Glyphosate for winter weed control in lucerne (*Medicago sativa* L.)

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Abstract of a Thesis submitted in partial fulfilment of the requirement for the Degree of Bachelor of Agricultural Science with Honours

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As lucerne stands age the plant population declines and perennial weed species such as dock, dandelion, twitch, white clover and ryegrass invade the resident lucerne. Existing winter weed control herbicides such as paraquat and atrazine are expensive and often inefficient at controlling these weeds. Therefore, three field experiments were conducted in Iversen Field, Lincoln University, to investigate the effectiveness of four rates (0, 1, 2 & 4 L/ha) of the herbicide glyphosate as a perennial winter weed control option for lucerne (*Medicago sativa* L.*)*. The objectives of this research were to determine the optimum date and rate for the use of glyphosate in an 8 year old lucerne stand, by quantifying the phytotoxicity effects of glyphosate on lucerne and the target weed species. Experiment 1 was topped to remove herbage on the 4/4/2019, with the glyphosate applied on the 29/4/2019. The delay between herbage removal and herbicide application allowed the regrowth of plant herbage (kg DM/ha). Thus, the application of herbicide was onto actively growing plants in Experiment 1 which increased effects of phytotoxicity on the lucerne and reduced its yield. Target weed species were suppressed, particularly by the 2 L/ha treatment. However, the suppression of twitch, dock and clover was temporary however. Experiment 2 was topped on the 31/5/2019 and the glyphosate applied on the 6/6/2019. Minimal lucerne phytotoxicity occurred, due to its dormancy and the low proportion of green leaf present on the stand at the time of application. Sufficient weed suppression was achieved of all plant species, particularly from the 2 L/ha treatment which reduced the weed content from 51% to 17%. Experiment 3 was topped on the 23/7/2019 and the herbicide was applied on the 24/7/2019. Severe phytotoxicity effects were observed on all plant species. This late application delayed spring regrowth and gave insufficient feed supply. The objectives of this study were met by concluding the optimum rate and date for glyphosate application on lucerne was 2 L a.i./ha at the beginning of June. From this date and rate, minimal phytotoxicity effects were observed from the lucerne as no herbage was present at the time application, and the perennial weeds were suppressed. The addition of atrazine to the glyphosate to control opportunistic spring annuals requires further investigation.

Keywords: Alfalfa, 1,1’-dimethyl-4,4’-bipyridinium, *Elytrigia repens,* herbicide, *Lolium perenne, Rumex obtusifolius, Taraxacum officinale, Trifolium repens*

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1 INTRODUCTION

Lucerne (*Medicago sativa* L.) is high quality forage legume promoted as the most suitable forage species for dryland New Zealand sheep production systems. This is due to its ability to withstand long summer dry periods and maintain a green, high protein feed when other forages fail (Langer 1967; Wynn-Williams 1982). Lucerne has an erect growth pattern which, paired with frequent defoliation of the resident stand, promotes growth of invasive weed species. This periodic defoliation exposes a bare soil surface which encourages the establishment of unwanted plant species. Weeds compete directly with the resident lucerne stand for resources and hence dramatically reduce yields of the high protein and high quality lucerne forage (Roux et al., 2014).

Existing methods for weed control include winter applications of atrazine and paraquat herbicides. These are commonly applied in New Zealand lucerne farm systems and control most winter active annual species and spring weeds. However, these chemicals are expensive, and as the area of lucerne grown on farms in New Zealand increases, farmers are looking for cheaper and more broad-spectrum weed control options. Late winter applications of atrazine and paraquat can increase the risks to lucerne as late herbicide application negatively affects spring growth (Moot et al., 2003). Several experiments (Cassells & Pritchard 1968; Forgie 1973; Logan & Arnst 1973; Moot et al. 2003) suggest a heavy defoliation of the lucerne stand in late June followed by an application of paraquat and atrazine 10 to 14 days' post grazing will achieve the desired outcome and minimise lucerne damage.

Glyphosate is a broad-spectrum herbicide first sold to farms in the 1970's and since then its use has increased over 100-fold (Myers et al., 2016). Plants exposed to glyphosate absorb the chemical N-(phosphonomethyl) glycine) through green plant tissue, predominantly leaf surfaces. Once adsorbed, the chemical flows towards the plant growing points, notably the apical meristem and root apex, where it interferes with the production of 5-enolpyruvylshikimate-3-phosphate synthase, a critical enzyme for plant growth and survival. However, glyphosate is not registered for use on lucerne but could provide a means of controlling invasive weed species whilst having negligible effect on the prevalence and crop health of the resident lucerne. Glyphosate has been observed to achieve these results, but damage to lucerne is observed when sprayed 4-5 weeks following grazing. This supports the need for an early application post-grazing (Davies et al*.,* 2003). Glyphosate is inexpensive and highly effective at controlling both annual and perennial rhizomatous and perennial invasive species including brown top (*Agrostis capillaris* L.) and yarrow (*Achillea millefolium* L.) which the traditional contact and residual herbicides such as paraquat and atrazine fail to control (Young, 2010). New Zealand legislation prevents the importation of glyphosate tolerant lucerne cultivars. This contrasts with the USA where glyphosate efficacy to control weed species has led to genetic development of glyphosate tolerant lucerne stands (Bouton 2012). Therefore, any recommendations to use glyphosate in New Zealand must use on-farm management practices and knowledge of chemical translocatability to eliminate risks of lucerne phytotoxicity effects following its application.

1.1 Aims and Objectives

The aim of this research is to determine the potential to utilise glyphosate to eliminate invasive weed species from a lucerne stand. This must be done whilst minimising phytotoxicity effects of the glyphosate on lucerne growth, development and longevity.

To do this there are three main objectives of this research:

> Objective 1: To determine the optimum date and rate for the use of glyphosate on older lucerne stands.
>
> Objective 2: To quantify the phytotoxicity effect of glyphosate on lucerne.
>
> Objective 3: To quantify the effect of glyphosate on target weed control.

To investigate these objectives glyphosate was applied in three different experiments in April, June and July at four different rates. The plant response to the herbicide was recorded through weekly GreenSeeker™ measurements, European Weed Research Society scoring, and visual plant population assessments. A herbage cut was taken in mid spring (September) to evaluate the dry matter and botanical composition of the lucerne, grass and broadleaf weeds. A review of relevant literature is presented which discusses the common weed species targeted for removal in lucerne stands, an analysis of current methods of weed control including the use of paraquat and atrazine, and the potential use of glyphosate.

2 REVIEW OF THE LITERATURE

2.1 Introduction

Glyphosate has been available since 1974 and is found world-wide, commonly known as “Roundup”. It is now present in approximately 90 New Zealand products including chemical mixtures (Douwes et al., 2003). The use of glyphosate internationally has accelerated over the past 10 years due to the introduction of genetically modified glyphosate-resistant crops such as soy bean and lucerne. However, genetically modified crops are illegal in New Zealand. Therefore, the use of glyphosate with lucerne has been limited. The development of glyphosate formulations was driven by the demand to replace previous herbicides which presented drift and crop damage, slipping efficacy and human health risks (Myers et al., 2016).

2.2 Weed Cycle

Common weeds present in lucerne stands throughout New Zealand include a mixture of annual and perennial grass and broadleaf weed varieties. Examples of these include summer annuals such as wireweed (*Polygonum aviculare),* fathen (*Chenopodium album),* shepherds purse *(Capsella bursapstoris)* and nightshade (*Solanum nigrum)* which are frequently present at establishment in spring. In subsequent years, winter annuals including storksbill *(Erodium cicutarium*) and subterranean clover *(Trifolium subterraneum)* invade and winter herbicide applications are used to control these. As stands age, perennial weeds such as dock (*Rumex obtusifolius),* Californian thistle *(Cirsium arvense),* the common dandelion (*Taraxacum officinale)*, couch or twitch grass (*Elytrigia repens)* become more prevalent. Palmer (1982) described the growth pattern of these weeds and supported they undulate according to Figure 2.1, whereby seasonal influence dictates percentage weed cover.

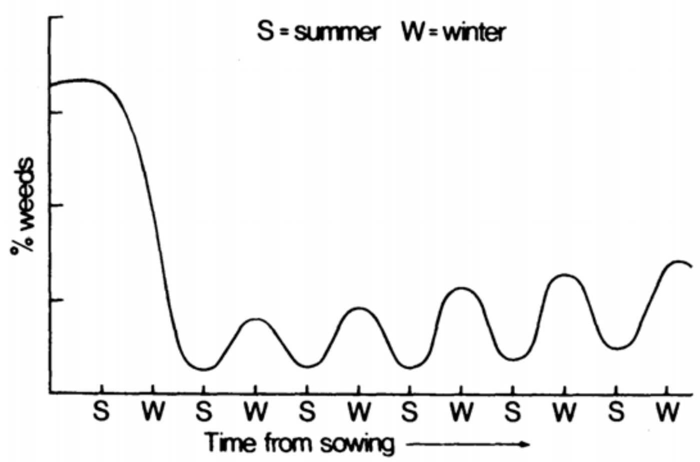

Figure 2.1 Seasonal pattern of weed content (%) in lucerne stands from the time of sowing (Palmer, 1982).

Figure 2.1 shows that weed abundance is most dominate immediately after sowing. These are usually spring annuals such as fathen that can be removed by defoliation. After this, the growth of lucerne in spring and summer outcompetes the growth of summer annual weeds, and therefore the percentage of weed content in a lucerne stand in summer is reduced. However, due to the reduced growth of lucerne in winter from lower temperatures and shorter days (Moot et al., 2003), winter annual weeds have lower competition from the lucerne plant and therefore are a greater percentage in the lucerne stand (Palmer, 1982). It is these winter annuals such as *Poa annua*, barley grass, and shepherds purse that the recommended annual winter spray targets. However, overtime the weed content is seen to increase in lucerne stands as the lucerne stand ages, and plants thin out to create greater areas of bare ground where the weeds can establish, with fewer lucerne plants per unit area (Palmer, 1982). When the gaps between the lucerne plants become large enough, summer weeds and perennial grasses establish such as perennial ryegrass (*lolium perenne)* and couch or twitch grass (*Elymus repens).* Perennial grass weeds are known to significantly depress lucerne yield (Cullen, 1965). It is recommended to remove perennial weeds as much as possible prior to sowing as these weed species can re-establish quickly, and are harder to remove once the lucerne is established. This includes Californian thistles, docks and dandelions.

Palmer (1982) described the weed process post sowing as lucerne plus summer annual weeds, to lucerne plus winter annual weeds. This is followed by pure lucerne in summer and lucerne plus annual weeds in winter, to lucerne plus perennial weeds, to perennial weeds without lucerne as the years advance. If factors such as low pH, wet soil or poor grazing management are exercised on a lucerne stand they reduce the competitive advantage of lucerne against the weeds, which will accelerate this weed invasion process.

2.3 Existing weed control methods

Winter annual weeds are shown to increase the feed production of lucerne stands during winter, therefore spraying out these weeds results in a reduction of total herbage yield. However, removal of such weeds increases the lucerne’s value in a cut and carry proposition. Comparatively, the removal of perennial weed species can permanently improve the lucerne as the basic cause of lucerne declines are corrected. For example, basic conditions which can be easily addressed include grazing management, soil fertility or wet soil.

2.3.1 Paraquat

Paraquat, also known as 1,1’-dimethyl-4,4’-bipyridinium salts, is a commonly used non-selective herbicide for weed control in established lucerne (Akhavein & Linscott, 1968). It is the most highly acutely toxic weed killer primarily for weed and grass control, killing green plant tissue on contact and becoming biologically inactive when it hits soil (Linscott et al., 1969). A common trade name for paraquat is Gramoxone, manufactured by Syngenta. The mode of action of paraquat is through absorption by the foliage of plants. Dinis-Oliveira et al., (2006) described how paraquat disrupts photosynthesis which allows water to escape, leading to rapid desiccation of foliage. The translocation of paraquat throughout plants also increases the possibility of residues in the targeted foliage (Dinis-Oliveira et al., 2006).

The World Health Organisation (WHO) classes paraquat as a Class 2 chemical, meaning moderately toxic. Paraquat poisoning is possible from skin exposure, inhalation or consumption, particularly from long exposures of concentrated paraquat, or if it enters the body from broken skin e.g cuts or grazes. The level of poisoning from paraquat is determined by the amount and duration of exposure, with corrosive penetration through cracks in the skin a common mode of access. Numerous deaths have occurred from exposure, either accidental or suicidal. Chronic effects from paraquat poisoning are caused from the extensive damage to the mitochondria of cells, with as little as a teaspoon of concentrated paraquat able to result in death (Watts, 2011).

From an environmental perspective, paraquat poses a risk to the health of aquatic and terrestrial organisms. The Environmental Risk Management Authority of New Zealand described paraquat as “very ecotoxic to the aquatic environment”. Various studies carried out on fish, rats, rabbits and birds concluded paraquat ranged from acute to chronic toxicity (Watts, 2011). Paraquat activity in the soil profile is also persistent, binding to clay and organic matter. This fixation to soil particles means paraquat is immobile in the soil, and therefore is unlikely to leach into waterways. However, soil fixation also means sufficiently clean surfaces are required during application (e.g knapsack sprayers must be free from dirt or contamination) to avoid adsorption of the paraquat before application onto the target vegetation (Beef +Lamb NZ, 2017). Water contamination may however occur due to soil run-off from slips or other such activity. Adsorption is noted to increase with increasing pH, therefore more acidic soils have lower adsorption. Field studies determined the half life (DT50) of paraquat was 7-8 years in the UK and 10-20 years in the USA (Watts, 2011). This difference could be due to the variation in climate between the typically wet UK compared to the dryer climate of the USA. Many factors influence the half life of a pesticide, including microbial activity, sunlight and water (Ney, 1995). Therefore, the increased rainfall typically of the UK could increase the nutrient cycling in the soil, consequently reducing the half life of paraquat in the soil. In relation to New Zealand, the UK climate could be compared to the West coast of New Zealand, which receives high annual rainfall. The USA climate could compare similar to that of Central Otago in New Zealand which is the coldest, driest part of New Zealand.

Paraquat, like most typical lucerne weed control sprays is applied in winter, between the months of June-August. The recommendation is for paraquat to be applied to established stands of a minimum 12 months old. Due to it’s mode of action, it is recommended that lucerne stands be eaten down to remove as much green leaf off the stand as possible, to minimise harm to the lucerne. Smith (1991) carried out an experiment with lucerne in Central Georgia, USA, which investigated the effect of paraquat on lucerne yield and crown carbohydrate content. Applications of paraquat at 0.28 and 0.56 kg of active ingredient/ha were applied to 5 and 4 year old alfalfa stands. Smith (1991) observed a linear relationship between the number of days post-harvest that the herbicide was applied, and the forage yield recorded. This experiment demonstrated that the greater the number of days between the harvest and the herbicide application, the lower the overall lucerne yield, thus illustrating a more effective application of paraquat if applied promptly post-harvest. The fewest number of days between harvest and herbicide application date was 4, yielding 3100 kg/ha in the 5 year old stand and 3700 kg/ha in the 4 year old stand. It was also concluded that applications at 0.28 kg/ha were sufficient to control grasses and broadleaf weeds when applied in early spring, and that lucerne yield was greater post herbicide application compared with non-treated plots (Table 1).

Table 1.0 Lucerne yields at first harvest following paraquat treatments applied in early spring on a 5 (Griffin) and 4 (Eatonton) year old lucerne stand. Application rate is kg of active ingredient per hectare (Smith, 1991).

