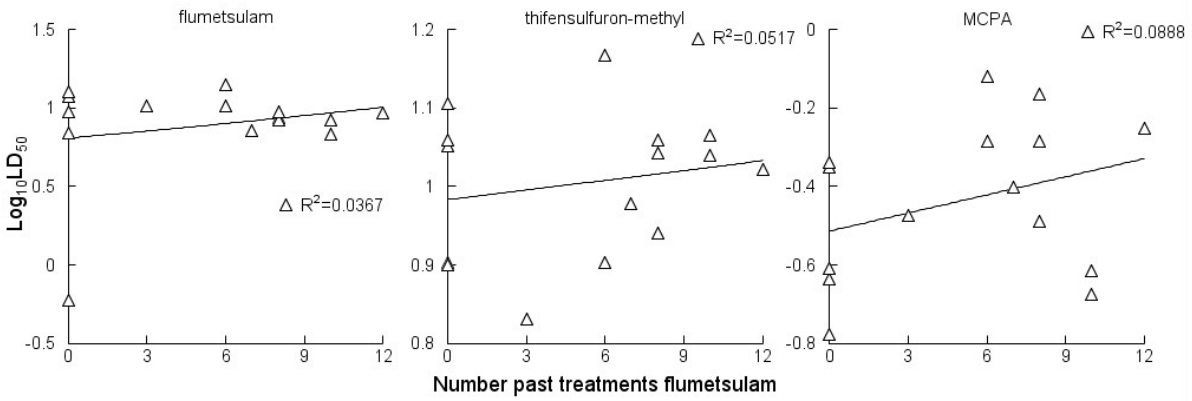

Figure 2.11 Correlation of Log10LD_{50}s for the 15 populations of giant buttercup with increasing past exposure to flumetsulam in Experiment 1. P values for flumetsulam, thifensulfuron-methyl and MCPA are 0.415, 0.494 and 0.281 respectively.

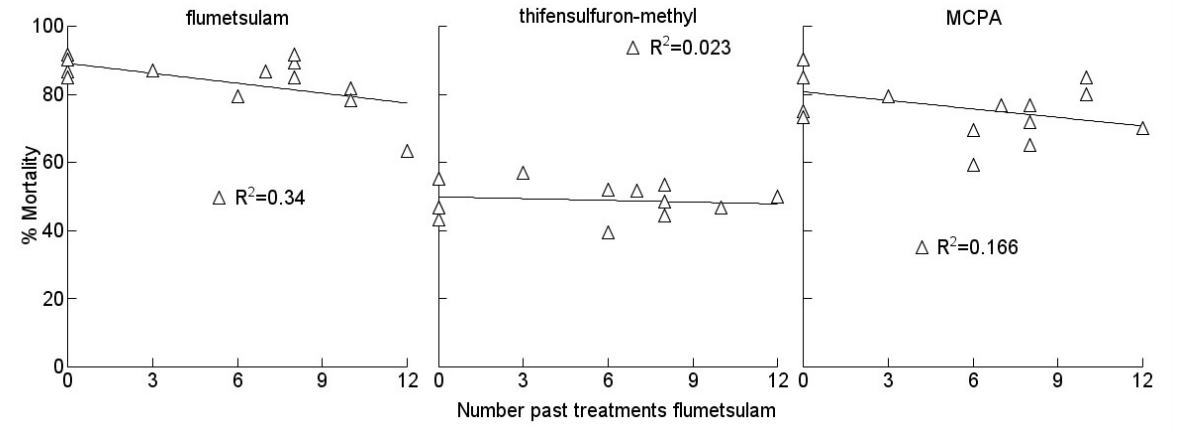

Figure 2.12 Correlation of percent mortality averaged over all five rates for the 15 populations of giant buttercup with increasing past exposure to flumetsulam in Experiment 1. P values for flumetsulam, thifensulfuron-methyl and MCPA are 0.023, 0.589 and 0.131 respectively.

2.5 Discussion

2.5.1 Objectives 1 and 2

In order to determine whether evolution of resistance due to historical exposure to flumetsulam has occurred (Objectives 1 & 2) it must first be shown that there are differences in the levels of mortality between the populations. In this study differences were evident in the numbers of individuals that survived in some of the populations across the range of rates of flumetsulam. However, as the numbers of survivors were low this could indicate that the frequency of resistant phenotypes is low and that resistance is just beginning to become evident in these populations.

Plants from Population 'G' were significantly more tolerant to flumetsulam ($p<0.05$) than all other populations tested here, when averaging mortality over all five rates (63% mortality compared to 79-91% mortality for the other 14 populations) (Table 2.11). This population had had the highest past exposure to flumetsulam of all other populations tested, having been treated every year for the last 12 years (Table 2.3). Twenty-five percent of buttercup plants from Population 'G' survived five times the recommended rate of flumetsulam compared to only 8% for 'Bh' and 'Br' which both had had less exposure to flumetsulam than 'G', and 0% for the other 12 populations (Tables 2.8 and 2.3). There was also a significant linear declining trend in percent mortality (averaged over all five rates) ($P<0.05$, $R^2 = 0.34$) when plotted against past exposure to flumetsulam (Figure 2.12). This provides some evidence that increasing past exposure to flumetsulam in this population has resulted in the selection of alleles conferring resistance to flumetsulam. The reason this correlation was not stronger may have been because most of the populations showed no resistance and there were only a few populations where there appeared to be a few individuals present with resistance genes, which may again indicate an early stage of resistance development. There was no linear trend, however, with average mortality over all rates and past exposure to flumetsulam for either thifensulfuron-methyl or MCPA treated plants (Figure 3.12) (R^2= 0.023 and 0.166 respectively). Plate 2.7 illustrates the higher survival of plants from population 'G' at rates above the recommended rate, compared to population 'C' with no past exposure (Plate 2.8), and both populations together (Plate 2.9).

Unlike the results for % mortality averaged over herbicide rates, the $\log_{10}LD_{50}$ values did not correlate well with past exposure to flumetsulam, according to the information I had collected during the farmer survey (Table 2.3 and Figure 2.11). If the hypothesis is correct that resistance has evolved to flumetsulam in giant buttercup populations due to past exposure, a significant increasing linear trend would have been expected when LD_{50} values for each population were plotted against past exposure to flumetsulam, but this was not the case (R^2=0.037) (Figure 2.11). This does not discount the idea that population tolerance to flumetsulam is influenced by past exposure to the herbicide, but may be because the LD_{50} values generated by the mortality curves in this study were poor estimates due to the poor fits of the data by the probit curves. In the case of flumetsulam the mortality curves did not fit well to the data because the rates chosen for the experiment gave a greater kill than expected so in many cases the LD_{50} values were off the scale of the graphs. Also, as mentioned in the methods the probit fit cannot accurately solve when more than one value is near 100% or if the dose-response curve is too steep and too few values are in the median range of mortality (Williams 1986). Attempts at fitting curves manually did not help because the mortality values for the lowest rate, particularly for flumetsulam and MCPA, were above 50% which made calculation of an LD_{50} impossible. In order to get better estimates of the LD_{50} values,

the experiment was repeated using a greater range of rates including lower rates, and with more replicates (see Chapter 3).

The biomass data indicate that past exposure to herbicides has resulted in an inherited tolerance in the buttercup populations to the herbicides (Figure 2.6, Table 2.2), and there is also some evidence of cross resistance between the two classes of ALS herbicide, thifensulfuron (sulfonylurea) and flumetsulam (triazolopyramadine), but not to the phenoxy herbicide MCPA (a different mode-of-action group) (Figure 2.7). The differences in biomass scores were apparent as early as 27 days after treatment and became more pronounced with each subsequent assessment as the herbicides took full effect.

Population 'G' proved to be tolerant to both of the ALS herbicides, flumetsulam and thifensulfuron-methyl, even though this population had never been treated with thifensulfuron-methyl in the past, indicating possible cross resistance. This was shown by the smaller reductions in biomass for plants treated with both flumetsulam and thifensulfuron-methyl for population 'G' compared to populations 'C' and 'R' with no past exposure to flumetsulam (Fig 2.7).

The mortality data also indicated cross resistance may be present as population 'G' had the highest number of plants surviving at the highest rate for both flumetsulam and thifensulfuron (Tables 2.7 and 2.8). In studies with other weed biotypes, responses are often similar between the sulfonylurea and triazolopyramadine classes (Tranel & Wright 2002), from which thifensulfuron and flumetsulam belong. Plate 2.7 illustrates the higher survival for population 'G' treated with flumetsulam and thifensulfuron, but apparent effective control with MCPA.

While cross-resistance between these two ALS inhibiting herbicides may be present, as indicated by results from some populations of giant buttercup in this study, overall mortality for thifensulfuron-methyl was low and resistance may only just be starting to show up, so the relationship was not clear-cut. Given that population 'R' was very susceptible to flumetsulam (after having a very high past exposure to phenoxy herbicides but not flumetsulam) multiple resistance is probably not a factor here.

Cross resistance has been reported widely amongst the five groups of ALS inhibiting herbicides in many weed species, but it is difficult to predict patterns because several different mutations leading to an altered ALS have been identified. For example cross resistance was found in populations of *Apera spica-venti* (Poaceae family; loose silky bentgrass) in the Czech Republic (Hamouzova et al. 2011), which was resistant to several different sulfonylurea herbicides. But in a tobacco (*Nicotiana tabacum*) example, cross resistance did not occur for all of the sulfonyl-

urea herbicides (Harms et al. 1992). So resistance to one herbicide in a particular mode-of-action group does not guarantee resistance to all chemicals in that group.

Although most cases of ALS resistance are due to an altered target site, there are also a few cases of increased herbicide metabolism resulting in detoxification of the herbicide and thus resistance to herbicides with very different modes of action (multiple resistance). For example resistant biotypes were found in rigid ryegrass (*Lolium rigidum* Gaud.) and blackgrass (*Alopecurus myosuroides* Huds.) (Christopher et al. 1991; Hall et al. 1994), but these were not selected for by ALS inhibiting herbicides. Instead, they were selected for by ACC'ase and Photosystem II inhibitors. The genetic basis for mutations and biochemistry of resistance mechanisms involved in these giant buttercup populations was beyond the scope of this study.

It is interesting to note that the two populations that had had high past exposure to MCPA ('R' and 'G') appeared more tolerant of MCPA than population 'C', which had never been exposed to MCPA (Fig 2.7). However, biomass was reduced to near zero at the recommended rate and above from 59 days onwards and overall mortality for MCPA was high for all populations (Table 2.9) indicating MCPA was quite effective overall on these young plants. This is an interesting result because it was previously thought that MCPA was generally ineffective on giant buttercup populations throughout Golden Bay because of evolved resistance. As the survey revealed that most farmers now have a control programme based mainly on flumetsulam and have largely stopped using the phenoxy herbicides, it may be that the resistant biotypes found widely in the 1980s (Bourdôt et al. 1990) have become less prevalent over time, as the selection pressure was reduced or removed. Experiments done previously predicted it would take 28 years for resistance to subside back to normal susceptibility if the selection pressure was removed (Bourdôt et al. 1996). However, as the formulations of MCPA used in the earlier studies and the present study were different (potassium salt and dimethylamine salt, respectively), and field use rates have changed also, rates cannot accurately be compared because it is possible that the newer formulation is more effective.

Current management practices for giant buttercup control in New Zealand dairy pastures have led to evolved resistance to the phenoxy herbicides MCPA and MCPB because of regular use over several years (Bourdôt & Hurrell 1988), and now possibly to flumetsulam as well, as indicated by these results. However further investigation is needed to confirm these results which are discussed in the following chapter.

2.5.2 Objective 3

The biomass and mortality data collected in this study enable an evaluation to be made about the efficacy of the newer ALS herbicides on giant buttercup compared with MCPA (objective 3).

The herbicides differed in both their overall effects on 4-month-old giant buttercup plants and the speed at which those effects took place. MCPA reduced the biomass of the weed quickly and effectively, while the two ALS herbicides were slower to act, taking about 2 months to reduce biomass to less than 10% of untreated at the recommended rates (Figure 3.5). This is not surprising given the mode of action of these two groups of herbicides and the differences in time for plant mortality to occur has been reported earlier (Moss 2002). This may be a consideration for dairy farmers when spraying because the expected increase in productivity from controlling the weed would be realised more slowly with the ALS chemicals, at a time when maximum pasture production is needed for dairy cows (spring/summer). Furthermore, thifensulfuron-methyl was not totally effective and biomass was never reduced to levels below about 10%, before regrowth occurred.

When comparing the effects of these herbicides at their respective label rates on giant buttercup plants, 101 days after treatment, flumetsulam and MCPA gave the greatest reduction in plant biomass (99% and 98%, respectively), while thifensulfuron-methyl was less effective (78% control) (Figure 2.5). The label rate of thifensulfuron-methyl would, according to these results, need to be at least doubled to achieve a similar level of control to flumetsulam. The label recommended rate of flumetsulam could be reduced somewhat, whilst still giving a high level of control of giant buttercup. In contrast, rates could not be reduced for either MCPA or thifensulfuron-methyl without sacrificing efficacy as rates below the recommended application rate gave very poor control. Although the middle rate of thifensulfuron used was slightly lower than the recommended application rate (10 g instead of 15 g ai/ha) this herbicide was still not very effective. Even at 2.24 times label rate (22.4 g ai/ha) mortality was lower overall for thifensulfuron (Table 2.8), compared to the other two herbicides applied at their recommended rates (Tables 2.7 and 2.9).