Treatment	Rate (kg/ha)	Griffin (kg/ha)	Eatonton (kg/ha)
Paraquat	0.28	2800	2800
Paraquat	0.56	2800	3000
Weed-free control		2700	3200
Non-treated		1800	2100
LSD (0.05)		300	600

In comparison, Foy and Witt (1993) conducted field experiments investigating the effects of paraquat on lucerne in Virginia. They conducted seven experiments, with various lucerne sowing dates and paraquat application dates. The weed varieties in each experiment varied also, with comparable weeds common in lucerne stands in New Zealand such as chickweed (*Stellaria media)*, shepherds purse*,* plantain *(Plantago major)*, dock, dandelion and red clover *(Trifolium pratense)*. The lucerne stands averaged between 3-8 cm tall at paraquat application date, with the weeds species being a similar height to this. Paraquat was confirmed as effective at suppressing the growth of perennial broadleaf weeds and grasses, as well as controlling chickweed and shepherds-purse (Foy & Witt, 1993). They concluded that the application of paraquat, either alone or in combination with Simazine, reduced the growth of some perennial species, but it did not increase lucerne yields in plots that were treated compared with plots without herbicide activity. In fact, reduced lucerne yields were observed from paraquat applications in early spring. This is contradictory to the results found by Smith (1991), who found Paraquat applications increased lucerne yield compared with non-treated plots. These opposing results could be due to the variation in Paraquat application rates, with Smith (1991) applying 0.28 kg/ha and 0.56 kg/ha of active ingredient compared with Foy & Witt (1993) which applied paraquat at 0.8 kg/ha. These differences in results could be due to the severity of the weed abundance also.

The difference in results could also be due to differences in time of applications. Moot et al., (2003) conducted a herbicide and grazing experiment on a 4-year-old lucerne stand at Lincoln University, New Zealand. Measurements were taken of winter management treatments including ungrazed to mimic a control treatment, grazed and sprayed plots. Paraquat and atrazine were sprayed on 31 July. From this research, it was concluded that the unsprayed and ungrazed crop had a linear growth rate of 70 kg DM/ha/d from mid-August and produced 2.8 t DM/ha by the first week of October, however 31% of this was composed of weeds. In comparison, the sprayed plots displayed similar linear growth rates, however delayed by approximately 14 days. This meant that by the first week of October, the sprayed crops were weed-free and consequently yielded less than the non-sprayed crops with only 2.4 t DM/ha. However, it was reported that two weeks later the crop cover had increased to 2.8 t DM/ha. Figure 2.2 shows the winter sprayed lucerne crops had greater total yield compared with the grazed or ungrazed and unsprayed plots.

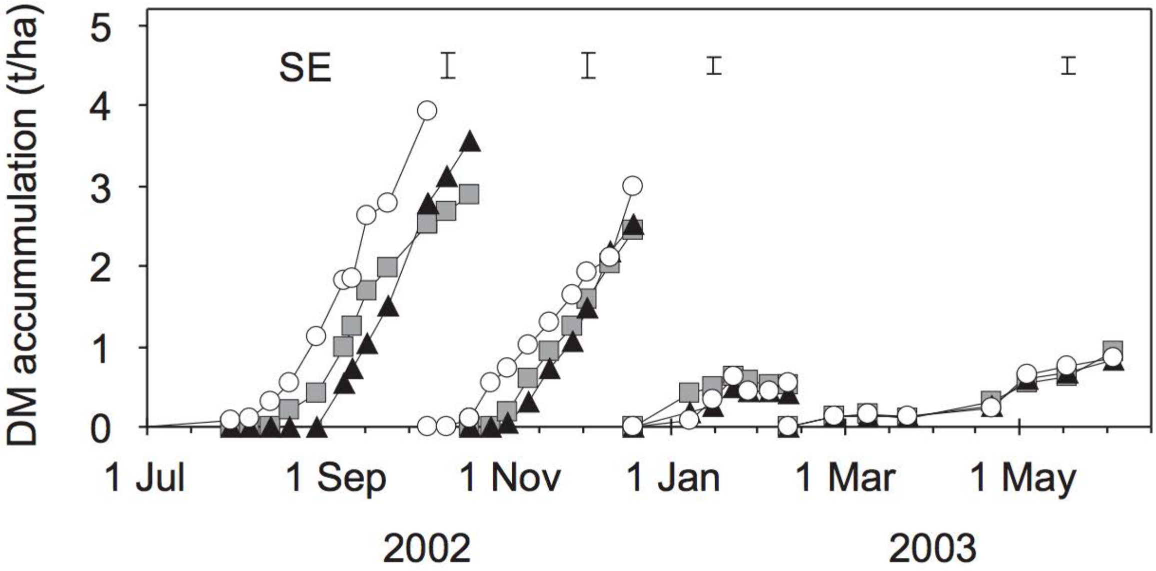

Figure 2.2. Dry matter (DM) accumulation over time for winter sprayed (O), grazed (▲) or ungrazed (■) dryland lucerne crops grown at Ashley Dene, Canterbury, New Zealand (Moot et al., 2003).

Moot et al., (2003) concluded that the application date of 31 July was too late in their environment and resulted in damage to the developing buds of the lucerne crop. The late winter grazing which followed contributed further to the decrease of lucerne yield by 25% due to the removal of the lucerne growing points. The time it took the crop to replenish these growing points allowed the increase of weed content by 50%.

2.3.2 Atrazine

Atrazine, also known as 2-chloro-4-ethylamino-6-isopropylamino-s-triazine (Lima et al., 2010), is a pre and post-emergent herbicide that selectively controls broadleaf weeds and grasses in established lucerne. Atrazine is taken up through the plant roots, where it affects the chloroplast within the plant cells by inhibiting photosynthesis in susceptible plants (Nel & Reinhardt, 1984). This leads to a reduction in carbon dioxide fixation by the plant (Ashton et al., 1981). Plants not affected by atrazine application absorb the chemical and metabolize atrazine without toxic consequences (Cheremisinoff & Rosenfeld, 2010). Atrazine is recommended for the control of broadleaf and grassy weeds in a large number of agricultural crops and pastures. Paraquat is reportedly responsible for 80% of the lucerne weed control market either on it’s own, or in 70% of cases in combination with Atrazine (Butler, 1982). More current figures are unavailable. Atrazine has been banned in some countries due to its inability to degrade and consequent accumulation in the environment (Lima et al., 2010).

Atrazine poses a threat to human health and safety from exposure, with warnings of skin reactions and organ damage from prolonged or repeated exposure (NRA for Agricultural and Veterinary Chemicals, n.d). Studies investigating the toxicity of atrazine have proven as little as 6320 micrograms of atrazine applied to rabbits has had a severe effect on eye and skin irritation (PubChem, 2019). Studies investigating the response of African clawed frogs determined that atrazine disrupted sexual development and concluded that species exposed to atrazine in the wild could also be at risk of impaired sexual development (Hayes et al., 2002). Research has also been carried out on goats, with 150 mg of atrazine administered for four days to observe the chemical’s effect on milk, urine and faeces (NRA for Agricultural and Veterinary Chemicals, n.d.). The milk tests observed reasonably consistent residue values, regardless of dosage period, at approximately 0.38 ppm and 0.25 ppm for each goat. Consideration must be made to the post-spray grazing of the crop however, as it is unlikely that animals would typically be grazing the crop immediately after the atrazine has been applied. The atrazine label specifies a withholding period for grazing of livestock of 28 days after application. Therefore, the administration of 150 mg of atrazine (NRA for Agricultural and Veterinary Chemicals, n.d.) is unrealistic for expected goat consumption. Provided the chemical recommendations are followed, the traces of atrazine then found in the animal milk, urine and faeces are unrealistic and not applicable for common grazing scenarios.

Atrazine does not bind to the soil as paraquat does. Rather, it travels easily through the soil profile and into surrounding waterbodies such as groundwater, rivers and lakes (Rosenfield & Feng, 2011). Atrazine is said to break down in the environment by micro-organisms as it is soluble. However, this process takes time and between the chemical entering the soil and this break down, the atrazine can be leached into waterways. The high mobility, combined with the heightened toxicity of atrazine to aquatic life, and long lasting effects, poses a risk to the biodiversity surrounding areas of application (PubChem, 2019).

Swan (1972) investigated the effect of atrazine on winter annual weeds in western United States. The weeds of interest of this experiment were species not commonly found in New Zealand lucerne stands such as tumble mustard (*Sisymbrium altissimum)* and prickly lettuce *(Lactuca serriola L.).* The atrazine was applied at 0.45, 0.9 and 1.8 kg/ha to established lucerne between 2 and 3 years old. It was observed that atrazine applications injured the lucerne the most, with no reduction in yield observed from the 0.45 kg/ha applications however damage observed from 1.80 kg/ha applications.

2.4 Glyphosate

Glyphosate is the active ingredient in herbicides for the control of weeds in both urban and rural areas. It is a broad spectrum, non-selective, pre and post-emergence herbicide that is popular because of it’s effectiveness for weed control and low price compared with other products (Chan & Mahler, 1992; De Roos et al., 2005). Glyphosate has the chemical formula N-(phosphonomethyl) glycine (Tu et al., 2001), and when applied in formulations with other substances, performs more effectively as a weed killer. Glyphosate is absorbed by foliage of a plant and translocated through the plant tissue to both above and below ground meristems (Duke, 2010; Van Bruggen et al., 2018). Within the plant, glyphosate inhibits an enzyme called EPSP synthase which stops the plant from producing particular aromatic amino acids essential for plant growth (Franz et al., 1997). Glyphosate is most effective when applied during peak active growth periods, with increased leaf area also increasing it’s effectiveness (Tu et al., 2001).

2.4.1 Reduced toxicity

The toxicity threat that glyphosate poses often confuses correlation with causation. Numerous studies defend the use of glyphosate, with the Agricultural Health Study (AHS) in 2001 finding “no statistically significant associations with glyphosate use and cancer”. The European Food Safety Authority in 2015 stated that the herbicide is “unlikely to pose a carcinogenic hazard to humans” (Andreotti et al., 2018). Glyphosate is claimed to be the least toxic pesticide, with lower acute toxicity than aspirin according to Giesy et al., (2000). Franz et al., (1997) supports this, by stating that EPSP synthase, is only present in plants and micro-organisms. Mammals, birds and reptiles do not possess this enzyme, therefore they are able to continue to obtain aromatic amino acids from their diets which are essential for growth. They concluded that the use of glyphosate does not have adverse effects on the health of other life forms.

The United States (US) Environmental Protection Agency (EPA) classifies herbicides for acute toxicity in four categories where “I” is the most toxic and “IV” is the least toxic. Based on oral rat tests, the EPA currently rates glyphosate as a Category IV herbicide for slight skin irritation and Toxicity Category III for mild eye irritation (EPA, 1998; Perron et al., 2017). This acute toxicity category placement was determined in 1998, which in terms of science and developed research surrounding glyphosate, is outdated research. The EPA is currently undergoing another review of this, based solely on independent studies and science while taking the WHO’s determination that glyphosate is a probable carcinogen into account, to determine if this classification should be altered (Plater, 2019).

The New Zealand Environmental Protection Authority (NZEPA) concluded that glyphosate was “unlikely to be genotoxic and carcinogenic” (Temple, 2016). This study reviewed the quality of the existing data and studies of glyphosate in 2016 to advise that it was not required to reclassify glyphosate as carcinogen or mutagen under Hazardous Substances and New Organisms (HSNO) (Temple, 2016). Conversely, Douwes et al., (2003), argues that the NZEPA process for evaluating the carcinogenicity of glyphosate is flawed. Douwes et al., (2003) suggested a possible conflict of interest between the NZEPA and the industry and management, describing a “lack of transparency about the relations”. Douwes et al., (2003) requested a reconsideration of the hazard classification for glyphosate under the Hazardous Substances and New Organisms Act 1996 to avoid compromising their reputation by undermining public confidence.

Finally, human risks involved with glyphosate and food production were addressed by tests carried out by the Ministry of Primary Industries (MPI) (2018) with milk and cream samples from New Zealand. MPI (2015) tested 60 commercially available processed fresh milk and cream samples for glyphosate or traces of AMPA, with all dairy samples coming back with negative results, testing less than 0.05 mg/L. The identified limit of quantification of this experiment was 0.01 mg/L and the limit identified of reporting was 0.05 mg/L. A larger sample size would have strengthened the reliability and accuracy of the statistics reported in MPI’s National Chemical Contaminants Program. A further study conducted in 2017 by MPI tested 308 raw milk samples, and concluded similar results. MPI (2017) stated an action limit of 0.01 mg/kg and recorded no glyphosate detections above this limit. Perron et al., (2017) reported that tests that analyzed human milk samples concluded that neither glyphosate nor AMPA were detected in the samples, with the glyphosate limit equaling 10 ppb and the limit of detection stated as 3.3 ppb. Conversely, an American article claims that the pesticides in milk can cause brain damage, claiming that the glyphosate applied to corn crops in America was contaminating the raw milk consumed by humans (Mercola, 2015). Further investigation finds this source labelled the number one natural health website. The claims made from Mercola, (2015) can not be compared with the statistics stated by MPI (2015, 2017 & 2018) as Mercola, (2015) reviews American grain-fed cattle, with no data or evidence of statistical analysis to support the claims stated. As well as glyphosate limits in place for dairy produce, New Zealand’s Ministry of Primary Industries also classifies a 10 maximum residue level for glyphosate accepted on fruit produced nationally. The limit of 0.01 mg/kg which is set at or about the limit of analytical quantification was issued under the Food Act 2014 (MPI, 2018). These legal limits were enforced to ensure human health is prioritized.

2.4.2 Glyphosate management for lucerne

Because glyphosate is a non-selective herbicide, there are phytotoxicity issues with application onto lucerne. This emphasizes the importance of management strategies such as defoliation and spray time. Research into the successful production of lucerne stands in New Zealand based farming systems has been extensive, yet despite this the uptake and incorporation of lucerne by farmers was reported to have steadily declined since the 1970's (Purves & Wynn-Williams, 1989). A survey completed by Kirsopp, (2001) throughout the South Island of New Zealand indicates 67% of farmers had incorporated lucerne into their systems yet it accounted for less than 20% of the land area. This conflicts with recommendations made by White (1982) that 40-60% of a dryland property should be in lucerne to maximise lamb liveweight gain and increase summer security. Evidently successful incorporation of lucerne has been challenging for New Zealand agriculturalists with potential reasons stemming from the increased management requirements and high susceptibility to weed invasion compared with other lower maintenance forages. More recent studies including Avery et al., (2008) and Anderson et al., (2014) have proven the profitability of lucerne through more efficient grazing management, making it adaptable for hill or high country farmers (Moot et al., 2003). This increased the amount of land area suitable for the productive growth of lucerne, increasing the total amount grown in New Zealand as shown in Figure #.

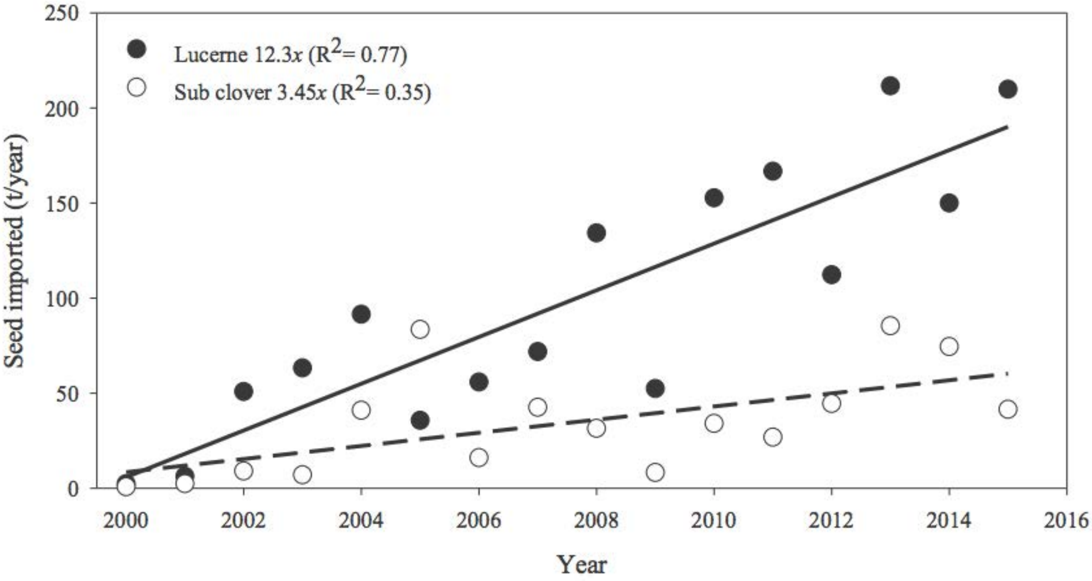

Figure 2.3 Lucerne and subterranean clover seed imports to New Zealand (Monk et al., 2016).

Lucerne seed imported in 2015 was recorded at 210 tonnes (Monk et al., 2016). This equates to approximately 25000 ha of lucerne sown annually (Monk et al., 2016).

Understanding seasonal growth patterns and requirements is vital to maintaining a pure and healthy lucerne stand. Ideally the goal of the farmer is to maximise growth and availability during periods of high stock demand particularly throughout summer. This is when the stand has mobilized energy from the roots stored over winter for node production and rapid growth with the onset of spring. In autumn, the stand partitions energy into root reserves for winter. At this time lucerne should be hard grazed, to remove aphids and foliage which exposes the under canopy of weeds which are sprayed off 7-14 days later (Moot et al., 2003). The hard grazing prior to spraying is deemed to be a key management practice in reducing lucerne leaf surface area likely to encounter the herbicide and suffer phytotoxic effects of the chemicals. Ideally farmers want to minimise phytotoxicity in lucerne post spraying whilst maximising interception of the spray onto invasive weed species thus reducing inter-specific competition with the lucerne in spring.

Successful future integration of lucerne into New Zealand farming systems is heavily dependent on increasing understanding around lucerne management. One aspect is the potential to utilise broad spectrum, inexpensive herbicides such as glyphosate which controls invasive weed species whilst having a negligible phytotoxic effect on the resident lucerne stand. This could improve the productivity, longevity and quality of lucerne particularly in areas when perennial weeds have invaded. If ease of lucerne weed management can be achieved, the potential incorporation into New Zealand farming systems may be stimulated.

An on-farm experiment of glyphosate was completed in Central Otago by Roux et al., (2003). They looked to determine the phytotoxicity effects of a glyphosate and atrazine combined spray on lucerne at different grazing and application dates. The 12.5 ha site was grazed between 8th and 12th of June by 1600 hoggets in a pre-winter clean up graze. The treatments can be broken down into three plots of additional grazing (6 September, 2 October and an ungrazed hay crop). The trial contained subplots of four times of herbicide application (unsprayed, 3 July, 22 August and 18 September 2012). The hay crop represented a control block and was left ungrazed between 12 June and 14 November over winter typical of most commercial properties in the area. The remaining plots underwent additional grazing and herbicide applications. Roux et al. (2014) determined that successful winter weed control of lucerne could be achieved with glyphosate and atrazine combined, during July and August when the lucerne was dormant and the crop cover was below 100 kgDM/ha. It was concluded that early spring grazing in early September, or a late herbicide application date (18 September) delayed the peak lucerne production by 10-30 days. This is important for the management of glyphosate application to ensure stock feed supply matches feed demand. Manipulation of glyphosate application date and rate could alter the pattern of spring feed depending on the location.

2.5 Conclusions

1. Existing methods for winter weed control in lucerne stands such as paraquat and atrazine are expensive and ineffective at removing perennial weed species from lucerne stands.

2. Glyphosate provides sufficient evidence for an alternative method for effective winter weed suppression in lucerne stands.

3. Further research is required to quantitatively support these findings.

3 MATERIALS AND METHODS

3.1 Site

Three herbicide experiments were carried out in Iversen Field 12, at Lincoln University, New Zealand. ‘Kaituna’ lucerne had previously been sown into a Wakanui silt loam soil (S-Maps, 2019) during the spring and summer of 2010. This herbicide experiment began in April 2019, when the stand was 8 years old. The three experiments used three different sowing dates at different locations in the paddock. Each experimental design was the same. For Experiment 1, Iversen Field 12 was a topped using a Fieldmaster triple head mulching mower on the 4 of April, with the experimental site marked out with pegs on the 8 April. The herbicide treatments were applied on the 29th April. For Experiment 2, the site was topped on the 31st of May and sprayed on the 6th of June. For Experiment 3 the area was topped on the 23rd of July, then the site pegged out and sprayed on the 24 July.

3.2 Climate

Daily climate data including temperature and rainfall were recorded approximately 0.5 km east of the experimental site. Temperature was recorded from data recorded every minute, with the averages logged every hour (Figure 3.1). Mean monthly air temperature was typical of the long-term average. The mean air temperature following the first herbicide application on the 29th of April was 10°C to the 1st of June. It was 7°C after the second spraying from the 6th of June to 23 July, and 8°C from 24th of July to the 27th of September. Average monthly rainfall from April to September 2019 for the duration of the experiment was 72.3 mm, slightly greater than the long-term average of 55.1 mm.

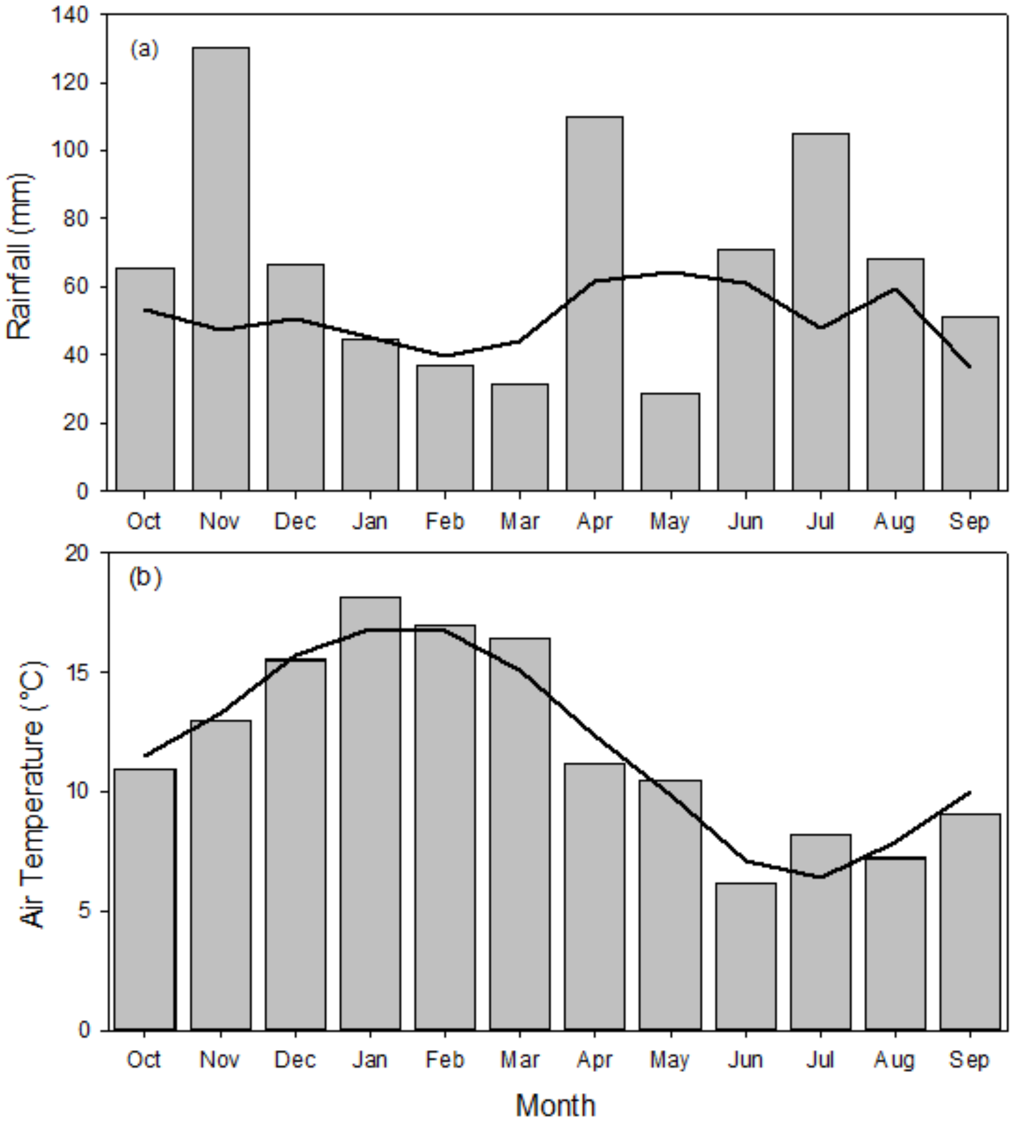

Figure 3.1 Monthly mean rainfall (a) and air temperature (b) at Iversen Field, Lincoln University, Canterbury from October 2018 to September 2019. The long term means (-) are for the period from 2000-2010.

3.3 Experimental design and treatments

Each experiment was a fully randomized complete block design (RCBD). The total area for all three experiments was 0.2205 ha, with an area perimeter of 35 x 21 m for each individual experiment. Each experiment consisted of 16 plots, measuring 8 x 4.5 m, with a 1 m wide buffer strips in between each plot (Appendix 1).

The herbicide was applied using purpose-build sprayer unique to Lincoln University (Plate 1). The hand powered plot sprayer had digital readouts of pressure and speed which allowed for accurate application of the agrichemicals via a 2m long boom. 5ml of Pulse penetrant was applied with every spray mix.

Plate 1 Picture indicating the boom sprayer used for all three experiments. Malcolm Smith was applying the glyphosate treatments for Experiment 3.

The target spray rates for the 4 treatments were 0, 1, 2 and 4 L a.i. /ha. During application, inconsistency with the concentration of active ingredient of glyphosate was encountered. Due to the nature of the sprayer used for the experiments, some of the herbicide mixture remained in the sprayer after each treatment application. Therefore, the actual concentration of active ingredient that was applied to the plots was determined by calculating the target application rate minus the proportion of active ingredient in the mixture that remained in the sprayer at the end of the application. This calculation was done for each experiment, and displayed in Table 3 below. Target application rate was not met for any experiments.

Table 2.0 Glyphosate active ingredient concentrations of the three experiments.

	Glyphosate used	0 L/ha	1 L/ha	2 L/ha	4 L/ha
Experiment 1: 29 April 2019	Synergy Glyphosate 360	0	0.5	1.3	3.0
Experiment 2: June 6 2019	Weedmaster Glyphosate 540	0	0.6	1.4	2.3
Experiment 3: 24 July 2019	Synergy Glyphosate 360	0	0.8	1.5	3.3

There was a mixture of annual and perennial grass and broadleaf weed species present at the time of spraying. These remained in the unsprayed control plots throughout the duration of the experiment but undulated population cover. The weeds included summer annuals such as wireweed and shepherds purse*,* winter annuals such as subterranean clover and predominantly perennial weeds including dock, the common dandelion, twitch or couch grass*,* perennial ryegrass and white clover (*Trifolium repens)*. The control of perennial weeds was the main focus of this review.

3.4 Measurements

Plant species (dandelion, clover, ryegrass, dock, twitch, lucerne) were visually scored each week following the glyphosate application of each trial using the European Weed Research Society (EWRS) scores (Table 3) to determine the phytotoxicity effects of the glyphosate. GreenSeeker measurements were also recorded every week using the Trimble GreenSeeker handheld crop sensor which used optical sensors to measure and quantify the ground cover of glyphosate applications (Kitic et al. 2019). The GreenSeeker has a sensor that emits red and infrared light and measures the amount reflected back of each type (AgriOptics, 2014). The strength of the light detected from the reflection indicates the level of greenery of the plant, representing the crops health. Any orange or brown dead plant material will reflect a weaker light, indicating poor plant health. This form of measurement was used as an indicator of plant canopy cover in each plot.

Visual plant population cover assessments were also recorded every week, using a 0.1 m^2 quadrat to measure the average percentage cover of each plant species and bare ground in each quadrat.

Table 3.0 The European Weed Research Society (EWRS) scale used to measure the severity of the plant phytotoxicity in response to the glyphosate application (Roux et al., 2014).

EWRS Score	Severity of symptoms	% of crop affected
1	Healthy plant	0
2	Very mild symptoms	0.1-2.0
3	Mild but clear recognizable symptoms	2.1-5.0
4	More severe symptoms but no effect on yield	5.1-10.0
5	Reduction in yield expected – Commercially unacceptable	10.1-18.0
6	Reduction in yield expected – Commercially unacceptable	18.1-30.0
7	Reduction in yield expected – Commercially unacceptable	30.1-45.0
8	Reduction in yield expected – Commercially unacceptable	45.1-70.0
9	Heavy damage to total kill – Commercially unacceptable	70.1-100

Yields and botanical compositions were recorded from a harvest on the 18th September using 0.2 m^2 quadrat per plot. This herbage was sorted into lucerne, broadleaf weeds and grasses. Samples were then dried in an oven at 60°C to constant weight.

3.5 Analysis

Data was analysed by repeated measures for each individual experiment due to the difference in active ingredient applied, using Genstat Version 16 (Laws Agricultural Trust, VSN International Ltd). Repeated measures was chosen to best analyse the patterns over time amoung treatments. Fishers Protected LSD separated means when significant at the α=0.05 level. For interactions, the most conservative LSD was used for separations and is reported in figures.

4 RESULTS

4.1 Experiment 1

4.1.1 GreenSeeker

All treatments from Experiment 1 showed an interaction (P<0.001) between herbicide rate and time. Initial GreenSeeker measurements taken on the 1st of May observed similar cover for all application rates with a mean of 0.40 ± 0.03. The GreenSeeker measurements deviated between application rates from the second measurement on the 8th May, 2019. At that time, the control and 1 L/ha had higher values in comparison with the 2 and 4 L/ha treatments which decreased. The GreenSeeker measurement for the control treatment increased from this point (0.45 ± 0.03) until the beginning of June (0.49 ± 0.03). From the start of June, the readings decreased over the duration of winter to be 0.45 ± 0.03 on the 26 July. From the start of August, readings for the control increased from 0.48 ± 0.03 to 0.61 ± 0.03 by the 27th of September. This pattern of GreenSeeker values reflects the temporal changes in canopy cover of the lucerne and weeds without herbicide.

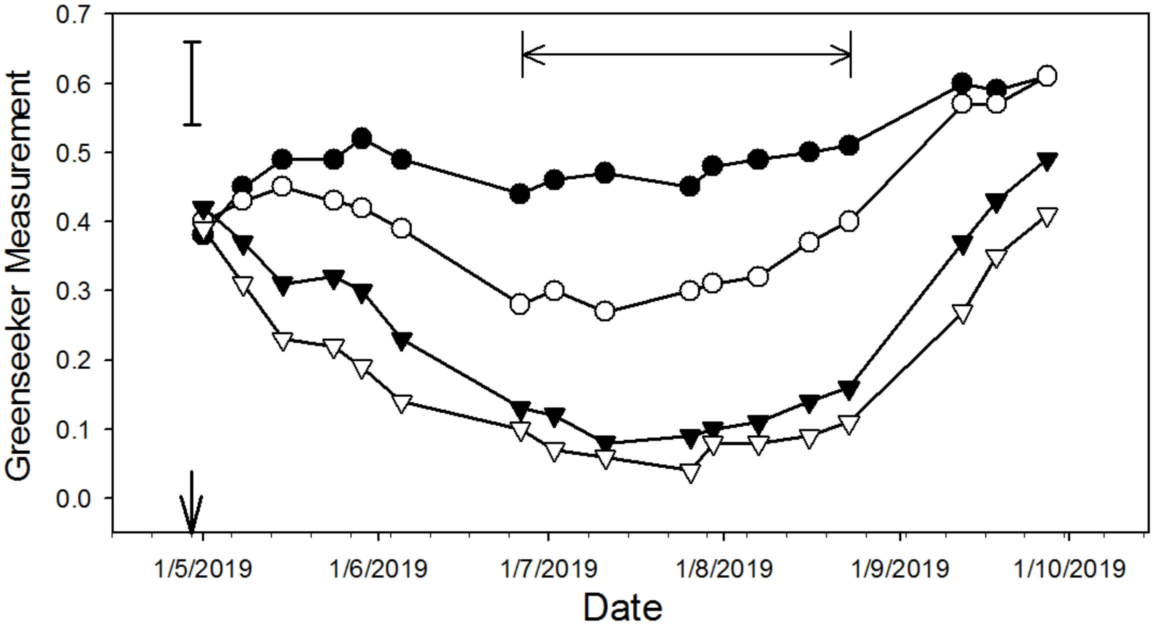

Figure 4.1 Experiment 1 GreenSeeker measurements recorded over time. The four glyphosate application rates were 0 (●), 1 (○), 2(▼) and 4 (▽) L/ha. Error bar represents the least significant differences (lsd) at α= 0.05 for comparisons amoung herbicide rates. Significant differences amoung treatments (←→) occurred between 26/6/2019 and 23/8/2019. Herbicide application date for Experiment 1 (↓) was 29th April 2019.