These results on 4-month old plants may only be an indication of their relative field efficacy against the species as results may differ for mixed-age plants in the field that may be more tolerant of herbicides. Also the plants in this study had been grown in a glasshouse for most of the 4 month period before being treated with herbicide, which may also have increased their sensitivity to herbicide treatment. However, the lowest recommended rates of MCPA for giant buttercup control were chosen for this study, as higher rates are recommended for more mature plants. Similarly, with flumetsulam, only one rate is recommended on the label for giant

buttercup no matter what age or growth stage, so results found here may be as expected in the field.

Plate 2.1 Tray of buttercup seedlings for one population growing in vermiculite soon after germination for use in Experiment 1. Plants were pricked out and planted into pots of potting mix at this stage.

Plate 2.2 Close up of giant buttercup seedlings soon after planting into pots (two seedlings per pot), growing in the glasshouse at Lincoln in Experiment 1.

Plate 2.3 Pots of giant buttercup seedlings soon after planting out in the glasshouse at Lincoln in Experiment 1.

Plate 2.4 Pots of young giant buttercup seedlings growing in the AgResearch Lincoln glasshouse prior to spraying (24th June 2010) in Experiment 1.

Plate 2.5 Pots of giant buttercup 2 weeks after treatment with MCPA (9th Sept. 2010), showing typical phenoxy herbicide treatment symptoms of bending and twisting of stems in Experiment 1.

Plate 2.6 Two pots of giant buttercup 2 weeks after herbicide application (9th Sept. 2010) in Experiment 1. An untreated control plant is on the left while the plant on the right is treated with 5 times recommended rate of MCPA. This photo shows the approximate size and developmental stage of buttercup plants at time of spraying.

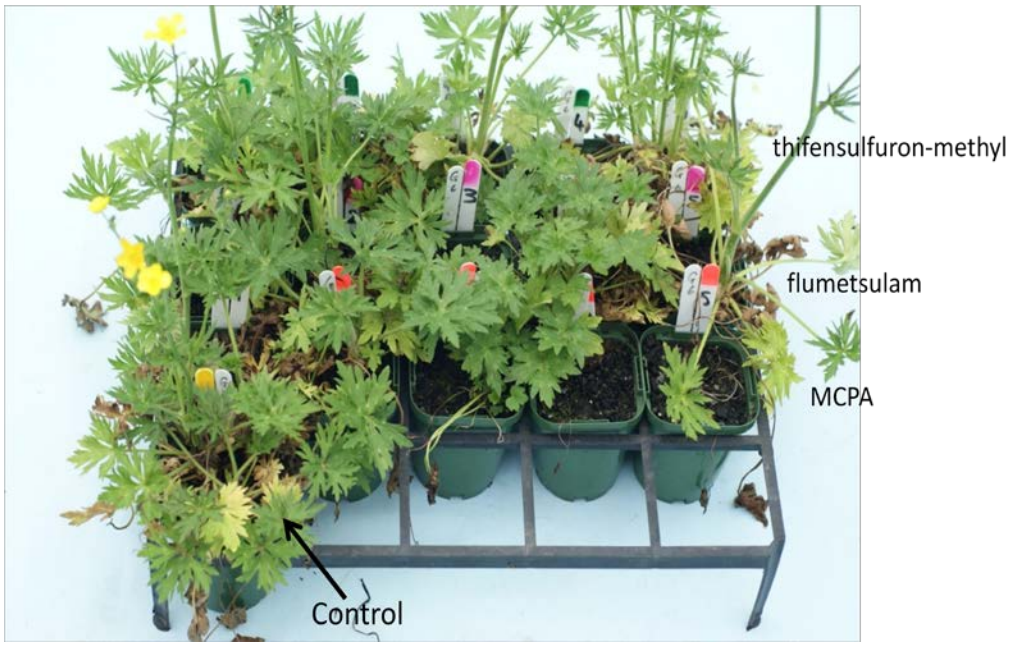

Plate 2.7 Pots of giant buttercup from one replicate, population 'G' 101 days after spraying (7th December 2010), Expt. 1. This population had high past exposure to flumetsulam during the 12 years prior to the experiment (Table 2.2). Plants are treated with five rates (increasing lowest to highest rate from left to right) of thifensulfuron-methyl (top row, green tags), flumetsulam (middle row, purple tags) and MCPA (bottom row, red tags), and control (bottom left corner, yellow tag).

Plate 2.8 Pots of giant buttercup from one replicate for population 'C', 101 days after spraying (7th December 2010) in Experiment 1. This population had not been exposed to any of the three herbicides (Table 3.2). Plants are treated with five rates (increasing lowest to highest from left to right) of thifensulfuron-methyl (top row, green tags), flumetsulam (middle row, purple tags) and MCPA (bottom row, red tags), and control (bottom left, yellow tag).

Plate 2.9 One replicate from each of population 'G' (top) and 'C' (bottom) treated with five rates of flumetsulam (increasing rate of 0.2- 5 times recommended rate from left to right) 101 days after spraying (7th December 2010) in Experiment 1.

Plate 2.10 Giant buttercup plant from population 'P' showing regrowth after spraying with 0.2 times recommended rate of flumetsulam, 101 days after treatment (7th December 2010) in Experiment 1. The original foliage was completely destroyed before fresh regrowth occurred from the rhizome.

Plate 2.11 Giant buttercup from population 'C' (top row) and 'G' (bottom row) treated with five rates of thifensulfuron-methyl (0.2-5 times recommended rates) with increasing rate from left to right and controls on far left (yellow tags) (7th December 2010) in Experiment 1.

Plate 2.12 Giant buttercup from populations 'G' (top row) and 'C' (bottom row) treated with five rates of flumetsulam (0.2-5 times recommended rates) with increasing rate from left to right (7th December 2010) in Experiment 1.

Chapter Three
Comparison of susceptibility to flumetsulam of two giant buttercup populations – Experiment 2

3.1 Introduction

In Experiment 1 (Chapter Two) giant buttercup populations were found to differ markedly in their susceptibility to treatments of flumetsulam in terms of the percentage mortality of their seedling progeny and this difference was found to be correlated with past exposure of the populations to flumetsulam. This study also showed that some resistant plants from some populations survived up to five times the recommended label rate of flumetsulam. The analysis of biomass data supported the mortality results, showing marked differences between populations in their responses to the herbicide and that seedling progeny of populations that had had a high past exposure tended to have less reduction in biomass.

However, the application rates of flumetsulam chosen in the earlier work were apparently higher than was required because even the low rates caused high mortality in most populations and as a consequence of that the probit curves generated in the data analysis were a poor fit to the data. This meant that determining the LD_{50} values from those poorly fitted probit curves, gave non-significant results when correlating them with past exposure to flumetsulam. Also the generated LD_{50} values did not support the results of the mortality data, in that some populations with high mortality (e.g. Population 'C') had higher LD_{50} values than populations with lower overall mortality (e.g. Population 'G').

The objectives of this next study were, therefore, to repeat the dose-response study using only flumetsulam, on the most resistant and most susceptible populations of giant buttercup determined from the previous experiment. This time a wider range of rates, together with greater replication would be used to derive more accurate dose response curves and LD_{50} values and thus enable the difference in the level of resistance to flumetsulam between these populations to be determined.

3.2 Methods

3.2.1 Seed germination and planting

On 23 December 2010, giant buttercup seeds from two populations (the one that appeared most resistant (G) and most susceptible (C) in Experiment One) were soaked overnight in 0.02% solution of KNO_3 (about 250 seeds from each population). These seeds were left over from the previous experiment and had

been stored in an airtight container under cool (4°C) conditions since collection in January 2010. On 24th December, approximately 24 hours later, seeds were dried on paper towels and dusted with a 50:50 w/w mix of Captan/Benlate fungicides, approximately 5 g seed to 0.5 g powder. The seeds were then sown into trays of damp vermiculite (one tray per population), lightly covered with vermiculite, then trays placed in clear plastic bags into a growth cabinet. This was set to cycle 20°C light for 12 hrs, then 10°C dark for 12 hrs. On 10 January 2011 the buttercup seedlings were pricked out one seedling each into 90 mm square (0.5 L) pots filled with bark, peat and sand potting mix plus 6-month slow-release fertilizer (Osmocote) (Appendix 2), watered and left in a shadehouse. Seedlings were all at the cotyledonary stage when planted.

3.2.2 Experimental design

On 11th January all pots were labeled, randomised, and organised into a split block design, as shown in Figure 1, with 7 blocks and 10 treatments (populations as main plots, treatments as subplots). The number of seedlings that came up allowed for 21 replicate pots per treatment (7 blocks x 3 pots per block) for Population 'G' (210 plants total) but only 14 pots per treatment (7 blocks x 2 pots per block) for Population 'C' (140 plants total). Plants were grown on for approximately 3 months in the shadehouse.

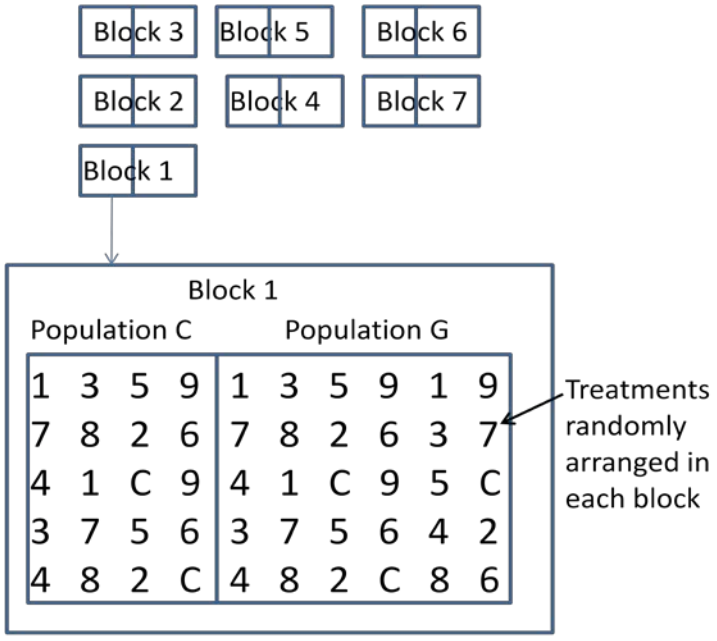

Figure 3.1 Diagrammatic illustration of layout of Experiment 2; a randomised block design with seven blocks, consisting of two populations with ten treatments (untreated control plus 9 rates of flumetsulam) randomly distributed within each block. Population 'C' has two replicates of each treatment within each block while Population 'G' had three.

Table 3.1 Rates of flumetsulam applied in Experiment 2

Treatment label	Proportion of recommended label rate	g ai/ha	g product/ha	mixing rate (g/L)
C	0.0	0.0	0.0	0.0
1	0.04	2.1	2.6	0.013
2	0.09	4.7	5.8	0.029
3	0.20	10.4	13.0	0.065
4	0.45	23.3	29.1	0.145
5	1 (label rate)	52.0	65.0	0.325
6	2.2	116	145.3	0.727
7	5.0	260	325.0	1.625
8	11.2	581	726.7	3.634
9	25.0	1300	1624.9	8.125

*field rate 65 g/ha in 200 litres water plus Uptake oil 500 ml/100L. Preside™ contains 800 g/kg flumetsulam = 52 g ai/ha @ 65 g Preside/ha. Adjuvant rate 1 L/ha = 5 ml/L.

On 14 April 2011, all plants were sprayed between 9-11am under cool (13^{o}C), overcast, still-air conditions. The treatments were nine rates of flumetsulam plus an untreated control (Table 4.1). Plants were sprayed in order of lowest to highest rate of flumetsulam applied using a moving conveyer-belt sprayer (belt speed 1.06 m/s) delivering 200 L/ha at 210 kPa through a single 8002EVS flat-fan TeeJet® (Spraying Systems Ltd) hydraulic nozzle mounted 30 cm above the plants.

Control plants were put through first using plain water. Spray equipment was flushed with water between treatments to prevent contamination between treatments. After spraying the plants were maintained in the shadehouse for two months from mid-April to mid-June and watered regularly throughout this time. The temperatures recorded in the shadehouse over the experimental period (Figure 3.2) were somewhat cooler than normal for April, about average for May and warmer for June, when compared to long term average data (source NIWA Virtual Climate Weather Station http://cliflo.niwa.co.nz/ accessed 22nd Sept. 2011).