The GreenSeeker values from 1 L/ha gradually decreased from 0.45 ± 0.03 on the 15th May to 0.27 ± 0.03 on July 11th. From then, the means increased to 0.40 ± 0.03 by the 23rd of August. A steeper incline was observed from 1 L/ha between the 23 August and the 27th of September with a GreenSeeker of 0.61 ± 0.03. GreenSeeker means were different from the 26th of June to the 23rd of August between 0 and 1 L/ha, and the other treatments. GreenSeeker means between the 2 and 4 L/ha treatments were not different throughout the duration of Experiment 1. Both treatment means followed a similar time course whereby values decreased from 1st May to the 26th of July, from 0.42 ± 0.03 to 0.09 ± 0.03 for 2 L/ha and from 0.39 ± 0.03 to 0.11 ± 0.03 for 4 L/ha. Of note, both rates showed an increase in GreenSeeker values up to 0.61 ± 0.03 on the 27th of September which meant values were not different to the control or 1 L/ha at the end of the measurement period.

4.1.2 European Weed Research Society (EWRS) score analysis

Experiment 1 EWRS of lucerne indicated all treatments had an interaction (P<0.001) between herbicide application and time. An immediate herbicide effect was observed from 2 L/ha and 4 L/ha at the first EWRS observation (15th May) with an initial mean of 6.8 ± 0.4.

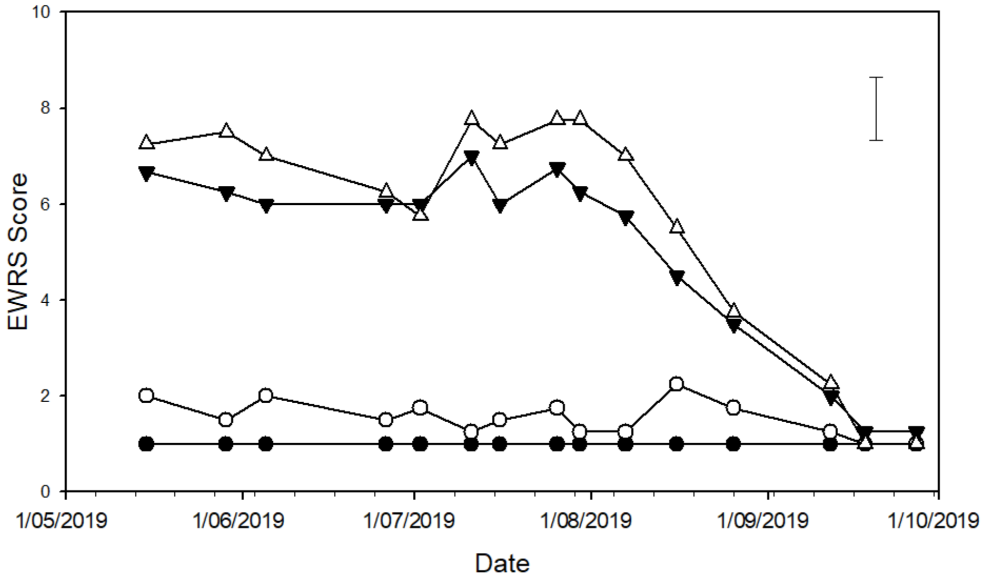

Figure 4.2 Experiment 1 European Weed Research Society score of lucerne over time. The four glyphosate application rates were 0 (●), 1 (❍), 2 (▼) and 4 (▽) L/ha. Error bar represents the least significant differences (lsd) at α= 0.05 for comparisons amoung herbicide rates over time.

The treatment mean for 0 and 1 L/ha (2.0 ± 0.4) was lower ($P<0.05$) than the 2 and 4 L/ha (6.0 ± 0.39) between the initial EWRS measurement on the 15th May and the 23rd August. From September 12th to the final EWRS measurement on the 27th September, treatment means were not different at 1.0 ± 0.4.

The EWRS of ryegrass in Experiment 1 indicated a trend that showed an interaction between herbicide and time ($P<0.10$). The treatment mean of 0 L/ha and 1 L/ha (1.5 ± 0.4) was different from the treatment mean of 2 and 4 L/ha (8.9 ± 0.4) between May 15th and the 23rd August.

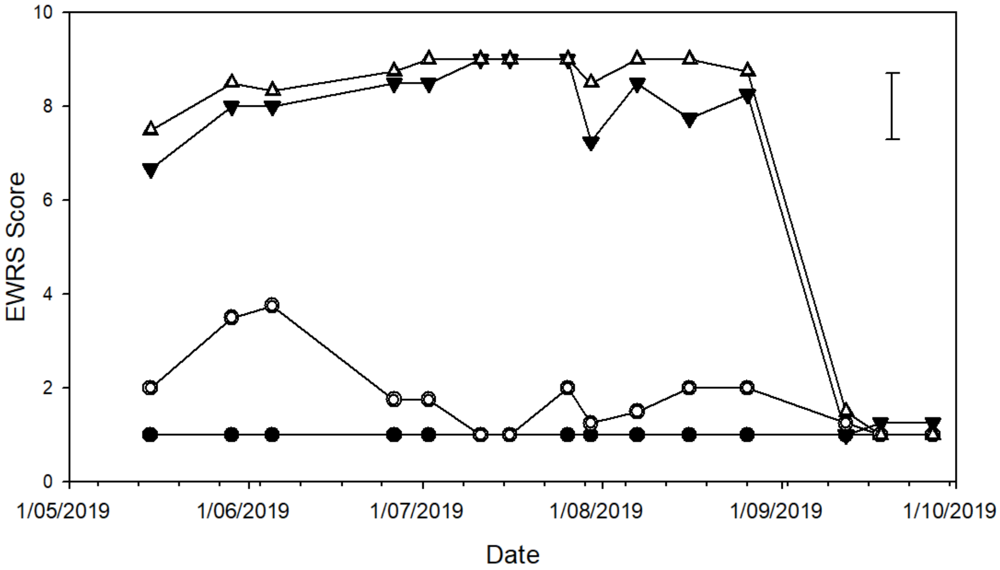

Figure 4.3 Experiment 1 European Weed Research Society score of ryegrass over time. The four glyphosate application rates were 0 (●), 1 (❍), 2 (▼) and 4 (▽) L/ha. Error bar represents the least significant differences (lsd) at α= 0.05 for comparisons amoung herbicide rates over time.

The trend ($P<0.10$) is shown by the steep decline in EWRS score of 2 and 4 L/ha treatments. Between 12th September and the 27th of September, there was no difference amoung the treatments, with a mean of 1.0 ± 0.4.

The EWRS score of dandelion from Experiment 1 (Figure 5.3) showed an interaction ($P<0.001$) between herbicide application and time.

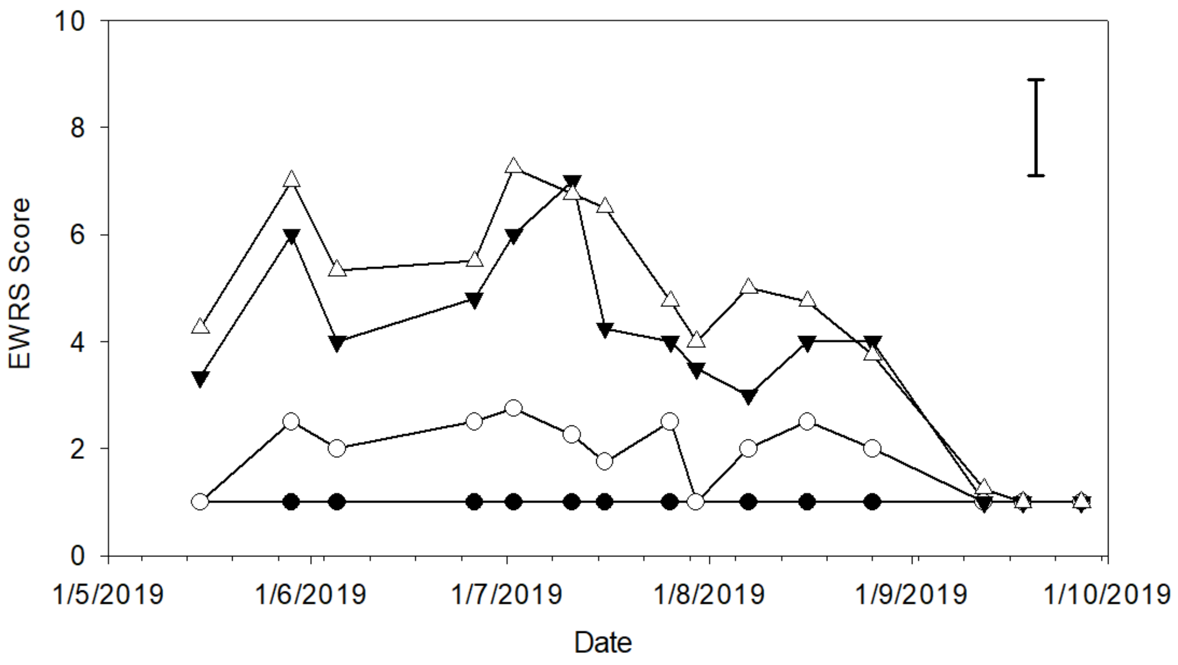

Figure 4.4 Experiment 1 European Weed Research Society score of dandelion over time. The four glyphosate application rates were 0 (●), 1 (❍), 2 (▼) and 4 (▽) L/ha. Error bar represents the least significant differences (lsd) at α= 0.05 for comparisons amoung herbicide rates over time.

For dandelion on the 29th May the 2 and 4 L/ha treatment mean of 7.0 ± 0.5 was higher than the 2.0 ± 0.5 from the control and 1 L/ha. Between the 26th June and the 16th of July, the treatment mean for 0 and 1 L/ha (1.0 ± 0.5) remained lower than for 2 and 4 L/ha (9.0 ± 0.5). From the 16th July to the 27th September, no treatment means were different.

For dock (Figure 4.5), there was also an interaction (P<0.05) between herbicide application and time. From the 15th of May to the 5th of June, the treatment mean of 2 and 4 L/ha was 5.0 ± 0.5 and higher than the 2.0 ± 0.5 for 0 and 1 L/ha. The maximum mean EWRS score for dock was 6.0 ± 0.5 from 4 L/ha. Between the 26th June and the 7th August there was no difference amoung treatments. On the 16thAugust there was a difference between 0 and 1 L/ha of 2.0 ± 0.5, and 2 and 4 L/ha of 5.0 ± 0.5. There was no difference amoung any treatment means from the 12th to the 27th of September.

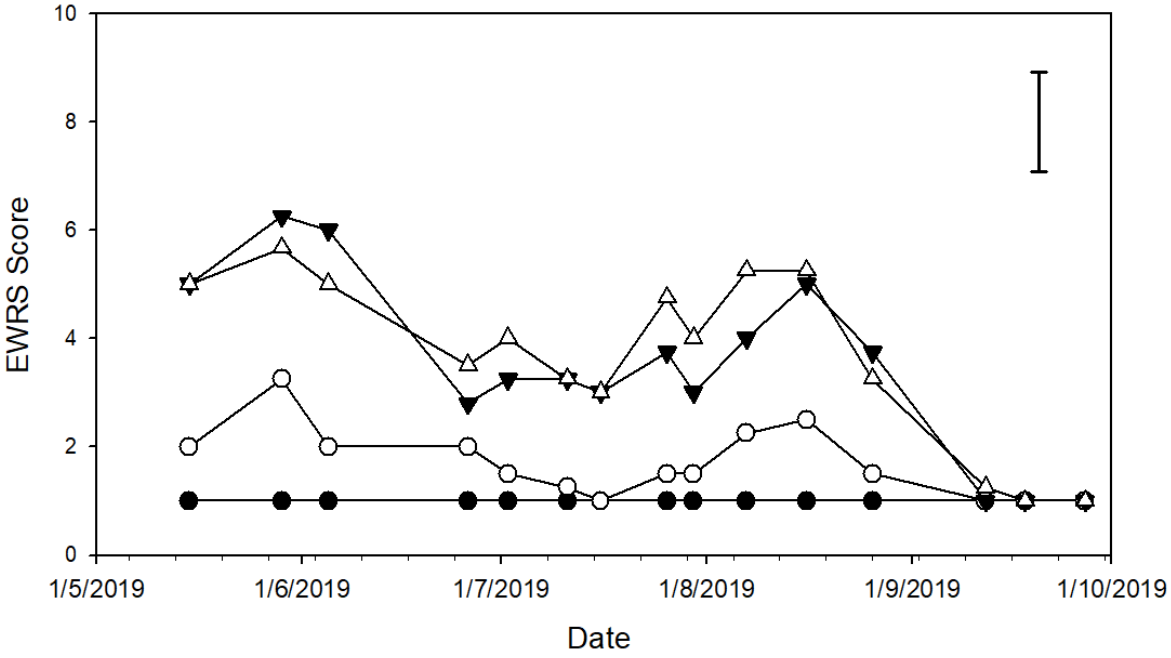

Figure 4.5 Experiment 1 European Weed Research Society score for dock over time**.** The four glyphosate application rates were 0 L/ha (●), 1 L/ha (❍), 2 L/ha (▼) and 4 L/ha (▽). Error bar represents the least significant differences (lsd) at α= 0.05 for comparisons amoung herbicide rates over time.

Plant species including twitch and white clover were present randomly throughout the experimental site in Experiment 1. Due to the inconsistency of the plant population, analysis of the EWRS score of these two species showed no significant interaction between herbicide application and time. The 1 L/ha application rate had no effect on twitch, with no difference observed compared to the treatment means of the control.

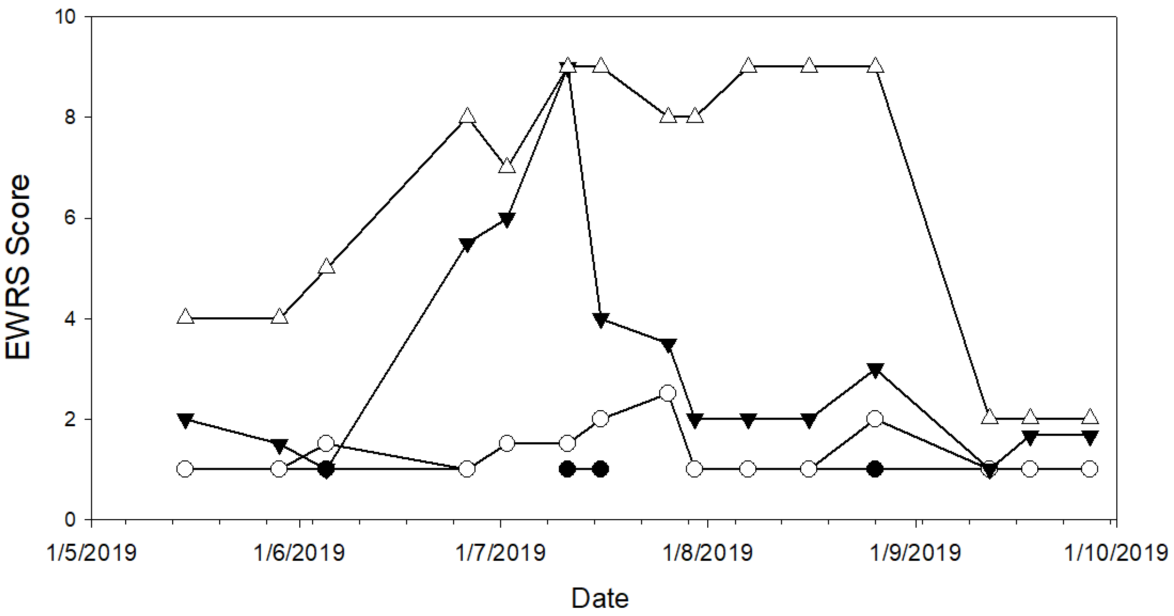

Figure 4.6 Experiment 1 European Weed Research Society (EWRS) score of twitch over time. The four glyphosate application rates were 0 L/ha (●), 1 L/ha (❍), 2 L/ha (▼) and 4 L/ha (▽). Error bar represents the least significant differences (lsd) at α= 0.05 for comparisons amoung herbicide rates over time.

The 4 L/ha treatment was slow to prove effective, with peak EWRS scores observed from the start of July. The 4 L/ha treatment observed effective suppression of the twitch until the start of September, with an EWRS mean of 9.0. The plant response to 2 L/ha glyphosate treatments were inconsistent, with a spike in EWRS mean score observed in early July. All treatment mean EWRS scores returned to approximately 1.0-2.0 by the end of the experiment.

The white clover from Experiment 1 was not analysed until the 1st of July (Figure 4.7). Observable treatment effects were seen from the 2 and 4 L/ha treatments. The mean EWRS score of the 4 L/ha treatment was greatest on the 11th of July at 6.3. The 2 L/ha treatment had a maximum EWRS score of 4.0 on the 16th of August. All EWRS treatments returned to a mean of 1.0 in September.

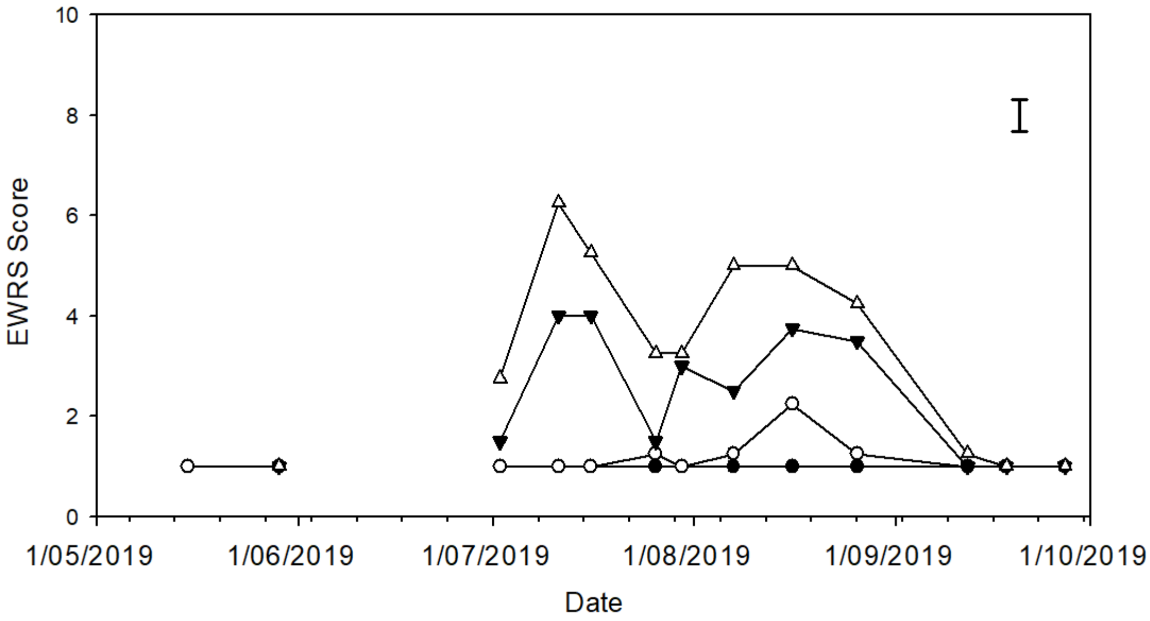

Figure 4.7 Experiment 1 European Weed Research Society (EWRS) score of white clover over time. The four glyphosate application rates were 0 L/ha (●), 1 L/ha (❍), 2 L/ha (▼) and 4 L/ha (▽). Error bar represents the least significant differences (lsd) at α= 0.05 for comparisons amoung herbicide rates over time.

4.1.3 Botanical composition analysis

Total dry matter (DM) harvested on September 18th ranged from a minimum of 545 kg DM/ha from 4 L/ha to 2637 kg DM/ha from the control treatment (P<0.001) which was higher than all other treatments (Figure 4.8). The 812 ± 170 kg DM/ha from 2 L/ha was similar to both 1 L/ha (1302 ± 170 kg DM/ha) and 4 L/ha (545 ± 170 kg DM/ha). However, the difference in total DM yield was not a function of lucerne contributions which was 562 ± 212 kg DM/ha (P<0.001) regardless of herbicide treatment. Broadleaf weeds contributed a minimum mean yield of 47 kg DM/ha from 4 L/ha, and a maximum of 543 ± 96 kg DM/ha from 0 L/ha. The proportion of broadleaf weeds in the control was five times greater (P<0.05) than in the herbicide treatments (107 ± 96 kg DM/ha). Grass weeds contributed a minimum of 63 kg DM/ha in 4 L/ha to a maximum of 1437 ± 297 kg DM/ha in the control. The mean 623 kg DM/ha of grass weeds from 1L/ha treatment was intermediate and did not differ from the other treatments.

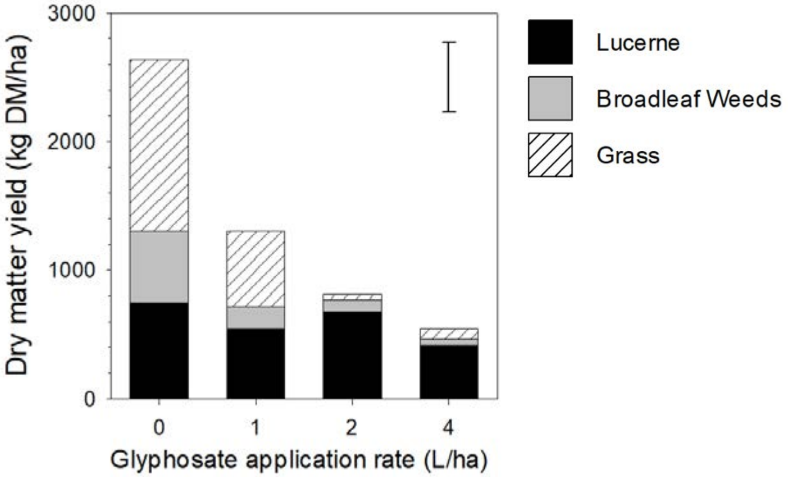

Figure 4.8 Experiment 1 botanical composition (kg DM/ha) of lucerne, broadleaf weeds and grass for each glyphosate treatment (0, 1, 2 & 4 L/ha). Error bar represents the least significant difference (lsd) for the total dry matter yield.

4.1.4 Visual plant population assessment

Large variability in plant appearance and density throughout the experimental site for all three experiments was observed. Consequently, the results and statistical analysis created from the visual plant population assessments were deemed an inadequate form of measurement to estimate canopy cover of each plant species.

Figure 4.9 and Figure 4.10 below indicate the effect of anomalies and inconsistent measurements on the trend of plant population cover. These inconsistencies were likely due to the superior age of the stand and the reliability and validity of the personal visual assessment. According to Figure 4.9, the lucerne plant population (%) under the control, 1 and 2 L/ha treatments observed no difference. The 4 L/ha rate observed a slight differentiation between the 15th of May and the end of July. This is proven inaccurate by the EWRS score (Figure 4.2) which indicated the effect of the 2 and 4 L/ha herbicide rates. The visual evidence provided by Plate 2 also proves the visual plant population assessment inaccurate whereby significant differences in lucerne plant abundance and density is evident. The coefficient of variation of 46% for lucerne supported the variation in distribution observed from the measurements also.

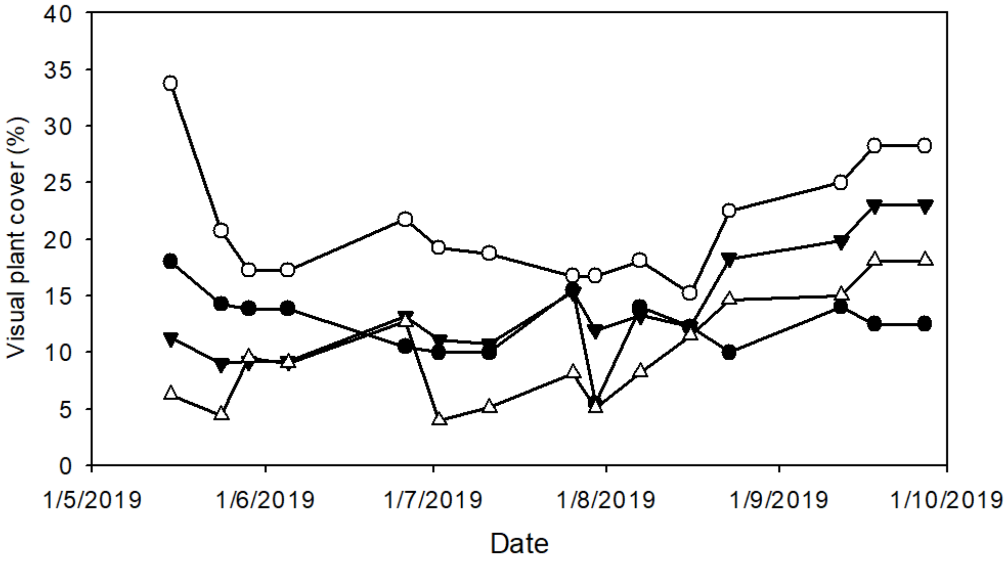

Figure 4.9 Visual plant population cover (%) of lucerne in Experiment 1 over time. The four glyphosate application rates were 0 L/ha (●), 1 L/ha (❍), 2 L/ha (▼) and 4 L/ha (▽).

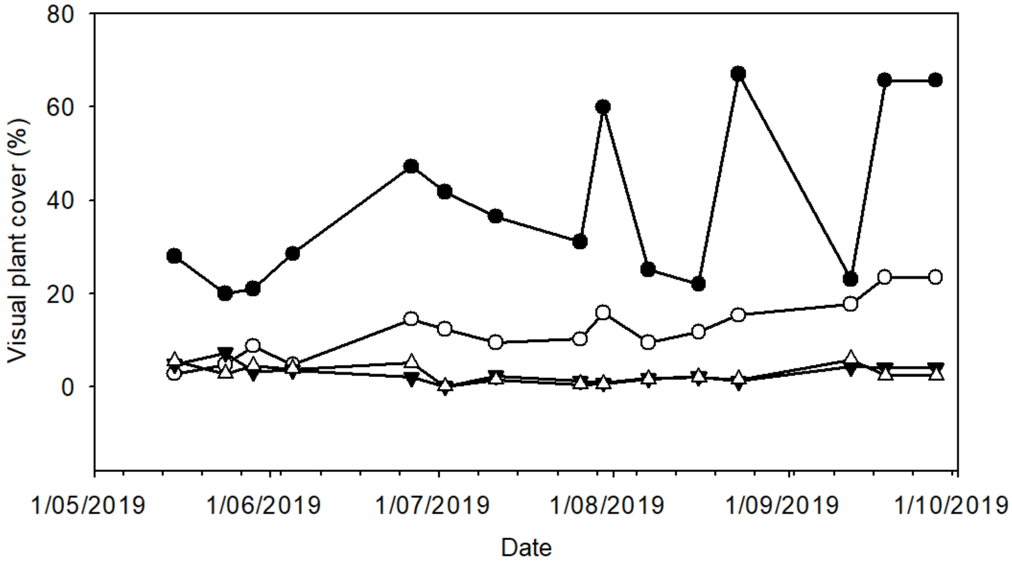

Figure 4.10 Visual plant population cover (%) of ryegrass in Experiment 1 over time. The four glyphosate application rates were 0 L/ha (●), 1 L/ha (❍), 2 L/ha (▼) and 4 L/ha (▽).

Figure 4.10 indicated variation in ryegrass plant population (%) cover. The undulating ryegrass yields observed from the 4 L/ha treatment, combined with the lack of observed trends from the control, 1 and 2 L/ha treatments, resulted in a coefficient of variation of 49%. Due to this, the GreenSeeker measurements were used to give more accurate representation of treatment effects on canopy cover.

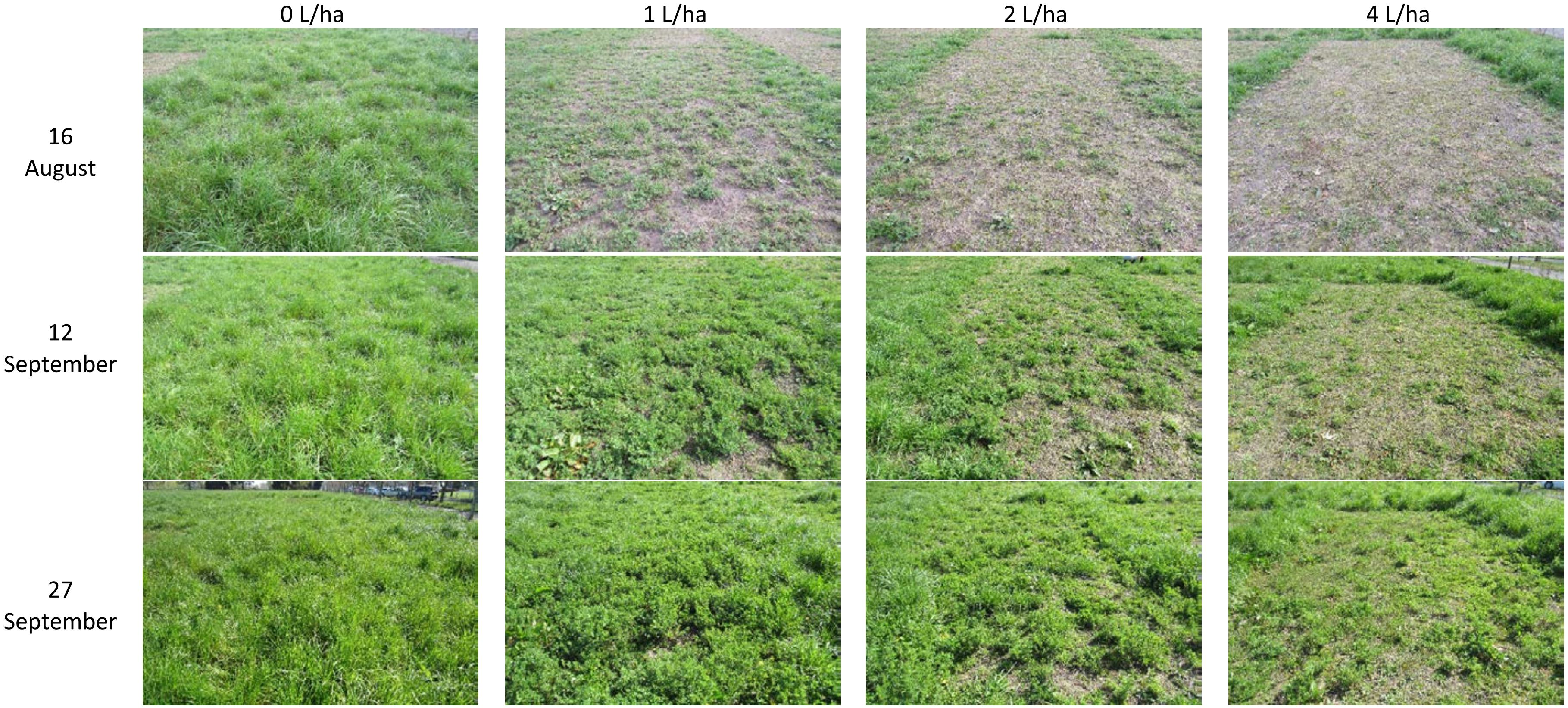

Plate 2 Experiment 1 pictures of plot number 13 (0 L/ha), 16 (1 L/ha), 15 (2 L/ha) and 4 (4 L/ha) from the 16th of August,12th of September and 27th of September. Glyphosate application was the 29th of April, 2019

4.2 Experiment 2

4.2.1 GreenSeeker

All treatments from Experiment 2 showed an interaction (P<0.05) between herbicide application and time. Initial GreenSeeker measurements taken on the 10th of June showed the mean cover for all herbicide application rates was 0.44 ± 0.03. Values decreased for all treatments between this initial measurement and the 17th June to a mean of 0.34 ± 0.03. From the 17th June, the control values were higher (P<0.05) than the sprayed crops and reached 0.61 ± 0.03 by the final date of measurement (27th of September).