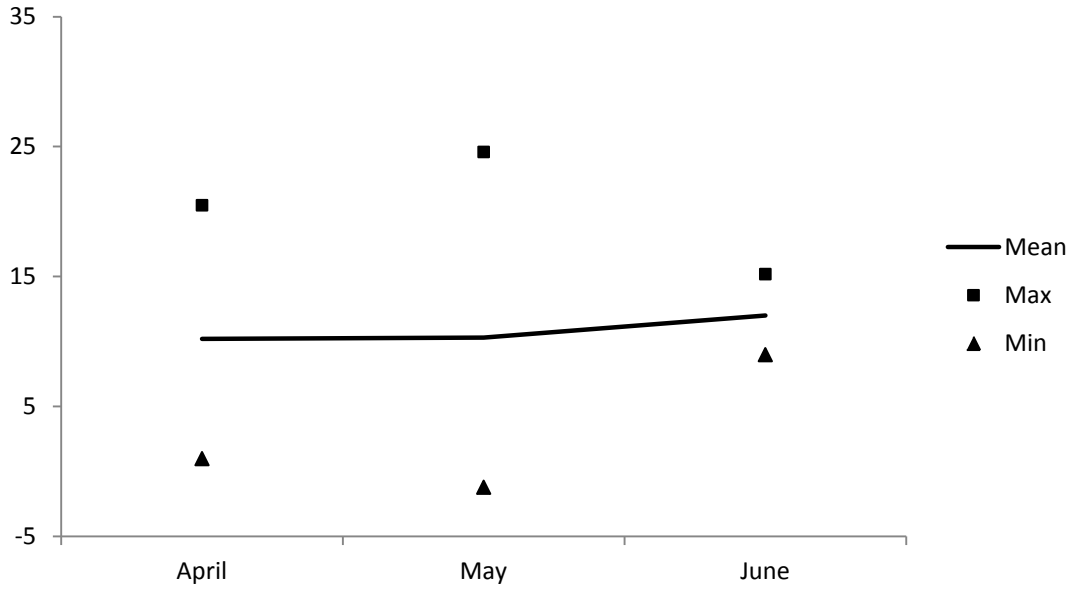

Figure 3.2 Min, max and mean monthly temperatures for the experiment period (14th April, to 14th June), recorded in the shadehouse throughout Experiment 2.

In mid-June the plants were shifted to an unheated glasshouse where the temperature was limited to a maximum of 18° C, to prevent damage from frosts. The plants were treated for aphids with pirimicarb (Pirimor® 50, Syngenta Crop Protection Ltd) on 27 June and with liquid fertilizer (Phostrogen, PBI Home & Garden Ltd) on 12 July.

3.2.3 Assessments

Plants were assessed for biomass (percent biomass of foliage compared to control) on 5 May, 24 June, 18 July and 4 August (21, 71, 95 and 112 days after treatment; DAT). This was done by comparing the size of each treated plant against the control plant for its respective population and replicate. Mortality was assessed on the 19th August (127 DAT) by recording whether each plant was alive or dead. A plant was deemed to be dead if it had no visible green tissue and the remaining brown dead plant material could be dislodged from the soil surface easily. All plants that were alive at this final assessment were producing new growth so their continued survival was considered certain.

3.2.4 Statistical analysis

The LD_{50} values were calculated in the same manner as for Experiment 1 (Chapter 2) only this time there were seven blocks and two populations in the analysis.

The percent mortality variable was analysed for the two populations and nine rates as a factorial with 21 replicates, using missing value estimation for the difference in the numbers of replicates, using a general ANOVA in Genstat. From this an LSD (5%)

was obtained for comparing the treatments. To compare between the two populations (21 vs 14 replicates, 'G' & 'C' respectively) the LSD was multiplied by √(1/21 + 1/14) ÷ √(2/21).

3.3 Results

3.3.1 Biomass

The biomass of plants from the 'G' and 'C' populations, treated with the recommended field rate of flumetsulam declined at a similar rate over time until about 70 days post-treatment (Figure 3.3). After that the biomass of the 'G' population began to increase as regrowth occurred, whilst the 'C' population continued to decline as leaf tissue was observed to die.

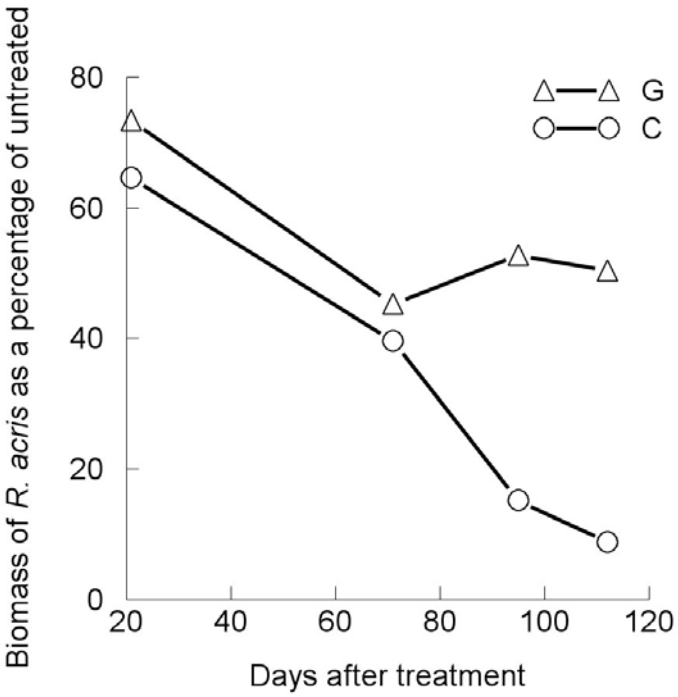

Figure 3.3 Reduction in biomass over time for giant buttercup plants treated with flumetsulam (52 g ai/ha) for populations 'G' and 'C' in Experiment 2.

Overall biomass of the 'G' population was consistently larger at all times of assessment at the field rate and above (Figure 3.4). It is noteworthy that neither population reached zero green biomass at the recommended rate by 112 days and only did so at rates of 5 times that recommended and above.

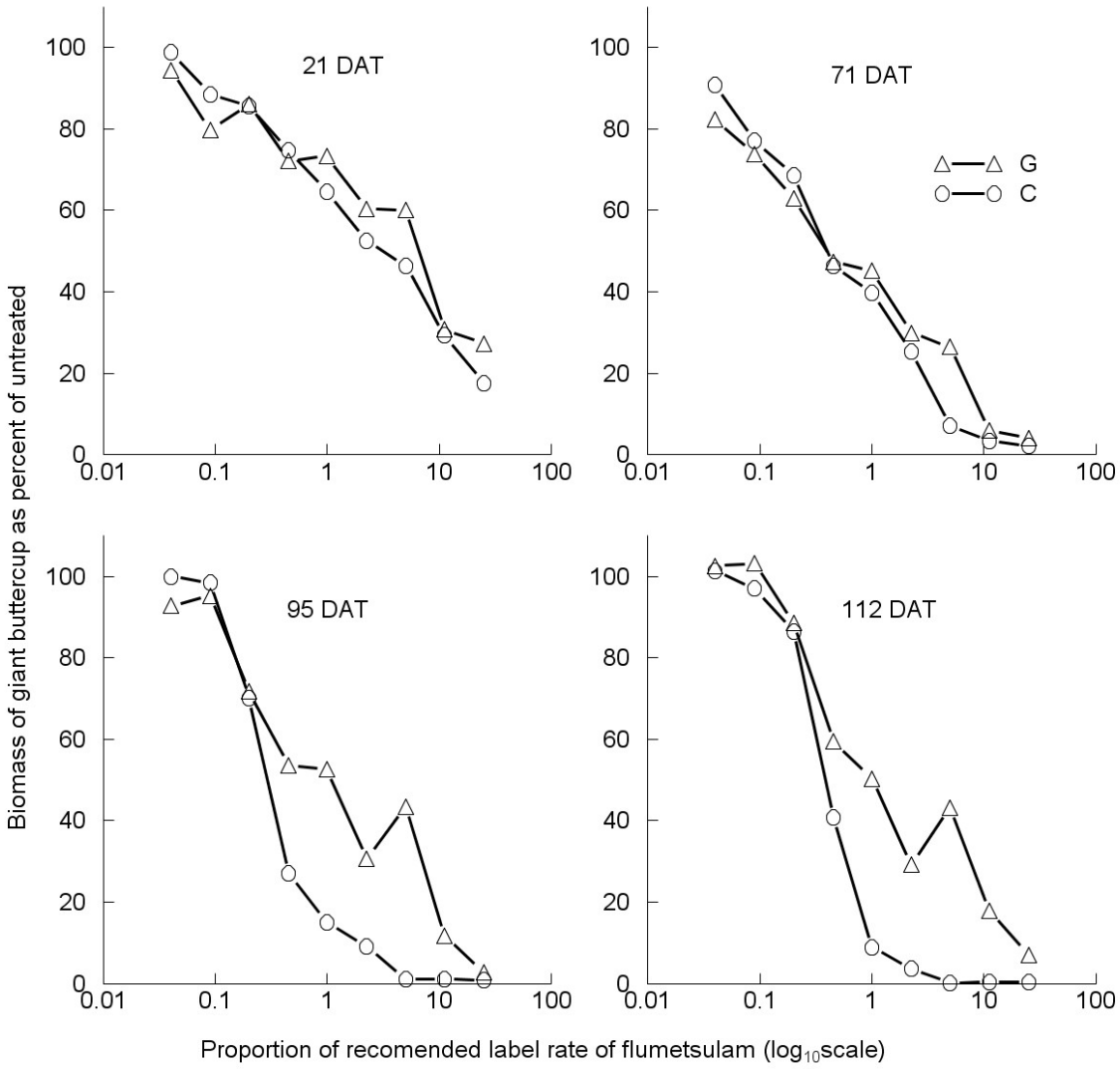

Figure 3.4 Reduction in biomass for giant buttercup plants treated with nine rates of flumetsulam at 21, 71, 95 and 112 DAT, for populations 'G' and 'C' in Experiment 2.

In population 'G' it was observed that six plants out of 21 (29%) survived each of the highest two rates (11.2 & 25 times recommended) when assessed 112 days after treatment. Biomass for these individuals declined to about 11% after 70 days but then increased markedly as they re-grew (Figure 3.5). Some plants were only damaged partially before re-growing while others showed complete dieback of foliage before regrowing from the rhizome (Plates 3.2 and 3.3).

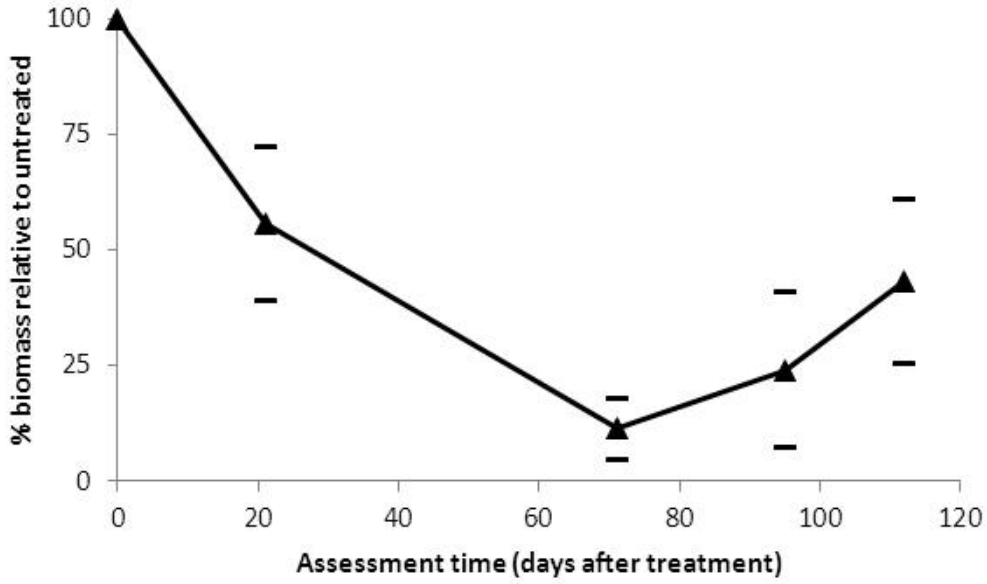

Figure 3.5 Change in biomass of the 12 plants ('G' population) that survived 11.2x and 25 times the recommended rate of flumetsulam in Experiment 2. Confidence intervals are shown (95%).

3.3.2 Mortality

The percent mortality of the two populations of giant buttercup ('G' and 'C') varied over the nine rates of flumetsulam but more plants survived from population 'G' overall (Table 3.2, Plates 3.1a,b,c,d). Furthermore, for the 'G' population the percentage of plants surviving was high at rates over the recommended application rate: 53%, 48% 29% and 29% at 2.2, 5, 11.2 and 25 times recommended rate, respectively (Table 3.2). This shows that a high proportion of individuals in the 'G' population are resistant to extremely high rates of flumetsulam.

Table 3.2 Percent mortality for populations of giant buttercup 'C' and 'G', treated with nine rates of flumetsulam at 112 DAT in Experiment 2.