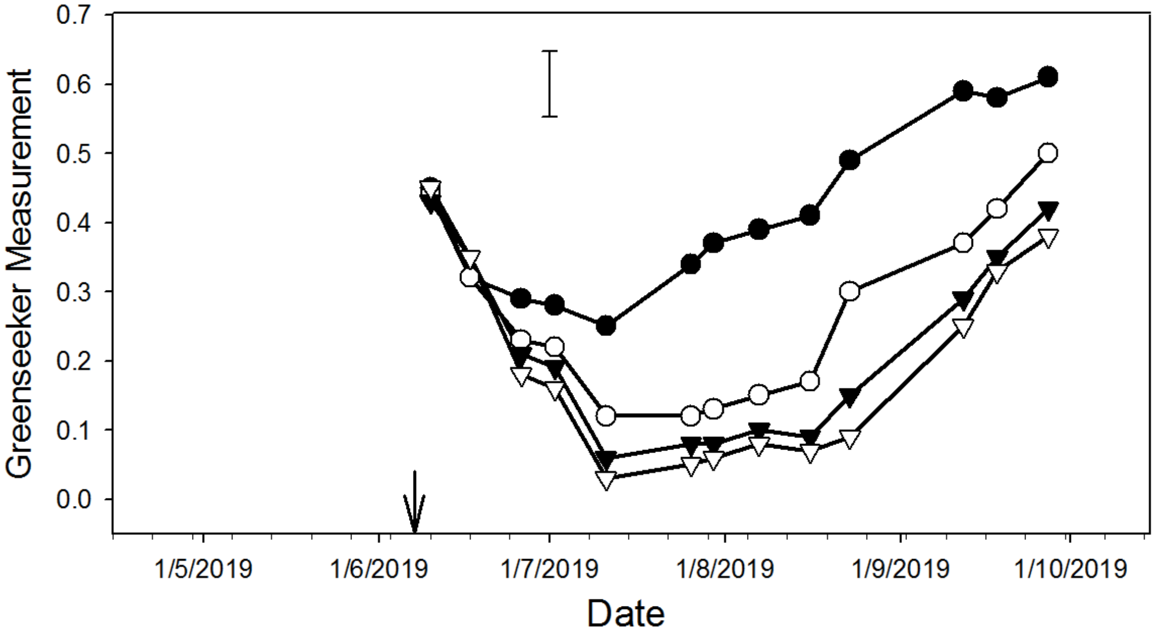

Figure 4.11 Experiment 2 GreenSeeker measurements recorded over time. The four glyphosate application rates were 0 (•), 1 (❍), 2 (▼) and 4 (▽) L/ha. Error bar represents the least significant differences (lsd) at α= 0.05 for comparisons amoung herbicide spray rates for each experiment. Herbicide application date (6th June) is indicated by (↓).

Sprayed treatments in Experiment 2 decreased at a greater rate than the control between the 17th June and 11th of July. From the 26th June, all treatments (1, 2 & 4 L/ha) decreased cover to the 11th July (0.12 ± 0.03, 0.06 ± 0.03 and 0.03± 0.03, respectively). From the 11th of July, the 1 L/ha treatment saw values increase to 0.50 ± 0.03 by the 27th September. The cover in the 1 L/ha was different (P<0.05) from 0, 2 and 4 L/ha on the 23rd of August, however not different from the 2 and 4 L/ha treatments on any other date from the 11th July to the 27th September. The 2 L/ha treatment means increased from 0.06 ± 0.03 on the 11th July to 0.42 ± 0.03 by the 27th September. The 4 L/ha treatment increased from 0.03 ± 0.03 on 11th July to 0.38 ± 0.03 by the final measurement but these values were not different from the 2 L/ha treatment at any time in Experiment 2.

4.2.2 European Weed Research Society (EWRS) score analysis

Experiment 2 EWRS of lucerne showed all treatments had an interaction (P<0.01) between herbicide rate and time. The maximum mean EWRS score of lucerne from Experiment 2 was 5.0 ± 0.5. Differences amoung treatments were observed from the first measurement (26th June) to the 11 July, when treatment means were not different from each other. The control treatment had an EWRS of 1.0 throughout the duration of the experiment. From the 11th July, the EWRS score for the 1 L/ha treatment increased from 2.0 ± 0.5 to 3.0 ± 0.5 by 26th of July, where it remained at until the 16th of August, before reducing back to 1.0 by the 12th of September.

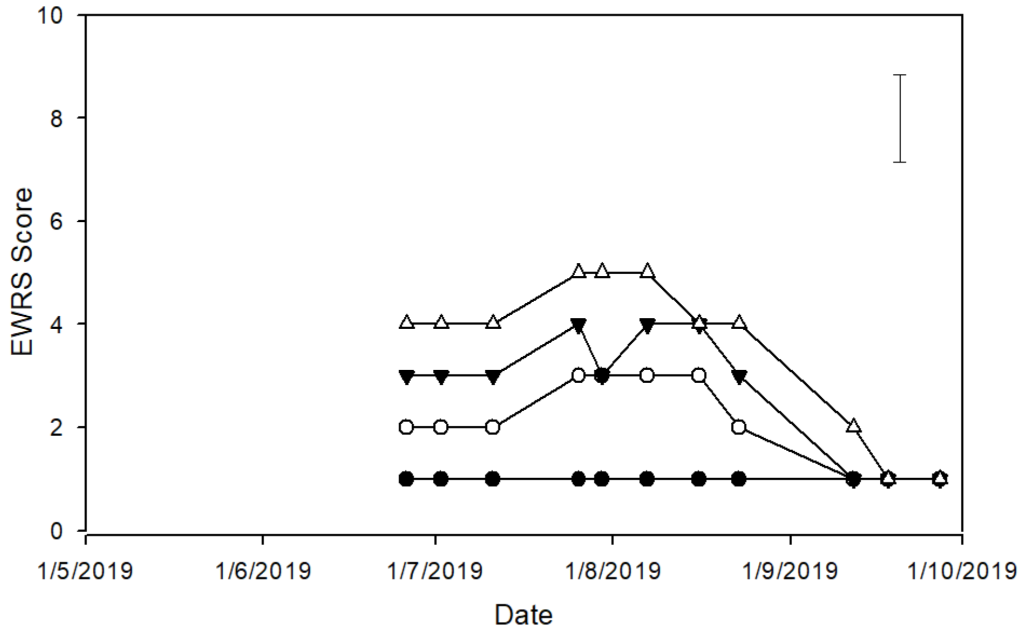

Figure 4.12 Experiment 2 European Weed Research Society (EWRS) score of lucerne over time. The four glyphosate application rates were 0 (●), 1 (❍), 2 (▼) and 4 (▽) L/ha. Error bar represents the least significant differences (lsd) at α= 0.05 for comparisons amoung herbicide rates over time.

The 2 L/ha treatment measured 3.0 ± 0.5 between the 26th June and the 11th of July. This then increased to 4.0 ± 0.5 by the 26th of July, before it decreased to be constant at 1.0 ± 0.5 from the 12th of September. The 4 L/ha treatment was 4.0 ± 0.5 from the 26th of until the 7th of August. It then decreased to 2.0 ± 0.5 by the 18th of September.

The EWRS of ryegrass in Experiment 2 showed an interaction (P<0.01) between herbicide rate and time. An immediate herbicide effect was observed from the herbicide treatments on the 26th June. The EWRS for the 1 L/ha increased from 4.5 ± 0.9 on the 26th of June to 6.0 ± 0.9 on the 26th of July, then decreased to 2.3 ± 0.9 on the 30th of July before increasing to 4.8 ± 0.9 by the 23rd of August. It remained constant at 1.3 ± 0.9 from the 12th of September. Between the 26th of June and 2nd of July, and between the 7th and 23rd of August, 1 L/ha was different (P<0.01) from all other treatments.

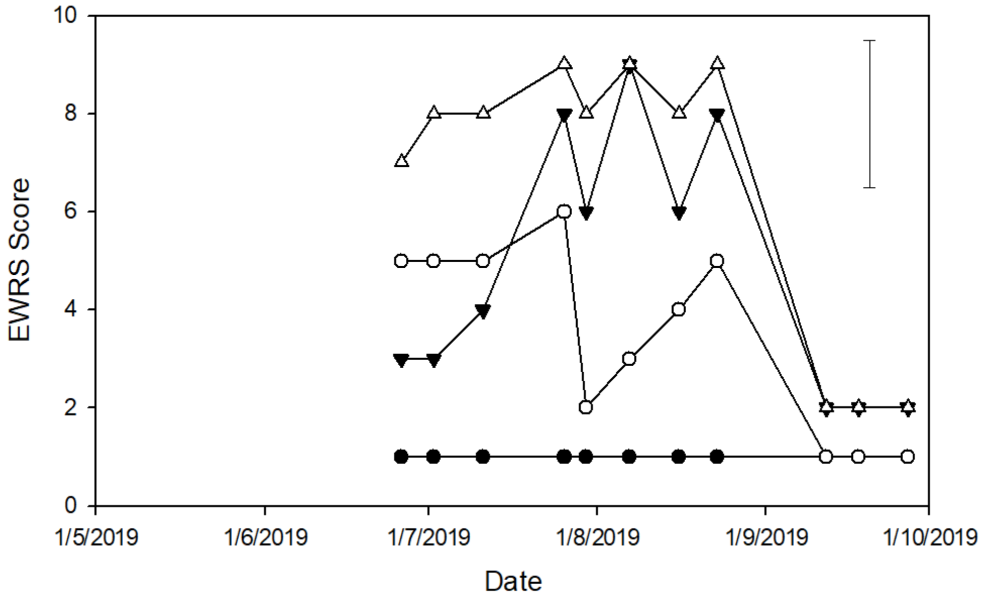

Figure 4.13 Experiment 2 European Weed Research Society (EWRS) score of ryegrass over time. The four glyphosate application rates were 0 (●), 1 (❍), 2 (▼) and 4 (▽) L/ha. Error bar represents the least significant differences (lsd) at α= 0.05 for comparisons amoung herbicide rates over time.

The ryegrass EWRS score for 2 L/ha was 3.0 ± 0.9 on the 26th of June and increased to 6.3 ± 0.9 by the 26th of July. Between the 26th July and the 23rd of August, the mean EWRS of 2 L/ha was 7.1 ± 0.9. During this period, the EWRS for the 2 L/ha treatment was higher (P<0.01) than the control treatment. The 2 L/ha treatment decreased to a mean of 2.3 ±

0.9 from the 12th to the 27th of September. The 2 L/ha treatment was only different (P<0.01) from the 4 L/ha treatment on the 26th of June. The 4 L/ha treatment had an EWRS of 6.7 ± 0.9 on the 26th of June, which increased to 8.4 ± 0.9 between the 26th of July and 23rd of August. It decreased to 1.8 ± 0.9 for September. The EWRS for ryegrass under 4 L/ha treatment was higher (P<0.01) than the control from the 26th of June to the 23rd of August.

The EWRS score of dandelion from Experiment 2 showed an interaction (P<0.05) between herbicide rate and time. The highest mean EWRS score was 5.3 ± 0.6 from 4 L/ha on the 16th August. All herbicide applications had similar initial measurements on the 26th June with a mean of 1.7 ± 0.6. The control treatment for dandelion remained at 1.0 ± 0.6 for the entire experiment.

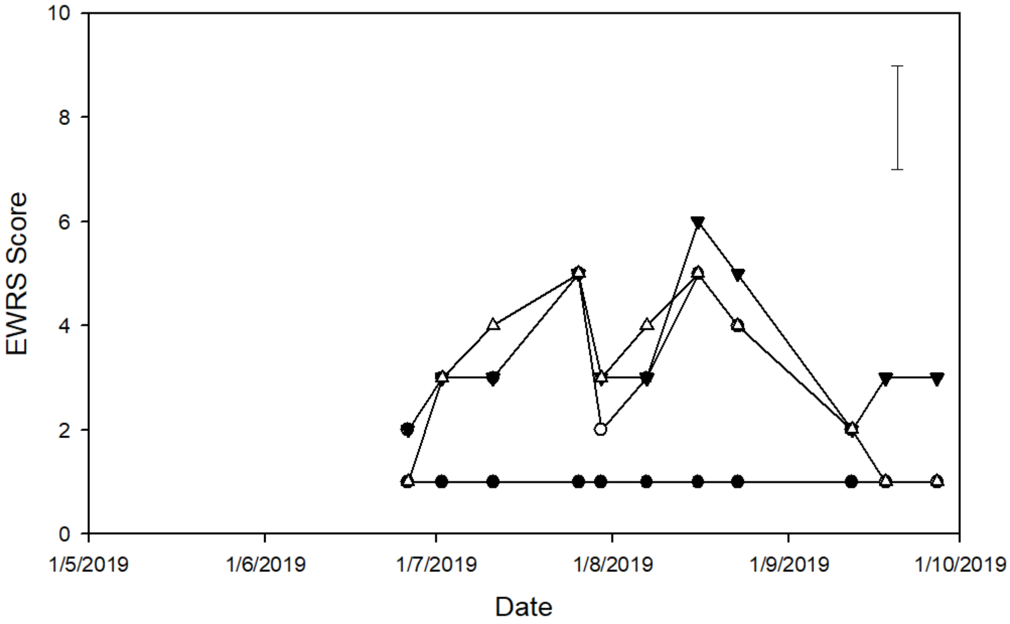

Figure 4.14 Experiment 2 European Weed Research Society (EWRS) score of dandelion over time. The four glyphosate application rates were 0 (●), 1 (❍), 2 (▼) and 4 (▽) L/ha. Error bar represents the least significant differences (lsd) at α= 0.05 for comparisons amoung herbicide rates over time.

The EWRS scores for dandelion treatments at 1, 2 and 4 L/ha were not different from each other at any time during the experiment. From the 26th of June, the 1, 2 and 4 L/ha treatments increased to 4.7 ± 0.6 by the 26th of July. At this point, the herbicide treatments were higher (P<0.05) than the control treatment. The herbicide treatments decreased to 2.8 ± 0.6 on the 30th of July, where they were not different (P<0.05) from the control treatment. All herbicide treatments increased to a mean of 4.2 ± 0.6 by the 23rd of August, where herbicide treatments were different ($P<0.05$) from the control treatment. From the 23rd of August, EWRS scores decreased to 2.4 ± 0.6 for all sprayed treatments until the 27th of September.

Experiment 2 EWRS of dock showed an interaction ($P<0.001$) between herbicide application and time. The highest EWRS score was 7.3 ± 0.6 on the 16th of August from the 4 L/ha treatment. The lowest EWRS score for dock was from the control treatment, which remained at 1.0 ± 0.6 for the entire experiment.

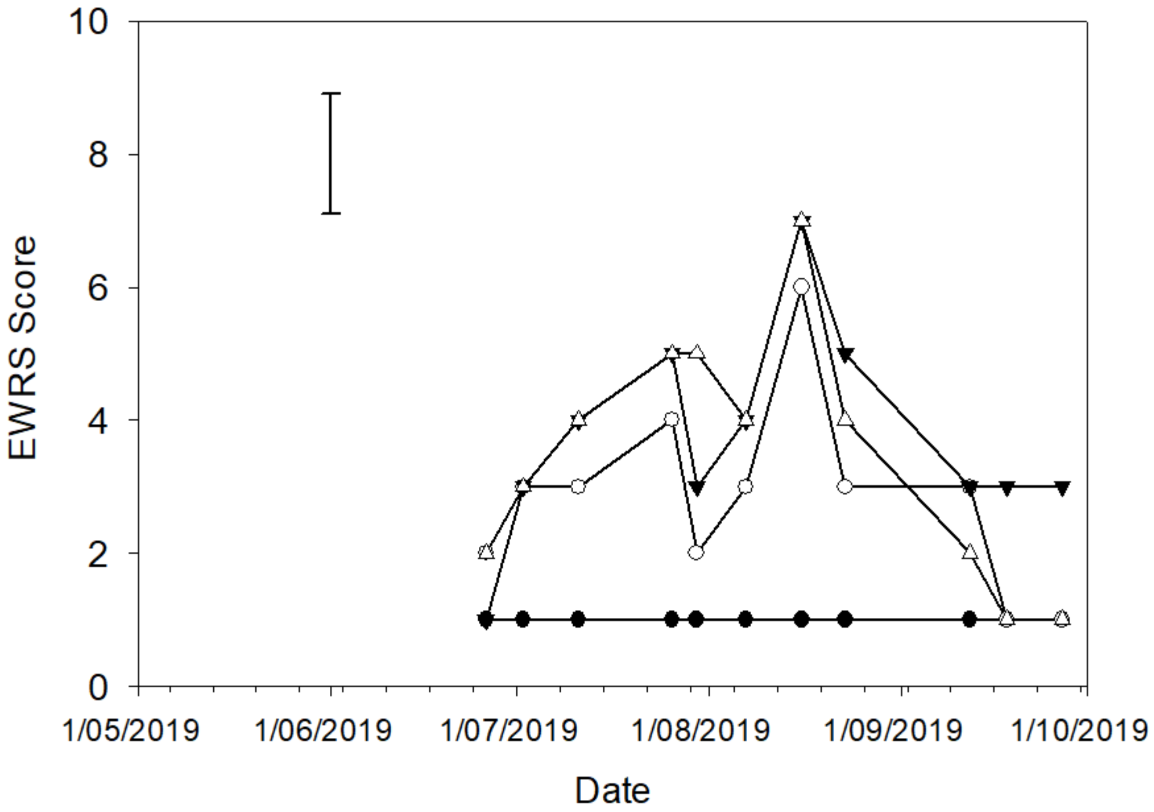

Figure 4.15 Experiment 2 European Weed Research Society (EWRS) score of dock over time. The four glyphosate application rates were 0 (●), 1 (❍), 2 (▼) and 4 (▽) L/ha. Error bar represents the least significant differences (lsd) at α= 0.05 for comparisons amoung herbicide rates over time.