	% Mortality	
Rate of flumetsulam (g/ha)	**G**	**C**
2.1	0	0
4.7	0	0
10.4	0	0
23.3	19.0	7.1
52.0	28.6	78.6
116	47.6	92.9
260	52.4	100
581	71.4	100
1300	71.4	100

The percentage mortality averaged over all rates was 32.3 and 54.4 (LSD 5% = 21.63) for the 'G' and 'C' populations, respectively. The difference was highly significant ($P < 0.001$). The back-transformed calculated LD_{50} values for the 'G' and 'C' populations were 222 g ai/ha (95% CI = 160 – 326) and 42 g ai/ha (95% CI = 34 – 51), respectively. This shows a 5.3-fold difference in the LD_{50} doses between the two populations. The Log_{10} (LD_{50}) values for 'G' and 'C' populations were 2.347 (95% CI = 2.20 – 2.51) and 1.62 (95% CI = 1.54 – 1.70), respectively (Figure 3.6).

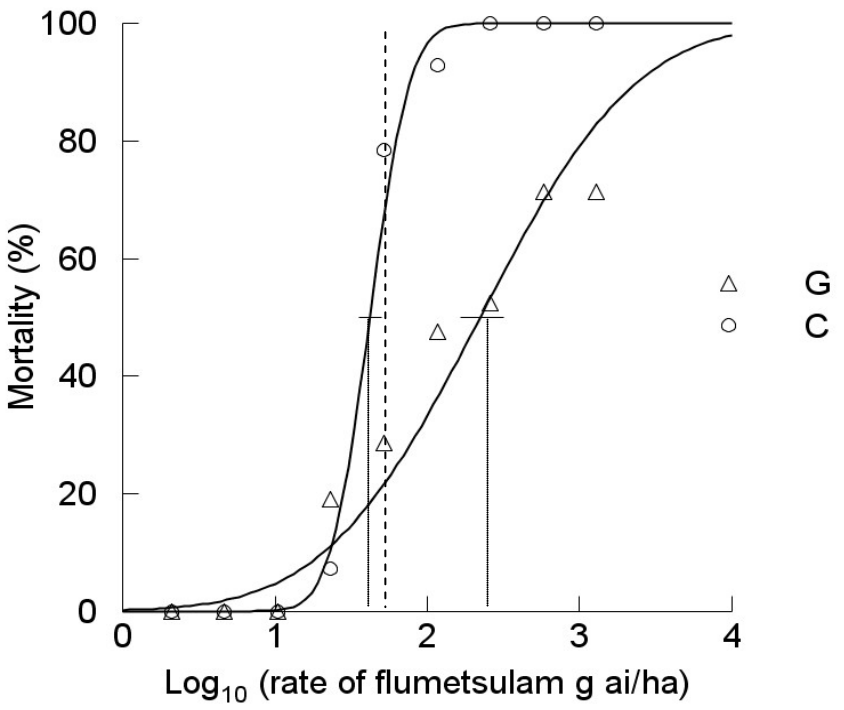

Figure 3.6 Percent mortality for the two populations of giant buttercup ('G' and 'C') treated with nine rates of flumetsulam in Experiment 2 with fitted Probit curves. Parameter estimates for the curves were generated in Genstat. Horizontal bars at the 50% mortality point show 95% confidence intervals for the LD_{50} values for each population. Vertical dotted lines indicate the log_{10} LD_{50} values; 1.62 and 2.35 for 'C' and 'G' respectively. Vertical dashed line is the middle rate (recommended field application rate).

3.4 Discussion

The results show a highly significant difference in both the percent mortality and the effective dose (LD_{50}) between the 'G' and 'C' populations. Population 'G' (high past exposure to flumetsulam) had a more than five-fold larger LD_{50} value than Population 'C' (no past exposure). The mortality results also showed that the proportions of survivors at rates above the label recommended field rate for population 'G', were similar (29 – 52% survival) (Table 3.2). This suggests that there is about one third or more of individuals in this population that are highly resistant to the herbicide, and that the remainder, have a somewhat lower level of resistance, as indicated by the survival at the field rate (71%) and at half the field rate (81%) (Table 3.2).

The probit curves fitted to data in this experiment were good fits and the LD_{50} values fell in the middle of the range of rates chosen so are well estimated. Also, in this experiment the level of mortality at the recommended application rate was lower than in Experiment 1, and very low at below the field rate. This differs from the result in Experiment 1 in which mortality was high at rates below the field rate. This factor also contributed to the better fit of the data in the probit analysis.

Factors that may have contributed to the difference in the responses of the populations in the two experiments are likely to be due to differences in the state of the plants at the time of spraying. Firstly, the plants in Experiment 1 were grown in the glasshouse over winter and brought out into a cool shadehouse in early spring, where they were suddenly exposed to frosts. Consequently, they were etiolated and tender (Plates 2.5 and 2.6, pg 77) and possibly more susceptible to herbicide injury, whereas in the second study plants were grown outdoors from late summer until autumn and they were hardy compact individuals at the time of spraying. Also, the time of spraying differed in that the spring-sprayed plants in the first experiment would have been actively growing under increasing temperatures (Fig. 2.4), whereas the autumn-sprayed plants in the second experiment would have been less actively growing as the days were shortening and maximum daily temperatures declining before winter (Fig. 3.2). It is generally accepted that plants growing more actively are more susceptible to flumetsulam herbicide and this is stated on the herbicide label (Young 2010). Another possible reason is a difference in the ages of the plants, (4 compared to 3 months at the time of spraying for Experiments 1 and 2 respectively), but this is less likely as the cause because the older plants were more susceptible than the younger plants, which is not what would be expected.

Although these results give a good indication that resistance to flumetsulam has evolved due to high past exposure it would be wise to test this on field populations

as prescribed in the criteria for the first determination of a herbicide resistant biotype (Heap 2011). This would entail spraying plots of giant buttercup, in a properly designed experiment, in pastures from the two populations where the seed was collected from.

Plate 3.1a & b show two different replicates of pots of giant buttercup treated with nine rates of flumetsulam (0.04-25 times recommended rate) on 11th August 2010 in Experiment 2. Pots are arranged with increasing rate from left to right with population 'G' (bottom three rows, green tags) and population 'C' (top two rows, purple tags) and control on far left (yellow tags).

Plate 3.1c & d show two different replicates of pots of giant buttercup treated with nine rates of flumetsulam (0.04-25 times recommended rate) on 11th August 2010 in Experiment 2. Pots are arranged with increasing rate from left to right with population 'G' (bottom three rows, green tags) and population 'C' (top two rows, purple tags) and control on far left (yellow tags).

Plate 3.2 A healthy plant from population 'G' that has survived rate eight (581 g/ha; 11.2 times recommended rate) of flumetsulam, 101 days after treatment (11th August 2010) in Experiment 2. In this case the original foliage was only partially damaged before fresh regrowth occurred.

Plate 3.3 A giant buttercup plant from population 'G' that has survived 25 times recommended rate of flumetsulam, 101 days after treatment (11th August 2010) in Experiment 2. The original foliage was almost completely destroyed before fresh regrowth occurred from the rhizome.

Plate 3.4 Two giant buttercup plants from population 'G', that have survived treatment with 11.2times (left) and 25 times (right) recommended rates of flumetsulam, 101 days after treatment (11th august 2010) in Experiment 2.

Chapter Four

Effects of MCPA, flumetsulam and thifensulfuron-methyl on newly sown and established pasture plants – Experiment 3

4.1 Introduction

Most farmers rely on herbicides for control of giant buttercup because of a lack of cost-effective or practical alternatives. During the survey (Chapter 2) some farmers had mentioned that all of the herbicides they use have often caused some pasture damage. MCPA (various trade names) damages clovers, as does thifensulfuron-methyl (Harmony, Chord, Ranger), according to the manufacturers' labels, when applied at the label rate (Young 2010). Damage to clover by MCPA is well documented and yields can be reduced by 50-80% (Thompson & Saunders 1984; James et al. 1993). Farmers are prepared to accept this outcome because of its relatively low cost of about $30-45/ha (current prices quoted from PGG Wrightson, includes GST) and rapid action during the spring milking season when maximum pasture production is needed to support lactating dairy cows. By contrast MCPB is not damaging to clovers but is not as effective on giant buttercup (Bourdôt et al. 2007) and has been shown to be less cost effective than MCPA (Popay et al. 1989). MCPB is not widely used by farmers because of its lack of effectiveness on giant buttercup. Because resistance has evolved in giant buttercup to MCPA and MCPB some biotypes require up to five times the recommended rate to achieve adequate control (Bourdôt & Hurrell 1988). Such high rates would increase control costs and potentially damage to clover, but generally these herbicides cease to be an option on those farms where resistance has evolved.

Thifensulfuron-methyl has been shown to damage both grass and clover and reduce pasture production for several months, and may cause a change in species composition (Rahman & Martin 1989; Gomand et al. 1991; Harris & Husband 1997). It is relatively inexpensive ($28/ha), but is slower acting than MCPA. During the survey of dairy farmers in Golden Bay (Chapter 2) the majority of farmers interviewed had never used thifensulfuron-methyl or had only used it once or twice because they said it was far too damaging to pastures to ever use again.

Although flumetsulam (Preside, Valdo) is claimed to be safe on pasture grasses and legumes, farmer anecdote suggests that it does have some damaging effects. Little scientific evidence exists to verify these observations. Only one study to date compares the effects of these three herbicides on pastures in a single experiment (Harris & Husband 1997) but interpretation of these results is limited as damage was assessed only by visual estimations of effects. At the recommended rate flumetsulam suppressed clover by an order of magnitude less than both

thifensulfuron-methyl and MCPA, (6% vs. 60 and 67% respectively). This may warrant its higher price $77/ha (including uptake oil), but must also be weighed up against its slower time to act at a time when maximum pasture production is needed.

Following on from the earlier work on giant buttercup resistance carried out in this thesis (Chapters 2 and 3), the aims of this study were to objectively compare the same three herbicides used (flumetsulam, thifensulfuron-methyl and MCPA) in terms of their effects on pasture production and plant species composition over five months after treatment. This was done by treating both newly sown (Experiment 3a) and established (Experiment 3b) grass/clover swards in pots with a range of rates of each herbicide, and measuring the dry weights of herbage components harvested at different times after treatment. This information could be used to determine whether there is scope for increasing rates of each herbicide in order to deal with resistance in giant buttercup, without compromising pasture quality. Although MCPB is also an option for control of giant buttercup in new pastures because it does not damage clovers and is used mainly for small weeds, it was not included in the study because the main focus of this research was on flumetsulam, thifensulfuron-methyl and MCPA.

4.2 Methods

4.2.1 Experiment 3a New Pasture

In June 2010, 64 square pots (0.5 L each) were filled with standard bark, peat and sand potting mix plus 6-month slow-release fertilizer, Osmocote® (Appendix 1) and sown with a seed mix of certified basic Nui perennial ryegrass (*Lolium perenne*) and Huia white clover (*Trifolium repens*), at a sowing rate equivalent to 20 and 3 kg/ha respectively, and then placed in a shadehouse. The pasture swards in pots were grown on for 2 months and trimmed once with scissors within this time to simulate natural grazing and to allow tillers to thicken.

The pasture swards in the pots were treated with a logarithmic series (2.236 multiplier) of five doses of thifensulfuron-methyl (Harmony, DuPont NZ Ltd), the dimethylamine salt of MCPA (Maestro 750) and flumetsulam (Preside) (both Dow Agrosciences NZ Ltd), each with two rates above and below the recommended label rates plus an untreated control, making 16 treatments in total as in Experiment 1, (Chapter 2) (Table 4.1). Pots were assigned to treatments randomly.

Table 4.1 Herbicide and dose rate treatments applied in Experiments 3a and 3b

Proportion of recommended rate	flumetsulam (g ai/ha)	thifensulfuron-methyl (g ai/ha)	MCPA (g ai/ha)
0.0	0.0	0.0	0.0
0.20	10.4	2.0	250
0.45	23.2	4.5	560
1.0 (label rate)	52.0	10.0	1250
2.24	116.3	22.4	2790
5.00	260.0	50.0	6250

Treatments were applied on 20 August 2010, 2 months after sowing, under cool, still air conditions. Treatment of all plants took from around 8-11am in which time the temperature changed from about 5^{o}C to 10^{o}C. All herbicides were applied by a conveyer-belt sprayer (belt speed 1.06 m/s) delivering 200 L/ha at 210 kPa through a single 8002EVS flat-fan TeeJet® (Spraying Systems Ltd) hydraulic nozzle mounted 30 cm above the top of the plants, as used in Experiments 1 and 2. The clover plants had several trifoliate leaves and the pasture swards were dense (about 15 cm in height on average) at the time of spraying (see Plate 4.2).

The pots in the experiment were arranged in a randomised block design, with four blocks of the 16 treatments. After treatment the pots were returned to the shadehouse and watered regularly and observed for 5 months. All green plant material was harvested from each pot to a height of 5 mm at 2, 3 and 5 months after treatment (allowing it to re-grow between harvests). The pasture swards measured 20-25 cm in height on average (from the soil surface to the top of the tallest grass shoots) at the time of each harvest, although plants were slightly taller and denser at the last harvest time due to warmer temperatures and rapid spring growth between the second and third harvests. On each occasion the harvested material was separated into ryegrass and clover before being dried at 65°C for 48 hours and then weighed.

4.2.2 Experiment 3b Established Pasture

This was carried out in exactly the same way as Experiment 3a, except that 75mm diameter cores were taken from an established perennial ryegrass and white clover pasture at the AgResearch Lincoln farm and grown in the same-sized pots as used in Experiment 3a. This pasture had been established for about 8 years without being cultivated (AgResearch farm worker, pers comm.). Pasture species appeared mainly to be perennial ryegrass and white clover, but other grasses, clovers and herbs were also present in small numbers, including subterranean clover (*Trifolium subterraneum*), cocksfoot (*Dactylis glomerata*) and narrow-leaved plantain (*Plantago lanceolata*). Experiment 3b ran concurrently with Experiment 3a in the

shadehouse and was treated using the same sprayer at the same time and with the same 16 treatments (Table 4.1).

Plant material was harvested on the same three occasions as in Experiment 3a, at 2, 3 and 5 months after treatment, but in this case the biomass was separated into grasses, clovers and weeds before being dried and weighed. The height of pasture was about 15-20 cm on average at the time of each harvest, although again slightly taller and denser at the last harvest time. Because the contribution of weeds was minimal, this component was excluded from the analysis.

4.2.3 Statistical Analysis and Design

The experimental design was a factorial with three herbicides and five application rates of each with four replicates of each treatment. Data for each experiment and for each time of harvest were analysed separately using GenStat's (Ver. 13 for Windows 2010) ANOVA procedure. All variables were analysed with herbicides and rates as a factorial design with the exception of the established clover dry weight. For that variable, treatments with a mean clover dry weight after spraying of less than 20% of the untreated were excluded from the analysis, to satisfy the ANOVA assumption of homogeneity of variance between treatments. The treatment means for established clover were highly variable, but this wasn't an issue with the grasses. The highest rates of MCPA were also excluded from the new clover analysis, for the same reason.

4.3 Results

4.3.1 Experiment 3a New pasture

Flumetsulam had reduced clover biomass significantly by 2 months after treatment by 43%, 75% and 63% at 0.2, 2.24, and 5.0 times the recommended rate respectively, but not at 0.45 and 1.0 times the recommended rate (Table 4.2). There was also evidence of a significant linear decline in biomass as the rate increased ($P<0.1$). At 3 months, the clover biomass was still significantly lower by 70 and 47% at 2.24 and 5.0 times the recommended rate but not at the lower three rates (0.2, 0.45, and 1.0 times recommended), but at 5 months there were no significant reductions at any rate. The total clover yield over the 5 month period was also significantly lower at 2.24 and 5.0 times recommended rate (by 39 and 32% respectively), but not at the lower three rates (0.2, 0.45 and 1.0 times recommended), (Figure 4.1a and Table 4.2). Plate 4.3 supports these results, as indicated by the amounts of clover visible in each pot for each rate of herbicide. Clover appears to be absent or greatly reduced in pots treated with the higher rates of flumetsulam.

Thifensulfuron-methyl reduced clover biomass significantly at all rates by between 41% and 90% at 2 months and by 56% and 90% at 3 months, but by 5 months biomass was only significantly reduced at the highest two rates (2.24 and 5.0 times recommended). Total clover yield over the 5 month period was significantly lower at all rates (P<0.05), by between 45% and 71%.

MCPA significantly reduced clover yields at all rates at 2, 3 and 5 months after treatment. Total production over the 5 month period was reduced by 46% and 80% for 0.2 and 0.45 times the recommended rate respectively, while at the field rate and above, clover was virtually eliminated (Table 4.2, last column, and Figure 4.2).

Table 4.2 Dry weight (g/pot) of clover harvested from pots of newly-sown pasture (Experiment 3a) at 2, 3 and 5 months after spraying, and the total dry weight of clover produced over the three harvests. Numbers in brackets were excluded from the analysis. * indicates a significant difference from the untreated control.

Treatment	Proportion of recommended rate	Months after Treatment			
		2	3	5	Total
flumetsulam	0.2	1.08*	1.44	5.00	7.51
	0.45	1.40	1.57	4.95	7.92
	1.0	1.55	1.66	5.10	8.31
	2.24	0.48*	0.69*	5.25	6.41*
	5.0	0.70*	1.19*	5.17	7.07*
thifensulfuron-methyl	0.2	0.73*	0.99*	4.00	5.71*
	0.45	0.49*	0.58*	3.70	4.77*
	1.0	0.38*	0.95*	4.83	6.15*
	2.24	0.26*	0.51*	3.55*	4.32*
	5.0	0.18*	0.21*	2.65*	3.04*
MCPA	0.2	0.55*	0.96*	4.15*	5.66*
	0.45	0.19*	0.16*	1.77*	2.12*
	1.0	(0.03)	(0.02)	(0.40)	(0.40)
	2.24	(0)	(0)	(0)	(0)
	5.0	(0)	(0)	(0.20)	(0.20)
Untreated	-	1.88	2.23	6.35	10.45
LSD (P<0.05)		0.77	0.79	2.81	3.29
Linear rate effect					
flumetsulam		10%	ns	ns	ns
thifensulfuron-methyl		ns	10%	ns	ns
MCPA		-	-	-	-

* = 5%, ** = 1% significant

Dry weights of grass were significantly reduced by the highest rate of thifensulfuron-methyl (5.0 times recommended rate) at 2 and 3 months but not for any other rate, herbicide or time (Table 4.3).

Total grass yields over the 5 month period tended to be higher than for untreated control pots for both flumetsulam and MCPA at all rates, but this difference was only significantly higher for the highest rate of MCPA and the lowest rate of thifensulfuron-methyl (Table 4.3 and Figure 4.1b). There was a highly significant linear decline (P<0.001) in total grass yield over the 5 month period with increasing rate for thifensulfuron-methyl.

Table 4.3 Dry weight (g/pot) of grass harvested from pots of newly sown pasture (Experiment 3a) at 2, 3 and 5 months after spraying, and the total dry weight of grass produced over the three harvests. * indicates a significant difference.

Treatment	Proportion of recommended rate	Months after Treatment			Total
		2	3	5	
flumetsulam	0.2	2.98	3.16	2.18	8.31
	0.45	3.12	2.71	2.30	8.12
	1.0	2.88	2.67	2.30	7.85
	2.24	2.88	3.13	2.28	8.28
	5.0	2.93	3.20	2.30	8.43
thifensulfuron-methyl	0.2	3.55	3.26	2.23	9.04*
	0.45	2.60	2.57	1.93	7.10
	1.0	2.48	2.57	2.10	7.15
	2.24	2.43	2.32	2.03	6.77
	5.0	1.80*	1.93*	2.18	5.90
MCPA	0.2	3.10	2.74	2.38	8.21
	0.45	3.08	3.12	2.43	8.62
	1.0	2.70	3.00	2.48	8.18
	2.24	2.73	3.02	2.23	7.97
	5.0	3.05	3.97	2.73	9.74*
Untreated	-	2.55	2.83	1.75	7.13
LSD (P<0.05)		0.73	0.86	0.70	1.65
Linear rate effect					
flumetsulam		ns	ns	ns	ns
Thifensulfuron-methyl		**	**	ns	**
MCPA		ns	*	ns	ns

* = 5%, ** = 1% significant

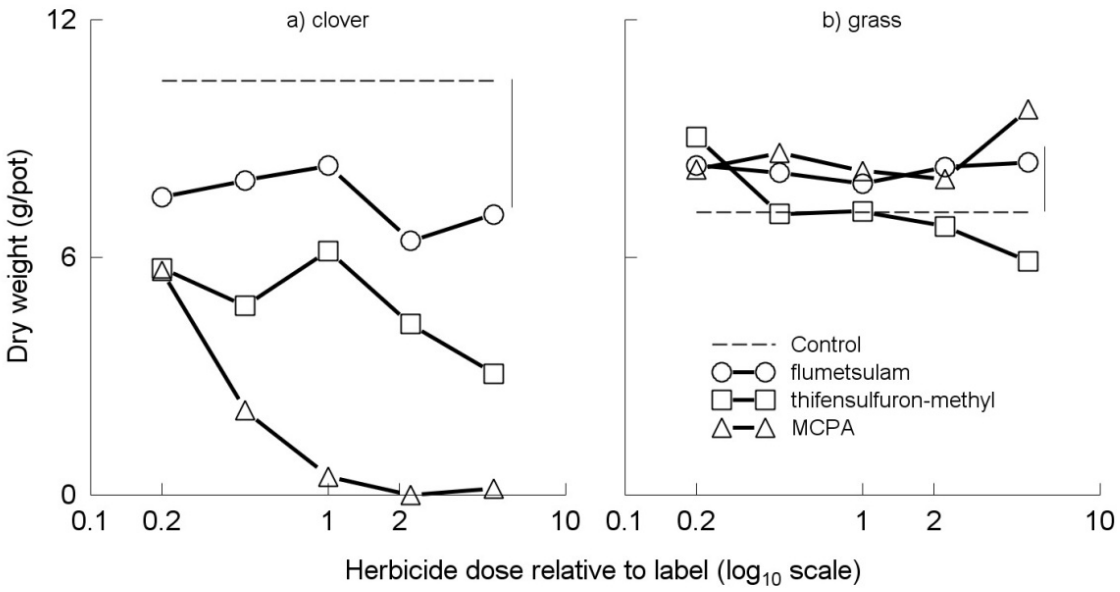

Figure 4.1 Dose responses of 2-month old (a) white clover and (b) perennial rye grass to flumetsulam, thifensulfuron-methyl and MCPA in Experiment 3a. Dry weights are the sums of harvests at 2, 3 and 5 months after treatment as given in Tables 4.2 and 4.3 (last columns). Vertical bar is LSD (5%).

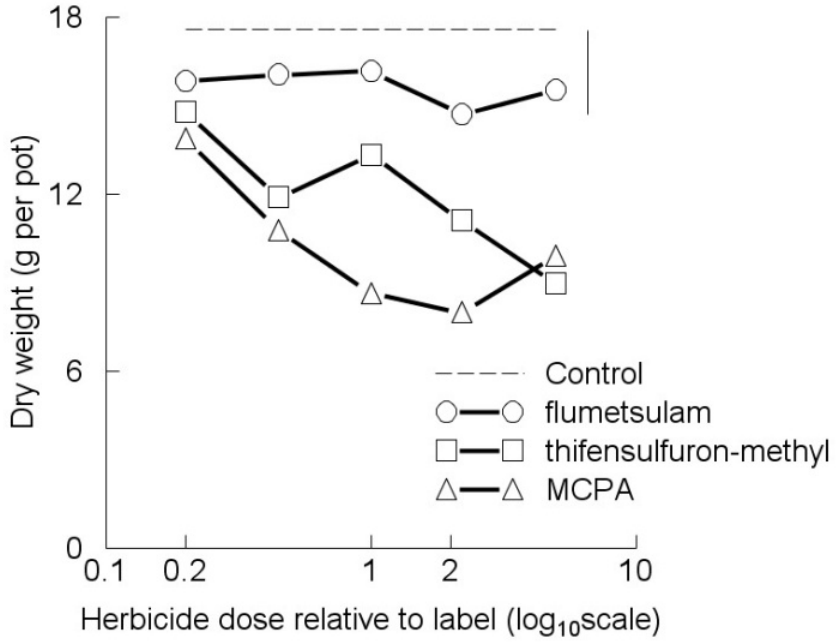

Figure 4.2 Dose responses of newly-sown pasture (grass and clover) to flumetsulam, thifensulfuron-methyl and MCPA in Experiment 3a, as given in Table 4.4, last column. Dry weights are the sums of harvests at 2, 3 and 5 months after treatment. Vertical bar is LSD (5%).

Flumetsulam had significantly reduced the total dry weight of pasture (grass and clover) at 2.24 times the recommended rate at 2 and 3 months, but not at any other rate or time (Table 4.4). Thifensulfuron-methyl had reduced the total dry weight of grass and clover at 0.45, 1.0, 2.24 and 5.0 times the recommended rate at 2, 3 and 5 months (except at the recommended rate at 5 months). It had also reduced the total yield over 5 months at all but the lowest rate (by 33 - 49%). MCPA reduced the total yield of grass and clover over the 5 month period at all five rates by between 21-55% (Table 4.4 and Figure 4.2).

Table 4.4. Dry weight (g/pot) of ryegrass and white clover harvested from pots of newly sown pasture (Experiment 3a) at 2, 3 and 5 months after spraying, and the total dry weight of grass and clover produced over the 5 month period. * indicates a significant difference from control.

Treatment	Proportion of recommended rate	Months after Treatment 2	3	5	Total
flumetsulam	0.2	4.05	4.60	7.18	15.82
	0.45	4.52	4.28	7.25	16.05
	1.0	4.43	4.33	7.40	16.16
	2.24	3.35*	3.82*	7.53	14.69
	5.0	3.63	4.40	7.48	15.49
thifensulfuron-methyl	0.2	4.28	4.25	6.23	14.75
	0.45	3.09*	3.15*	5.63*	11.86*
	1.0	2.85*	3.52*	6.93	13.30*
	2.24	2.69*	2.83*	5.58*	11.09*
	5.0	1.98*	2.14*	4.83*	8.94*
MCPA	0.2	3.65	3.70*	6.53	13.87*
	0.45	3.26*	3.28*	4.20*	10.74*
	1.0	2.73*	3.02*	2.88*	8.62*
	2.24	2.73*	3.02*	2.23*	7.97*
	5.0	3.05*	3.97*	2.88*	9.89*
Untreated	-	4.42	5.06	8.10	17.58
LSD (P<0.05)		0.98	1.07		2.94
Linear rate effect					
flumetsulam		10%	ns	ns	ns
Thifensulfuron-methyl		**	**	ns	**
MCPA		ns	ns	**	**

* = 5%, ** = 1% significant

4.3.2 Experiment 3b Established pasture

The biomass of the components of the cores were reduced in a similar way to the effects seen in Experiment 3a, however the responses were more variable.