For dock, the 1 L/ha treatment increased from 1.8 ± 0.6 on the 26th of June to 5.8 ± 0.6 by the 16th of August. The 1 L/ha treatment then decreased back down to 1.0 by the 18th of September and remained there for the entire experiment. Between the 2nd of July and the 26th of July, and again between the 7th of August and the 23rd of August, the 1 L/ha treatment was different (P<0.001) to the control treatment. The 2 and 4 L/ha treatments increased from a mean EWRS score of 1.3 ± 0.6 on the 26th of June to 7.1 ± 0.6 on the 16th of August. The 4 L/ha treatment decreased to 1.0 ± 0.6 by the 18th of September. The 2 L/ha treatment decreased to 2.8 ± 0.6 by the 18th of September where it remained for the entire experiment. The 2 and 4 L/ha treatments for dock were not different at any point in Experiment 2.

The EWRS score of twitch from Experiment 2 showed an interaction (P<0.001) between herbicide rate and time. At the first measurement on the 26th of June, the control treatment was lower (P<0.001) than the 2 and 4 L/ha treatments, but not different from the 1 L/ha treatment.

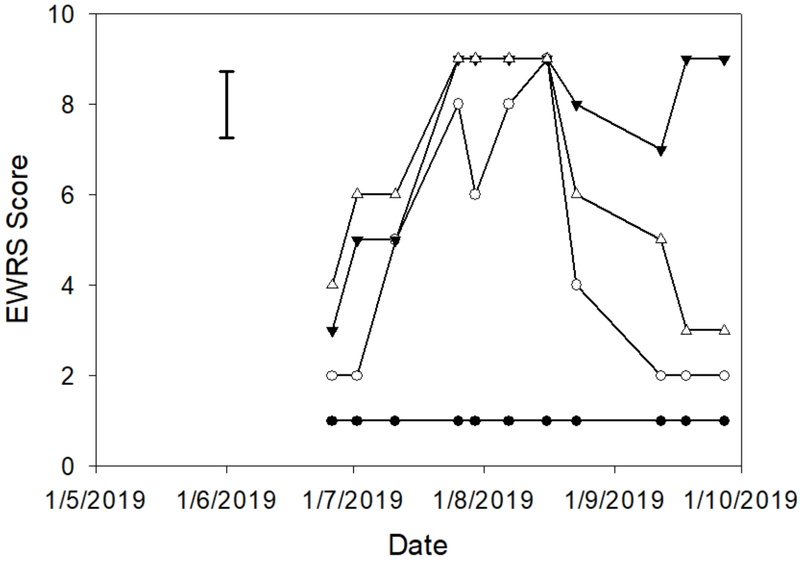

Figure 4.16 Experiment 2 European Weed Research Society (EWRS) score of twitch over time. The four glyphosate application rates were 0 (●), 1 (❍), 2 (▼) and 4 (▽) L/ha. Error bar represents the least significant differences (lsd) at α= 0.05 for comparisons amoung herbicide rates over time.

For twitch, the herbicide treatments increased the EWRS scores from the 26th of June 9.0 ± 0.7 to the 26th of July. Between the 26th of July and 16th of August the herbicide treatments (8.42 ± 0.7) were different (P<0.001) from the control treatment. From the 23rd of August to the 27th of September the herbicide treatments deviated. The 2 L/ha remained at 8 .0 ± 0.7, and was different (P<0.001) to all other treatments during this period. The 4

L/ha treatment decreased to 3.0 ± 0.7 by the end of September, and was different (P<0.001) from the control treatment, 1 and 2 L/ha treatment. The 1 L/ha treatment reduced during this time to 2.0 ± 0.7.

The EWRS score of white clover from Experiment 2 showed an interaction (P<0.001) between herbicide rate and time. The highest EWRS score for white clover 5.8 ± 0.3 was recorded from 4 L/ha on the 16th of August. On the 26th of July the EWRS for 1 and 2 L/ha increased from 1.9 ± 0.3 to 4.5 ± 0.3 by the 16th August. Treatments 1 and 2 L/ha reduced to a mean EWRS of 2.0 ± 0.3 on the 12th September. The 1 L/ha treatment decreased further to 1.0 ± 0.3 from the 18th to 27th of September, however the 2 L/ha treatment remained at 2.0 ± 0.3 for this period.

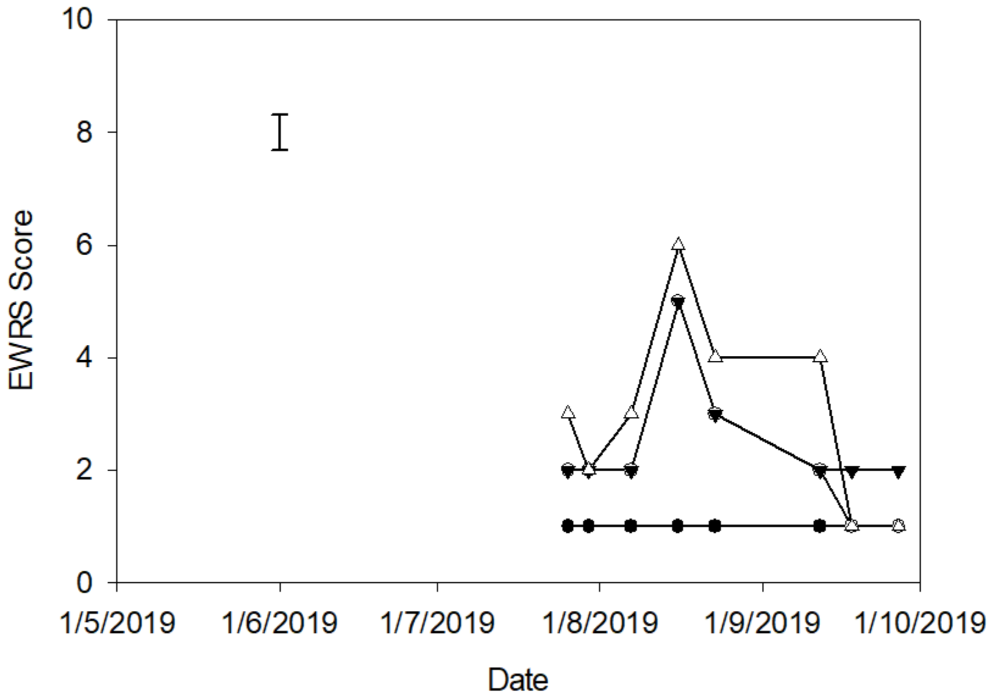

Figure 4.17 Experiment 2 European Weed Research Society (EWRS) score of white clover over time. The four glyphosate application rates were 0 (●), 1 (❍), 2 (▼) and 4 (▽) L/ha. Error bar represents the least significant differences (lsd) at α= 0.05 for comparisons amoung herbicide rates over time.

At the first white clover EWRS measurement on the 26th of July, 4 L/ha at 2.8± 0.3 was higher (P<0.001) than the control, however not different from 1 and 2 L/ha. The 4 L/ha treatment increased to 5.8 ± 0.3 on the 16th of August, then decreased again down to 1.0 ± 0.3 by the 18th of September.

4.2.3 Botanical composition analysis

Total dry matter (DM) harvested (September 18, 2019) ranged from a minimum of 929 kg DM/ha from 4 L/ha to maximum 2200 kg DM/ha from the control (P<0.001). The mean control yield of 2200 ± 163.8 kg DM/ha was higher than all other treatments. There was no difference in yields amoung the other treatments (1, 2, & 4 L/ha)

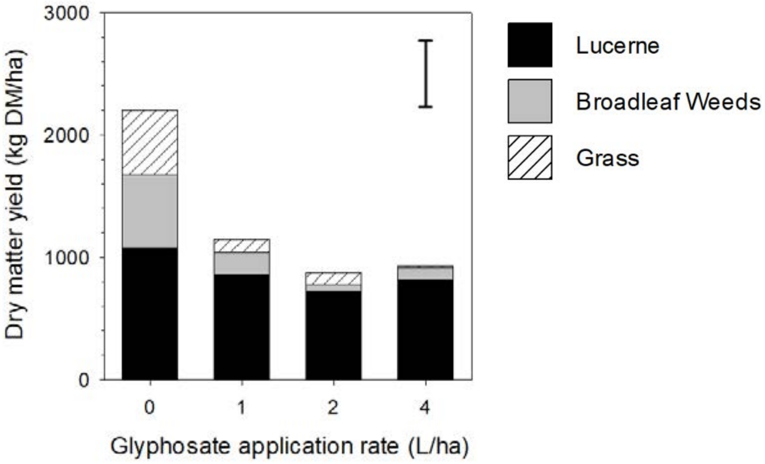

Figure 4.18 Experiment 2 botanical composition (kg DM/ha) of lucerne, broadleaf weeds and grass for each glyphosate treatment (0, 1, 2 & 4 L/ha). Error bar represents the least significant difference (lsd) for the total dry matter yield.

However, the difference in total DM yield was not a function of lucerne contributions which was 871± 198.7 kg DM/ha (P<0.001) regardless of herbicide treatment. Broadleaf weeds contributed a minimum mean yield of 83 ± 77.9 kg DM/ha from 4 L/ha and a maximum mean yield of 618 ± 77.9 kg DM/ha in the control. The proportion of broadleaf weeds of 618.5 ± 77.9 kg DM/ha in 0 L/ha was six times greater (P<0.01) than in the herbicide treatments 92.2 ± 77.9 kg DM/ha. Grass weeds contributed a minimum of 14 kg DM/ha to a maximum of 557 ± 118.6 kg DM/ha (P<0.05). The grass weeds were 556.7 ± 118.6 in 0 L/ha were seven times greater (P<0.05) than in the herbicide treatments 71.6 ± 118.6 kg DM/ha. This was visually observed in Plate 3 also.

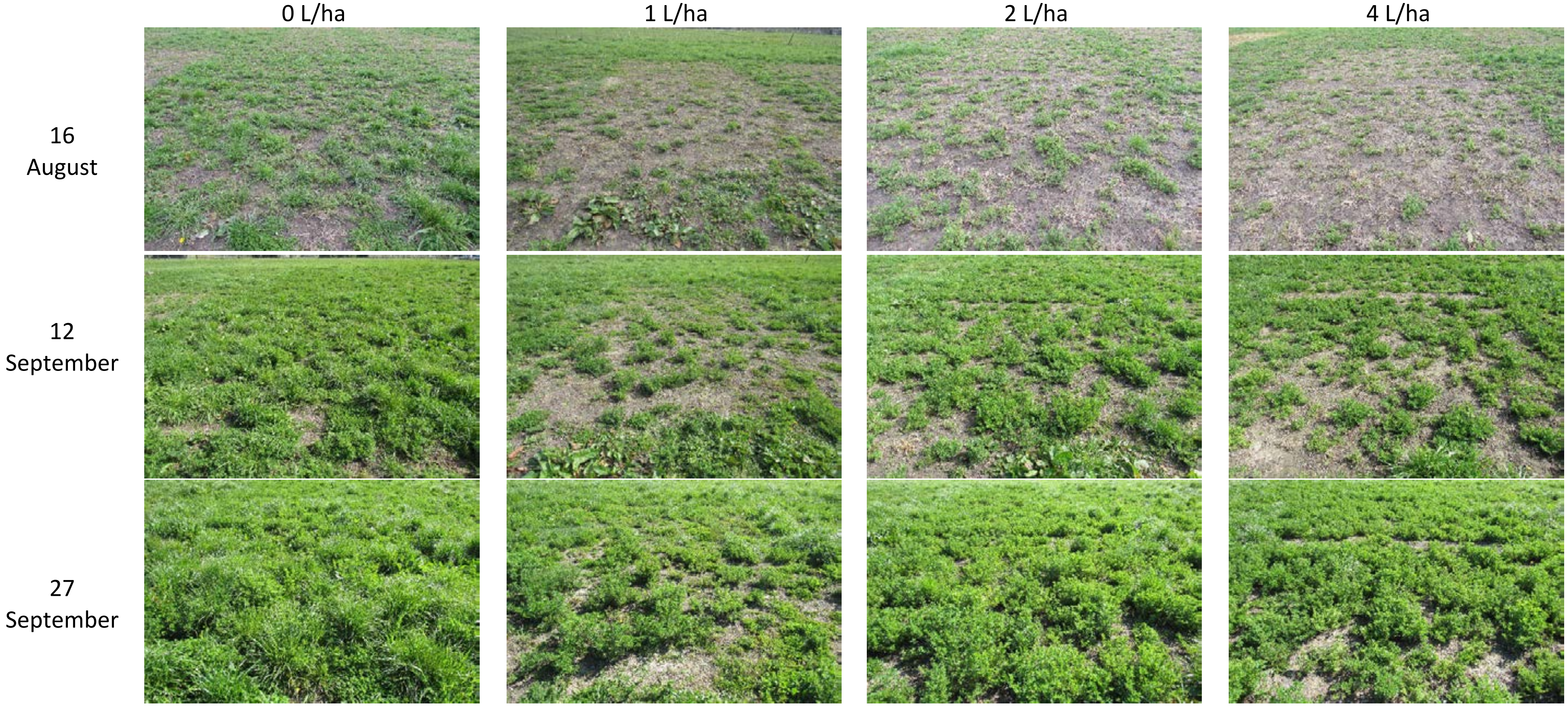

Plate 3 Experiment 2 plot pictures of plot number 11 (0 L/ha), 2 (1 L/ha), 9 (2 L/ha) and 8 (4 L/ha) on the 16th of August, 12 of September and 27th of September.

4.3 Experiment 3

4.3.1 GreenSeeker

All treatments from Experiment 3 showed an interaction (P<0.001) between herbicide rate and time. Initial GreenSeeker measurements taken on the 24th of July showed no difference in cover for all herbicide rates with a mean of 0.51 ± 0.03. All values then decreased to a mean of 0.23 ± 0.03 on the 30th of July. On the 7th of August, treatments differed. At this time the control treatment increased from 0.31 ± 0.03 to 0.62 ± 0.03 by the final measurement on the 27th of September. Between the 16th of August and the 27th of September the control treatment was different (P<0.001) from the herbicide treatments.