Flumetsulam significantly reduced clover yield at 5.0 times the recommended rate at 2 months (by 92%) and at 2.24 and 5.0 times recommended rates at 3 months (by 75-78%), but there were no significant reductions at any other rate or time (Table 4.5). There was a significant linear decline ($P<0.05$) in clover yield with increasing rate of flumetsulam at 2 and 3 months, and in the total clover yield produced over 5 months. Thifensulfuron-methyl reduced clover dry weight at all rates at 2 months (78-96% reduction) and 3 months (80-97% reduction), but only at 0.2 times the recommended rate at 5 months. The total clover yield over the 5 month period was reduced at 0.2, 0.45 and 5.0 times the recommended rate. MCPA reduced clover dry weight at all rates at 2 months (52-100% reduction) and 3 months (73-100% reduction) but only at 5.0 times the recommended rate at 5 months. MCPA reduced the total clover yield over 5 months at 0.45 times the recommended rate and above (by 73-98%) (Table 4.5 and Figure 4.3a).

Table 4.5 Dry weight (g/pot) of clover harvested from pots of established pasture (Experiment 3b) at 2, 3 and 5 months after spraying, and the total dry weight of clover produced over the three harvests. * indicates a significant difference between the treatment and the untreated. (Treatments where the mean was less than 20% of the untreated mean were excluded from the statistical analysis so that the analysis of variance assumption of homogeneity of variance was satisfied; this is indicated by putting the corresponding treatment means in brackets.

	Proportion of recommended	Months after Treatment			
Treatment	rate	2	3	5	Total
flumetsulam	0.20	0.60	1.13	3.44	5.17
	0.45	0.55	0.87	2.41	3.83
	1.00	0.50	0.74	3.27	4.52
	2.24	0.20	0.28*	1.85	2.33
	5.00	(0.05)	0.25*	1.30	1.60*
thifensulfuron-methyl	0.20	(0.03)	(0.03)	(0.10)	(0.10)
	0.45	0.14*	(0.20)	0.63	0.98*
	1.00	(0.05)	(0.22)	1.77	2.04
	2.24	(0.06)	(0.11)	1.75	1.92
	5.00	(0.03)	(0.04)	(0.20)	(0.20)
MCPA	0.20	0.30	0.30*	1.57	2.17
	0.45	(0.05)	(0.15)	1.14	1.34*
	1.00	(0.04)	(0.08)	1.32	1.44*
	2.24	(0.03)	(0.08)	(0.40)	(0.50)
	5.00	(0.00)	(0.00)	(0.10)	(0.10)
Untreated	-	0.63	1.14	2.97	4.74
LSD (P<0.05)		0.48	0.82	2.46	3.06
Linear rate effect					
flumetsulam		-	*	ns	*

ns = not significant; * = 5% significant; ** = 1% significant

Grass biomass was not significantly affected by any of the herbicides, as they all yielded the same or more than the untreated control (Table 4.6). These results showed that where the clover was reduced the grass tended to compensate with increased yields.

Table 4.6 Dry weight (g/pot) of grass harvested from pots of established pasture (Experiment 3b) at 2, 3 and 5 months after spraying, and the total dry weight of clover produced over the three harvests. * indicates a significant treatment difference.

Treatment	Proportion of recommended rate	Months after Treatment			Total
		2	3	5	
flumetsulam	0.2	1.95	0.76	1.10	3.81
	0.45	1.93	0.72	1.00	3.66
	1.0	2.35	0.80	1.08	4.23
	2.24	1.50	0.78	1.20	3.48
	5.0	2.68	0.97	0.99	4.64
thifensulfuron-methyl	0.2	3.70	1.05	1.25	6.00
	0.45	3.03	0.66	0.93	4.61
	1.0	2.10	0.86	0.93	3.88
	2.24	2.35	0.64	0.79	3.77
	5.0	2.13	0.79	1.13	4.04
MCPA	0.2	3.20	0.92	0.88	4.99
	0.45	3.63	1.34	1.53	6.49
	1.0	2.33	0.75	1.03	4.11
	2.24	1.85	0.87	1.07	3.79
	5.0	2.20	0.77	1.12	4.08
Untreated	-	2.25	0.72	0.90	3.87
LSD (P<0.05)		1.16	0.373	0.476	1.66
Linear rate effect					
flumetsulam		ns	ns	ns	ns
Thifensulfuron-methyl		**	ns	ns	ns
MCPA		**	10%	ns	*

* = 5%, ** = 1% significant

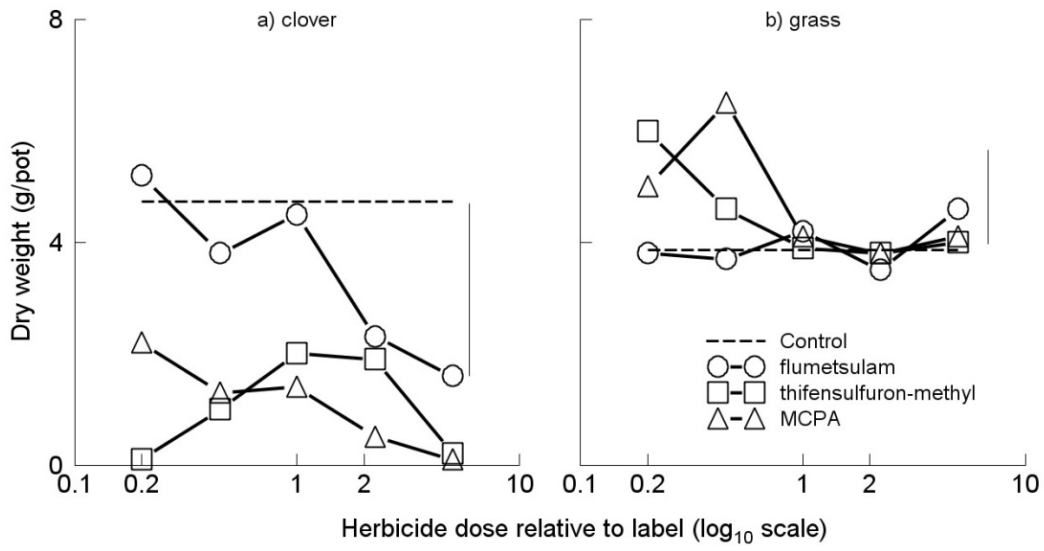

Figure 4.3 Dose responses of (a) white clover and (b) perennial ryegrass to flumetsulam, thifensulfuron-methyl and MCPA in Experiment 3b, for established pasture. Dry weights are the sums of harvests at 2, 3 and 5 months after treatment (from Table 4.7). Vertical bar is LSD (5%).

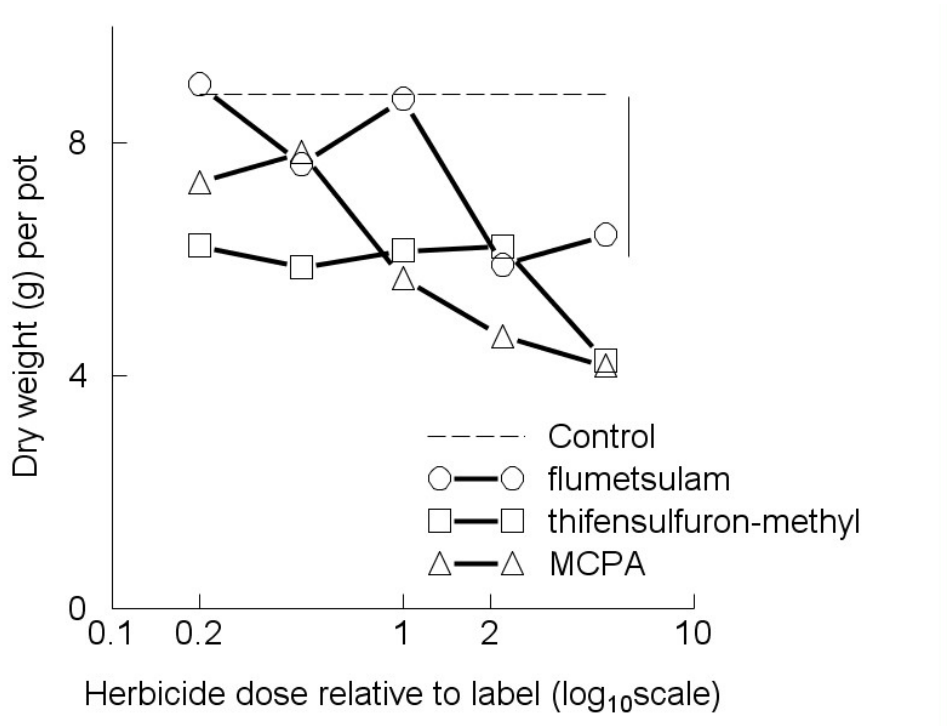

Figure 4.4 Dose responses of established pasture (grass and clover) to flumetsulam, thifensulfuron-methyl and MCPA in Experiment 3b, for established pasture. Dry weights are the sums of harvests at 2, 3 and 5 months after treatment (from Table 4.7). Vertical bar is LSD (5%).

Flumetsulam reduced the total dry weight of the pasture (grass and clover) at 2.24 times the recommended rate at 2 months (by 41%) and 3 months (by 42%), but not at any other rate or time (Table 4.7). The total yield of grass and clover over 5 months was also significantly reduced at 2.24 times the recommended rate by 33% (Table 4.7, last column and Figure 4.4).

Thifensulfuron-methyl reduced the total pasture dry weight at all rates by 43-59% at 3 months and at 0.2, 0.45 and 5.0 times recommended rates at 5 MAT, but at no other rate or time. The total yield over 5 months was reduced at rates of 0.45 and 5.0 x recommended rate (Table 5.7 and Figure 5.4). MCPA reduced the total pasture dry weight at all rates (except 0.45 times recommended rate) at 3 months (43-59% reduction), and at 2.24 and 5.0 times recommended rates at 5 months (57-70% reduction). The total yield over 5 months was reduced at the recommended rate and above by 36-53% (Table 4.7, last column, and Figure 4.4).

Table 4.7 Total dry weight (g) of grass and clover harvested from pots of established pasture (Experiment 3b) at 2, 3 and 5 months after spraying, and the total dry weight of pasture produced over the 5 month period. * indicates a significant difference from control.

Treatment	Proportion of recommended rate	Months after Treatment			Total
		2	3	5	
flumetsulam	0.2	2.56	1.90	4.54	9.00
	0.45	2.52	1.63	3.47	7.62
	1.0	2.85	1.55	4.35	8.75
	2.24	1.72*	1.09*	3.07	5.89*
	5.0	2.85	1.24	2.32	6.41
thifensulfuron-methyl	0.2	3.75	1.08*	1.38*	6.21
	0.45	3.21	0.92*	1.71*	5.84*
	1.0	2.27	1.12*	2.75	6.14
	2.24	2.61	0.78*	2.81	6.20
	5.0	2.15	0.83*	1.27*	4.25*
MCPA	0.2	3.55	1.25*	2.50	7.30
	0.45	3.67	1.49	2.67	7.83
	1.0	2.36	0.84*	2.46	5.66*
	2.24	1.95	1.02*	1.69*	4.66*
	5.0	2.20	0.77*	1.19*	4.16*
Untreated	-	2.95	1.90	3.98	8.82
LSD (P<0.05)		1.19	0.62	1.99	2.85
Linear rate effect					
flumetsulam		ns	**	*	*
Thifensulfuron-methyl		**	**	ns	ns
MCPA		**	ns	ns	**

* = 5%, ** = 1% significant

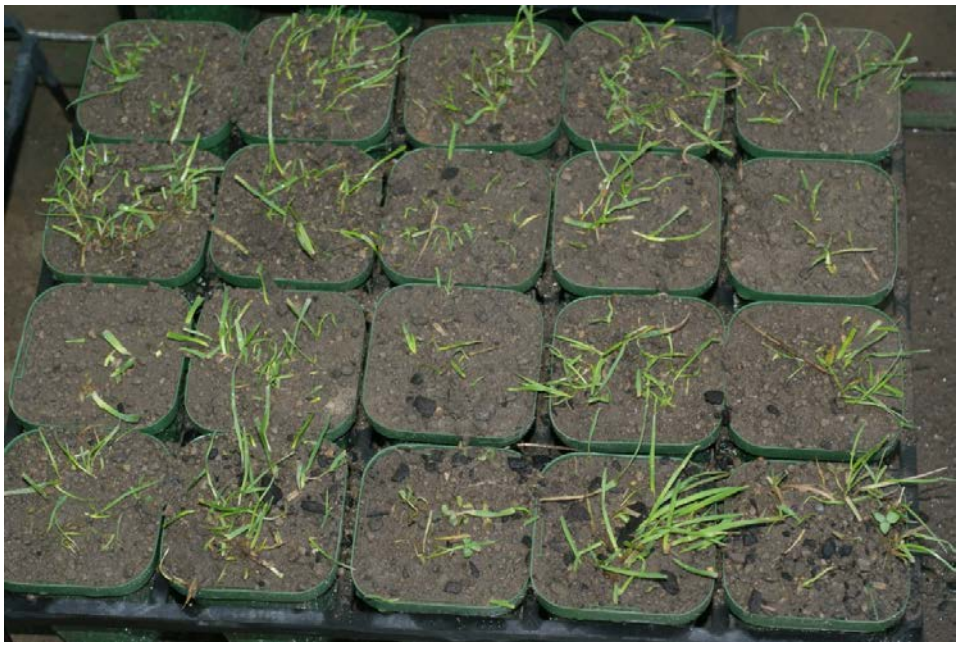

Plate 4.1 Pasture cores taken from the Lincoln farm and planted into square pots for established pasture Experiment 3b, 24th June 2010.

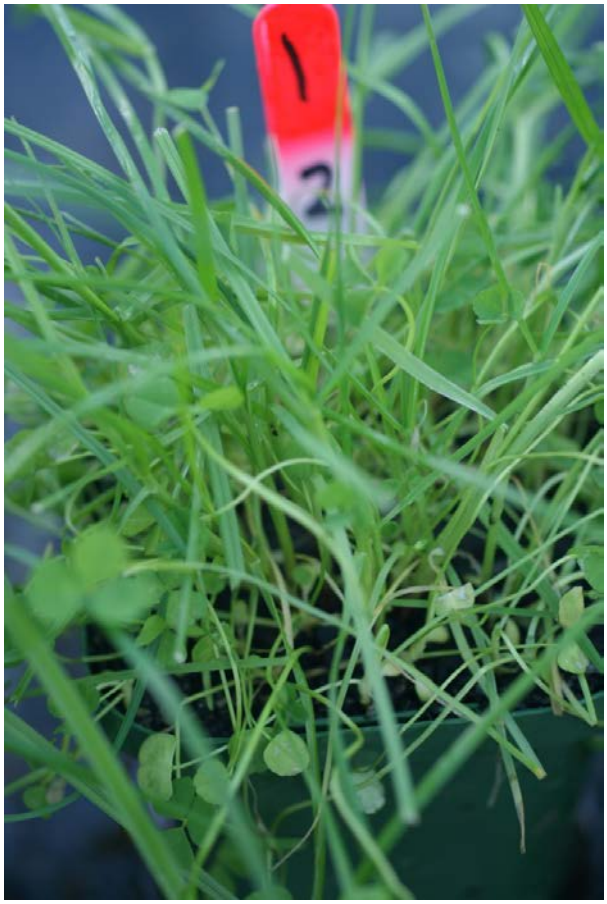

Plate 4.2 One pot of newly sown pasture soon after treatment with 0.2 times recommended application rate of MCPA in Experiment 3a. This photo indicates the approximate developmental stage of white clover plants at time of spraying.

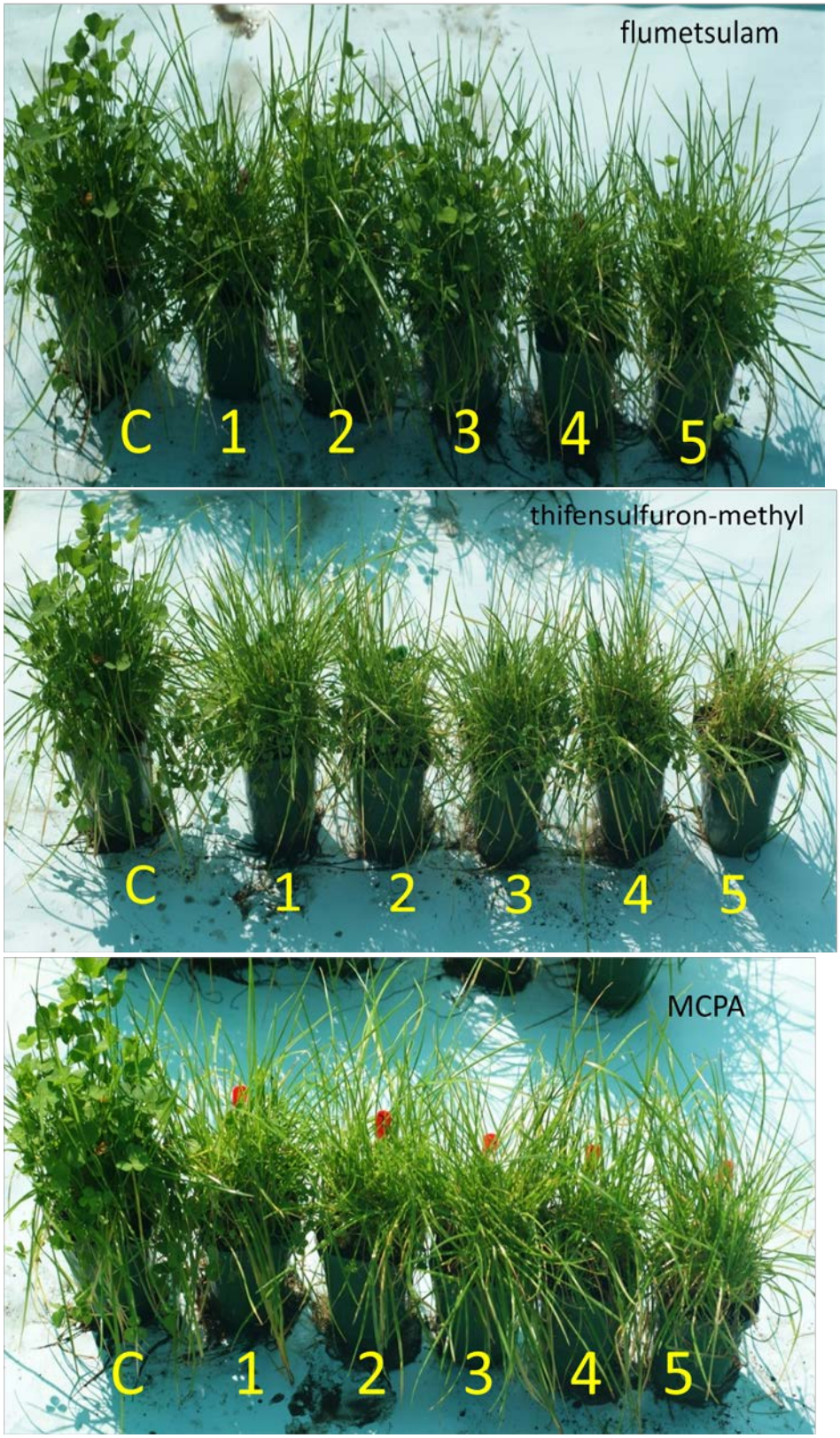

Plate 4.3 a, b & c These three photos show one replicate of the five treatments (rates) of each herbicide, plus an untreated control, taken 2 months after treatment in 'new' pasture pots (Experiment 3a). Rates are arranged lowest to highest L-R (0.2-5 times recommended rates) with control on far left. From top to bottom flumetsulam, thifensulfuron–methyl and MCPA.

4.4 Discussion

Both thifensulfuron-methyl and MCPA, at their respective recommended rates, reduced the total productivity of both the new and established pasture swards (Figs. 4.2 and 4.4) in the 5 months following treatment, mainly by reduction in clover content (Fig. 4.1a, 4.2a). These results are consistent with past research (Gomand et al. 1991; Harris & Husband 1997). By contrast, flumetsulam did not affect the clover or grass productivity at its recommended rate, so is the best herbicide choice for farmers (of the three tested here) to avoid pasture damage.

These results concur with those of the previous study by Harris and Husband (1997) but show that the damage from flumetsulam at above recommended rates is greater than they reported. The earlier study found that white clover was reduced by 18% at 1-2 months after treatment with flumetsulam (100g ai/ha) in established pastures, but in the present study (3 months after treatment with flumetsulam 116 g ai/ha in established pasture), clover was reduced by 75% (Table 4.5 and Figure 4.3). Newly sown pasture showed similar levels of clover reduction in the present study at 2 and 3 months after treatment with this same rate of flumetsulam (70-75% reduction), (Table 4.2), but this effect was not evident after 5 months. The difference in results between the two studies may be partially due to the earlier study being carried out in the field while the present study was carried out in a shadehouse using transplanted pasture cores taken from the field. Although a field environment may be more realistic in some ways, this experiment was carried out under controlled conditions with automatic watering, simulated grazing, standardized soil type and nutrient addition. This would reduce the variability inherent in field situations, including uneven topography, defoliation by stock, soil type, nutrient availability, drainage, wind, rain, drought and impact of pests and diseases. A search of the literature found no other studies on the effect of flumetsulam on white clover but studies on other medics, red clover and subterranean clover showed some damage at field rates in some cases (Bowran et al. 1993; Gilmour 1996; Ceballos et al. 2005).

The established pasture was far more variable in its response than was the new pasture, probably because pots began with varying quantities of clover and grass species (Plate 4.1), whereas the new pasture pots were sown with a consistent rate of seed. The yield from established pasture cores was also much lower than for the new pasture pots at all harvest times, because they did not grow back as vigorously after being harvested. These low data values may also have contributed to the higher variability and higher than expected reductions in yield in the established pasture cores.

The Preside (flumetsulam) label recommends spraying new pasture after the clover second trifoliate leaf has emerged (Young 2010), which is normally 3-4 weeks after emergence (S. Monk, pers. comm. AgR Grasslands technical officer). This research indicates that flumetsulam can damage clover severely at above label rates, when sprayed 2 months after sowing (with several trifoliate leaves) and this damage persists for 3 months (Table 4.2). At the recommended rate it had 20-25% less clover at 2 and 3 months in both new and established pasture, but these were not significant results.

MCPB is the only other selective herbicide option (besides the three tested here) available that is known to be safe on new clover, but this was not included in the study as it was not the main focus of this research. MCPB is known to be safe to use on new pastures (Harris & Husband 1997) for control of giant buttercup, and this study indicates that flumetsulam may be safe too. Bentazone has a recommendation for giant buttercup in established pasture also, but it must be used with either MCPA or MCPB applied first, and it doesn't have a specific recommendation for giant buttercup in new pasture.

The white clover component of pastures is important for New Zealand dairy farmers who rely on it for high milk productivity (Harris et al. 1998), unlike overseas farmers who often supplement feeding with other high energy food sources such as corn. Clover is also an important nitrogen fixing element (Charlton & Stewart 2000), and therefore if its content in the pasture is reduced, additional sources of nitrogen (e.g. urea) must be added in order for the grass to grow well, which will increase production costs (Andrews et al. 2007).

Harmony (thifensulfuron-methyl) has a label warning indicating that damage to pasture species is likely and recovery of clover vigour can take up to 4 months. In this study thifensulfuron-methyl damaged clover at all rates and the effects were still apparent after 5 months (Table 4.2). Also, it was the only herbicide that affected grass productivity as well as clover. However, the effect on grass productivity was confounded by the compensatory effects of removing/reducing the clover component of the swards.

Maestro (MCPA) has a label warning that high rates may cause clover suppression but doesn't warn about using on new pasture. This chemical almost completely eliminated clover from both new and established pasture, even at the lowest rates, for at least 3 months. MCPA is marketed in mixtures with MCPB (e.g. Select, Tandem, Thistrol Plus) and recommended for use on seedling giant buttercup in new pasture. These products at their recommended rate contain MCPA equivalent to 100 g a.i./ha. In this study, the lowest rate of MCPA (250 g a.i./ha) caused significant damage to clover productivity (45 % reduction over a 5 month period),

(Table 4.2 and Figure 4.1). Clover yield was reduced even more at this low rate at earlier times (70% and 57% reduction at 2 and 3 months after spraying). In light of these results it is possible that these mixtures may still be damaging to young clover, but rates used here were not as low as recommended in these mixtures.

Although significant reductions in clover productivity seen here only occurred at 2.2 and 5 times the recommended rate of flumetsulam, which would not theoretically be applied to pastures, in reality this may occur. When applying herbicides with a vehicle-mounted boom the swath may overlap partially or completely, when crossing tracks and can also occur if the water rates or mixing rates are not correct. Farmers have also reported sometimes increasing the dose of herbicide to achieve better control of giant buttercup (Golden Bay dairy farmer, Graham Ball, pers comm.). These results indicate that the rates of flumetsulam cannot be increased above recommended rates, should resistance to giant buttercup evolve or to achieve better control, without causing pasture damage.

Lack of pasture persistence is a problem in dairy pastures (Nie et al. 2004), and newly sown pastures that typically contain perennial ryegrass and clover often appear to quickly revert to grass weeds and other broad-leaved weeds. Degrading of pasture quality is often brought about by weakening as a result of drought, overgrazing or pugging in winter, and it has been shown that in these degraded swards recruitment into the gaps is usually by weedy species rather than desirable pasture species (Tozer et al. 2010). This is due to the lack of seeds of sown species, and a correspondingly high proportion of weed seeds in the seedbank. The results of this study have shown that the use of two herbicides marketed as ‘selective’ (thifensulfuron-methyl and MCPA) can have a serious detrimental effect on the pasture composition over a 5 month period, when applied at the recommended rate. Although the grass tended to partially compensate for the removal of the clover there was, overall, significantly less total biomass produced (25-51% reduction for the two herbicides respectively, Figs. 4.2 and 4.4). This, together with the removal of giant buttercup (which can be up to 60% cover at its peak) would inevitably result in an opening up of the sward which could promote the ingress of giant buttercup and other weeds by allowing recruitment of seedlings. Therefore, removing the weed without any follow-up to prevent seedling establishment may be only a short-term solution to improve pasture production.

When farmers are contemplating spraying pastures for giant buttercup control they need to carefully consider the consequences of using herbicides which can damage the pasture and weigh this up against the benefits of removing the weed. Also, they need to consider the optimum timing of spraying to maximise these benefits. Both flumetsulam and MCPA have recommendations for spraying giant buttercup in spring or autumn. It may be preferable to spray in the autumn at the end of the

milking season (provided pasture is not drought or frost damaged) so that pasture quality is not compromised during the peak of milk production and to allow the herbicide time to remove the weed before the next season begins. It has also been found that early rather than late winter applications of phenoxy herbicides cause less reductions in white clover and total pasture dry matter yields (Honore et al. 1980), but August rather than May applications were found to be more effective on giant buttercup (Popay et al. 1989b). Spring and autumn applications of flumetsulam were found to be equally effective on giant buttercup (Harris & Husband 1997) however, it is generally thought that spraying is best done when pasture is actively growing (such as in spring) so it can grow out of the damage quickly, and is more effective for weed control at this time too. One way of overcoming the problem of pasture damage could be to apply the herbicides using wick boom technology, where pasture is not exposed to the herbicide, but this needs further investigation.

Chapter Five
Final Discussion and Conclusions

Results from the two experiments on giant buttercup (Experiments 1 and 2 in Chapters 2 & 3 respectively) both indicated that giant buttercup seedling progeny from populations with high past exposure to flumetsulam have a greater tolerance of this herbicide. While in the first experiment results were not conclusive because the ranges of herbicide rates chosen were too high, resulting in poorly fitting probit curves and LD_{50} estimates, the second experiment using a wider range of rates and more replicates gave a higher level of confidence in the results. These latter results showed a 5.3-fold difference in the LD_{50} values between the two populations tested (no versus high historical exposure), giving good evidence for resistance evolution. However, according to the criteria for initial confirmation of a resistant weed and its inclusion in the database (Heap, 2011), field trials need to be carried out to validate the conclusions derived from glasshouse dose-response tests (Criteria No. 2; Data confirmation of resistance). These field trials would entail spraying plots in the susceptible and resistant populations in the grazed dairy pastures that the seed was collected from, using a similar wide range of rates as in the glasshouse studies.

The first experiment suggested that the application rate of flumetsulam could be reduced without sacrificing efficacy, but the second experiment showed this was probably not the case and that the recommended application rate is required to get a satisfactory level of kill on a susceptible population. Also, in the field, plants would be of mixed age and perhaps not as susceptible as the young plants used in this study, so reducing the application rate would probably not be advisable. One reason for the difference between the two experiments could be due to the timing of application, whereby spring rather than autumn application gave a higher level of mortality. This was previously shown by Harris and Husband (1997) who found spring applications to be more effective than in autumn, in the first year of treatment. The second experiment also showed even higher doses of flumetsulam may be required on more resistant populations to achieve a similar level of control. But the results also showed that raising the rate of flumetsulam in a population that was showing resistance (e.g. population ‘G’) would not achieve a higher level of kill because the proportion of survivors at these higher rates did not change (Table 3.2) and remained at about 29% survival for both 11.2 and 25 times recommended rates. This also implies that the most resistant population was made up of a proportion of highly resistant individuals tolerant of any realistic rate and others that were susceptible at recommended rates and below. So, this leaves no scope for managing resistance through altering the use rate of flumetsulam. Furthermore there is little scope for increasing rate in order to get a higher level of kill because the pasture tolerance becomes problematic for flumetsulam at above label rates (Figure 4.1) and the cost of control would be excessive.

It was widely believed that many giant buttercup populations in Golden Bay were resistant to phenoxy herbicides; which may be the reason most farmers say they are now using flumetsulam (as indicated in the survey in Chapter Two). The research in this study seems to suggest that many of these populations are now

susceptible to MCPA because there were few surviving plants above the recommended application rate. However, directly comparing the susceptibilities of giant buttercup populations in the earlier study to MCPA with those in the present study is not appropriate because they are not the same populations. One possibility for the apparent higher susceptibility is that the selection pressure has been reduced or removed and these populations have reverted to a susceptible state during the two decades since ALS inhibiting herbicides became available. If this is the case, that resistance to MCPA has diminished, then that leaves open the possibility for herbicides to be rotated in order to delay or prevent further development of resistance.

Given that one population in this study (population 'R') appeared susceptible at the recommended rate of MCPA, despite being a heavy user of MCPA for several decades up until the present, this does not support the hypothesis about reversion to the susceptible genotype. A possible explanation for the apparent susceptibility of the 'R' population is related to the seed collection. Often when researchers test for resistance they collect seed from populations that have been recently treated with the chemical in question. In those cases all the susceptible plants would have recently been killed, leaving only resistant individuals from which to collect seed, and in doing so over-estimating resistance as a consequence. If the 'R' population had been treated with an ALS herbicide, rather than MCPA, in the previous season there may have been more MCPA susceptible plants present to collect seed from, because the selection pressure for MCPA would have been lower. This farmer had never used flumetsulam but did admit to using thifensulfuron-methyl on two occasions which may have been in the year prior to seed collection.

There is also the possibility that the newer formulation of MCPA (dimethylamine salt) is more active on giant buttercup than the potassium salt formulation which had been used in previous dose-response studies when resistance was first discovered in the 1980s. However, this explanation is unlikely to account for the difference of 5-fold in susceptibility that was found by Bourdôt and Hurrell (1990) and the higher level of susceptibility in this study. A third, and more likely, explanation is that the plants differed in their susceptibilities between the first and second experiments, because of their growth stage. Since the plants were grown from the same seed lines in both experiments it is possible that the MCPA-treated plants would have responded similarly to the flumetsulam-treated plants in Experiment 2. The mortality of plants treated with flumetsulam was lower in the second experiment than in the first suggesting that the plants in the first experiment were more susceptible because they were weakened by being young, tender glasshouse-grown individuals. If the same explanation were true for MCPA, this gives some support for why the mortality of plants was high in Experiment 1. Furthermore, it has been shown that plants treated with MCPA in spring are more susceptible than those treated in autumn (Popay et al 1984), which gives further support for this explanation.

The four herbicides registered for giant buttercup control in New Zealand pastures (MCPA, MCPB, flumetsulam and thifensulfuron-methyl) fall into only two mode-of-action groups, and cross-resistance within the groups occurs. Populations of giant buttercup that are resistant to MCPA are also resistant to MCPB (Bourdôt et al. 1994), and the current study indicates that populations resistant to flumetsulam may also be resistant to thifensulfuron-methyl (Figure 2.7). The implications of these results are that current management practices for giant buttercup using regular applications of currently available herbicides are unsustainable, because resistance has evolved to both chemical groups in different populations. Therefore it is possible that populations could evolve resistance to both groups leaving few options for selective chemical control in that farming system.

In the meantime, the importance of proper herbicide resistance management practices must be stressed to dairy farmers who use annual herbicide applications to control giant buttercup, in order to prolong the usefulness of herbicides on their farms. This includes rotating herbicides from different chemical groups from year to year, for example MCPA one year and flumetsulam the next, and skipping treatments where possible to allow seed set from susceptible plants and thus the 'dilution' of resistant seeds in the seedbank (Taskforce 2010). However it is also important to use the herbicides at rates which achieve a high kill of the weed, because low rates or rates that give a poor kill can lead to the evolution of polygenic resistance (Renton et al. 2011), through a build-up of resistant alleles. Lower than required doses can also be unintentionally applied through using herbicides on the wrong growth stage of plants, miss-timing applications, poor application technique or applying to plants which are under environmental stress.

More research is needed on refining control practices with less reliance on chemical control and integrating more non-chemical management approaches. Mowing during flowering (after grazing) may reduce the dominance of the weed (Lamoureaux & Bourdôt 2007) and also rotating pasture with crops (e.g. maize) for at least 12 months (Tuckett 1961). Grazing by sheep, which eat the buttercups, could be an option but is not very practical for dairy farmers to implement.

Alternatively, new herbicides could be found from different chemical groups. However the development of new herbicides may not provide an easy solution because resistance has shown to evolve quickly to many of these newer groups soon after their introduction (Moss 2002). Also, this is an unlikely possibility because few new herbicides are discovered nowadays. There are some herbicides from different groups used overseas that have shown effectiveness on giant buttercup but have not been trialed in New Zealand (Bourdôt 2011). For example dicamba (Group O2), aminopyralid and picloram (Group 03) show activity but they are selective to only grasses and damage legumes. Also there are some chemicals in New Zealand from different groups which have shown effectiveness on buttercup but are damaging to pastures (e.g. glyphosate, Group G and oxadiazon, group E). Some of these, for example glyphosate, dicamba, aminopyralid and picloram could be applied by a wick wiping technique. Giant buttercup may be a good candidate for wick wiping because of its tall height (can grow up to 1m), and cattle graze selectively around it. But farmers have reported trying wick wiping, using some of

these products, and have suffered pasture damage because the grass grows up through the buttercup plants at times of the year and is damaged by the herbicide. More research is needed on developing this idea, in particular, testing these herbicides against giant buttercup and also some of the newer wick applicators that follow uneven contours often found in dairy pastures.

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Appendix One

This letter was mailed to farmers on 10th December 2009.

Dear…

Your name has been randomly selected from a list of dairy farmers in Golden Bay to participate in a survey on control of giant buttercup. The information from this survey will be used as part of my Masters project with Massey University and AgResearch.

This project aims to determine whether giant buttercup is developing resistance to the newer-generation herbicides flumetsulam (Preside, Valdo) and thifensulfuron-methyl (Harmony, Ranger, Chord) on dairy farms in Takaka.

Background: Giant buttercup has evolved resistance to the phenoxy herbicides such as MCPA and MCPB as a result of use over several decades. Currently, flumetsulam, and to a lesser extent thifensulfuron-methyl, are the herbicides of choice by farmers for broad acre control because they are the only herbicides with label claims for giant buttercup in dairy pastures. Since they are both ALS inhibitors (a mode-of-action highly prone to resistance), giant buttercup populations are very likely to evolve resistance as they did to the phenoxy group through repeated use. Farmer anecdote suggests that this has already occurred on some farms. Data from this study could be used to support the case for more research into an alternate control for giant buttercup.

I ask that you take some time to answer the questions on the following page on your experiences with these two herbicides, such as history of use over the last decade and how effective you have found them to be both initially and longer term. I will then telephone you in a few days to discuss your answers. We may then ask if we could collect some buttercup seed from your farm sometime in January 2010 to test for possible resistance.

Thanks very much for your participation and co-operation,

Sincerely,
Carolyn Lusk
Science Technician (Weeds Group)

Questionnaire

1. Do you have giant buttercup on your farm?

2. How many paddocks are on your farm?

3. How many of these paddocks contain giant buttercup?

4. Have you used herbicides in the last 12 years to control it?

5. Have you used Preside or Valdo?

6. Have you used Harmony, Chord or Ranger?

7. For each of the herbicides listed above that you have used, can you provide the following:

    a. How many times have you used it, at what application rate, and what time of year?

    b. What was your overall opinion of each chemical, does it do the job?

    c. Have you noticed any change in the way the herbicide has performed over the years? (that is, is it any less effective now than it was when you first started using it?)

8. Have you used any other herbicide(s) in the last 12 years or before that? If so what herbicide, number of times used, application rate and time of year?

Appendix Two

Plant and Food Lincoln potting mix analysis. This was the potting mix used for all of the experiments and is the standard mix used by Plant and Food, Lincoln.

60% Hort-grade wood bark chips
40% washed crusher dust (residue from road chip crushing)
1.0 kg dolomite lime
0.6 kg agricultural lime
1 kg Osmocote© Exact©, 5- 6 mth slow release
Osmocote contains NPK 15+9+12+2MgO + TE (0.02 B, 0.055 Cu, 0.45 Fe, 0.06 Mn, 0.02 Mo and 0.02 Zn)
0.35 kg superphosphate
0.45 kg zeolite
0.1 kg $Ca(NO_3)_2$
0.1 kg K_2SO_4

Appendix Three

Link to published paper on work presented in this thesis.

Lusk CS, Bourdôt GW, Harrington KC and Hurrell GA 2011. Pasture tolerance and efficacy of three herbicides used against giant buttercup (*Ranunculus acris* subsp. *acris* L.). New Zealand Plant Protection. 64: 86-92

http://www.nzpps.org/journal/64/nzpp_640860.pdf