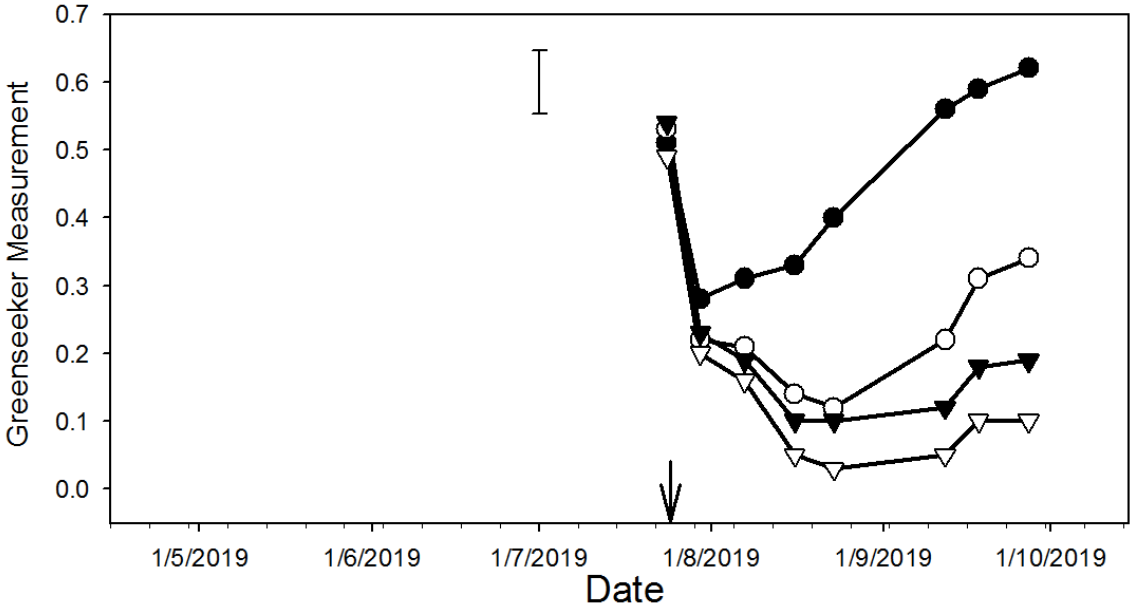

Figure 4.19 Experiment 3 GreenSeeker measurements recorded over time. The four glyphosate application rates were 0 (●), 1 (○), 2 (▼) and 4(▽) L/ha. Error bar represents the least significant differences (lsd) at α= 0.05 for comparisons amoung herbicide spray rates for each experiment. Herbicide application date (24th July) is indicated by (↓).

The 1 and 2 L/ha treatments decreased from 0.20 ± 0.03 on the 7th of August to 0.11 ± 0.03 on the 23rd of August. From the 23rd of August, the 1 L/ha treatment increased to 0.34 ± 0.03, and the 2 L/ha increased to 0.19 ± 0.03, by the 27th of September. There was no difference between the 1 and 2 L/ha treatments during this period. The 4 L/ha decreased from 0.20 ± 0.03 on the 30th of July to 0.03 ± 0.03 by the 23rd of August, and was not different from the 1 and 2 L/ha treatments during this time. From the 23rd of August, the 4 L/ha treatment increased to 0.10 ± 0.03 by the 27th of August but lower ($P<0.001$) than the 1 L/ha treatment.

4.3.2 European Weed Research Society (EWRS) score analysis

The Experiment 3 EWRS scores from each treatment depicts similar temporal patterns for each plant species (lucerne, ryegrass, dandelion, dock, twitch, and white clover). For lucerne all treatments had an interaction ($P<0.001$) between herbicide rate and time. The lucerne under the control treatment remained with an EWRS score of 1.0 ± 0.3 for the entire experiment. The EWRS score of the herbicide treatments increased from 1.1 ± 0.3 on the 30th of July to 4.0 ± 0.3 by the 16th August. From the 16th of August, 1 L/ha increased to 4.3 ± 0.3 on the 23rd of August, and then decreased back to 2.5 ± 0.3 by the 27th of September.

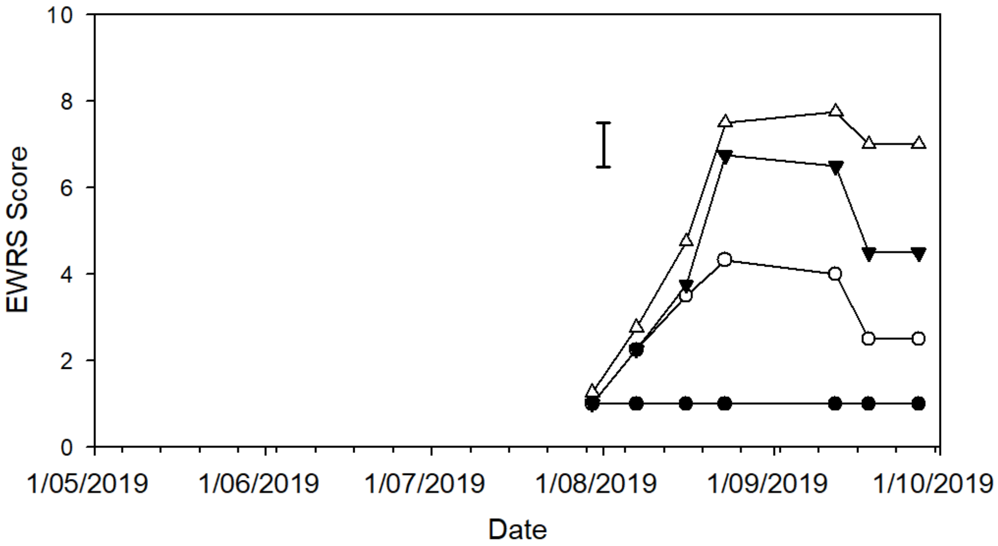

Figure 4.20 Experiment 3 European Weed Research Society (EWRS) score of lucerne over time. The four glyphosate application rates were 0 (●), 1 (❍), 2 (▼) and 4 (▽) L/ha. Error bar represents the least significant differences (lsd) at α= 0.05 for comparisons amoung herbicide rates over time.

From the 16th of August, the 2 L/ha treatment increased to 6.8 ± 0.3 by the 23rd of August and then reduced back down to 4.5 ± 0.3 by the 27th of September. The 4 L/ha treatment increased to 7.8 ± 0.3 by the 12th of September and was still 7.0 ± 0.3 on the 27th of September.

The ryegrass EWRS for Experiment 3 indicated that all treatments had an interaction (P<0.001) between herbicide rate and time. The control treatment for ryegrass remained at 1.0 ± 0.6 throughout the entire experiment. All herbicide treatments on the 30th of July were not different from each other with a treatment mean of 1.4 ± 0.6.

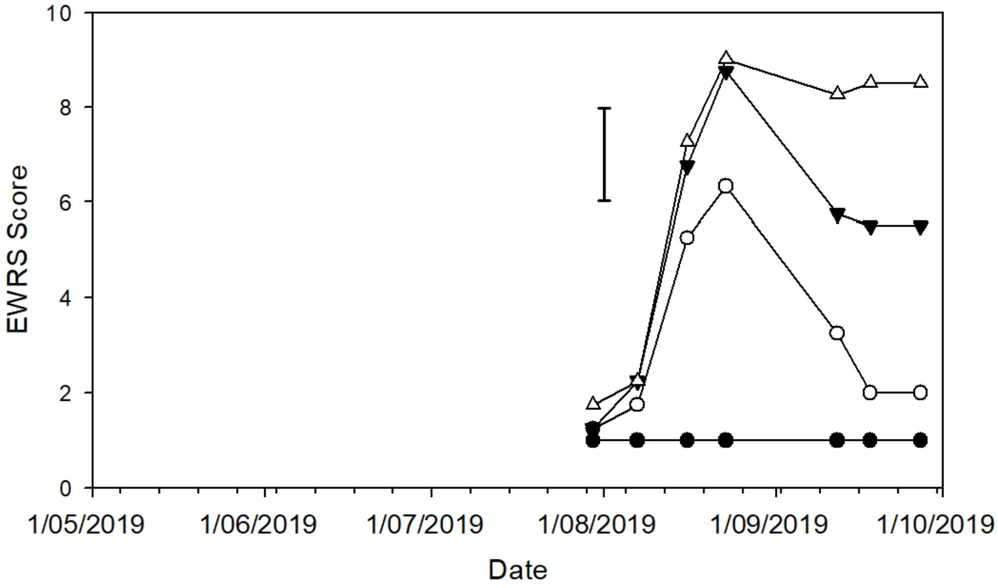

Figure 4.21 Experiment 3 European Weed Research Society (EWRS) score of ryegrass over time. The four glyphosate application rates were 0 (●), 1 (❍), 2 (▼) and 4 (▽) L/ha. Error bar represents the least significant differences (lsd) at α= 0.05 for comparisons amoung herbicide rates over time.

The maximum EWRS score for ryegrass was 9.0 ± 0.6 from 2 and 4 L/ha on the 23rd of August. The 1 L/ha treatment increased to 6.3 ± 0.6 by the same date. All herbicide treatments decreased between the 23rd of August to the 12th of September. The 1 L/ha decreased to 2.0 ± 0.6, the 2 L/ha decreased to 5.5 ± 0.6 but the 4 L/ha remained high at 8.5 ± 0.6. All treatments remained at those respective EWRS scores till the 27th of September.

The EWRS scores for dandelion followed similar temporal pattern to the lucerne in Experiment 3. All treatments had an interaction (P<0.05) between herbicide rate and time. The dandelion under the control treatment remained at 1.0 ± 0.6 for the entire experiment.

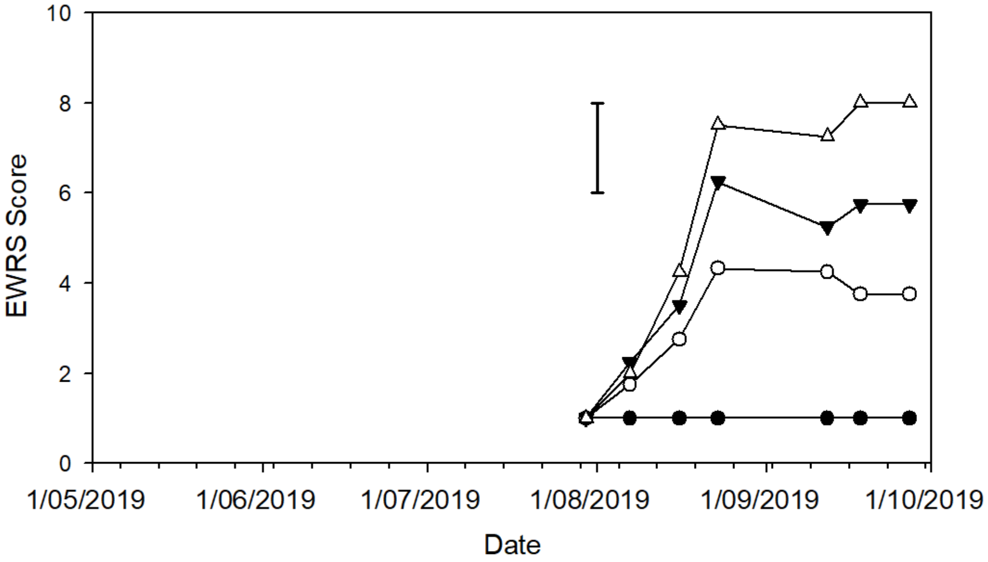

Figure 4.22 Experiment 3 European Weed Research Society (EWRS) score of dandelion over time. The four glyphosate application rates were 0 (●), 1 (❍), 2 (▼) and 4 (▽) L/ha. Error bar represents the least significant differences (lsd) at α= 0.05 for comparisons amoung herbicide rates over time.

The EWRS score of the herbicide treatments on dandelion increased between the initial measurement on the 30th of July and the 23rd of August. The 1 L/ha treatments decreased from 4.3 ± 0.6 on the 23rd of August to 3.8 ± 0.6 by the 27th of September. The 2 L/ha treatment decreased from 6.3 ± 0.6 on the 23rd of August to 5.8 ± 0.6 by the 27th of September. In contrast the 4 L/ha treatment increased from 7.5 ± 0.6 on the 23rd of August to the 8.0 ± 0.6 by the final measurement on September 27th.

The dock and twitch EWRS mean scores from Experiment 3 showed there was not an interaction between herbicide rate and time. The herbicide plant response to each treatment followed a similar temporal pattern to the dandelion and lucerne.

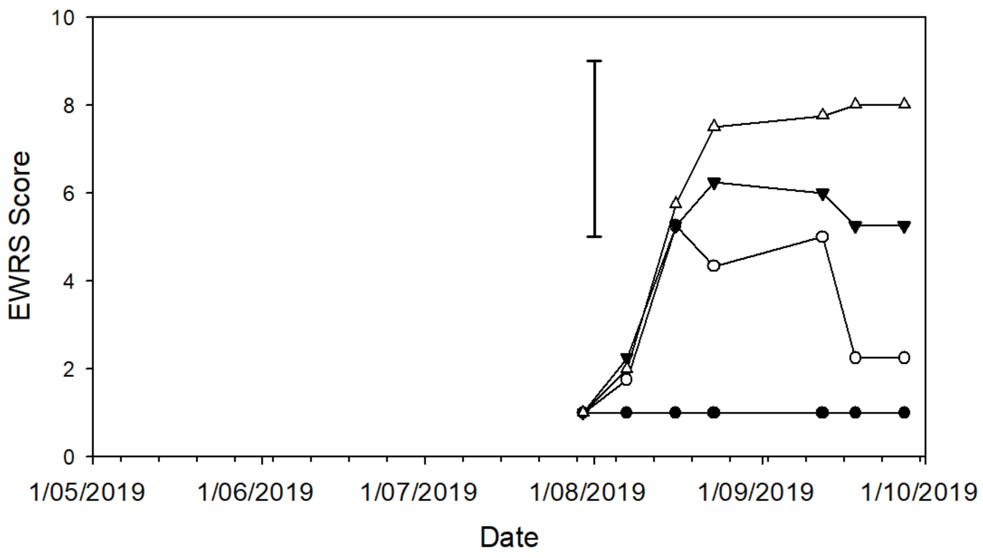

Figure 4.23 Experiment 3 European Weed Research Society (EWRS) score of dock over time. The four glyphosate application rates were 0 (●), 1 (❍), 2 (▼) and 4 (▽) L/ha. Error bar represents the least significant differences (lsd) at α= 0.05 for comparisons amoung herbicide rates over time.

Herbicide treatments for dock were observed from Experiment 3 on the 16th of August where treatment means differed. The 4 L/ha treatment remained at a mean EWRS of 7.6 ± 1.2 from the 23rd of August. The 2 L/ha treatment decreased from 6.3 ± 1.2 on the 23rd of August to 5.2 ± 1.2 on the 27th of September. The 1 L/ha treatment decreased from 5.3 ± 1.2 on the 23rd of August to 2.3 ± 1.2 on the 27th of September.

The twitch EWRS score for 4 L/ha reached a maximum score of 8.0 on the 12th of September and did not differ by the 27th of September. The 1 L/ha treatment observed a maximum EWRS score of 4.5 on the 12th of September and decreased to 3.0 by the end of the experiment. There was no difference between the 2 L/ha and control treatment means for EWRS.

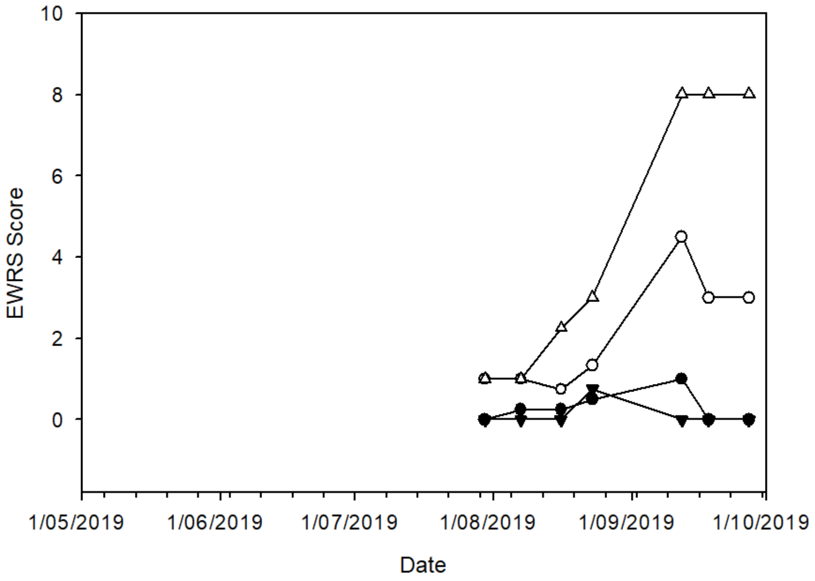

Figure 4.24 Experiment 3 European Weed Research Society (EWRS) score of twitch over time. The four glyphosate application rates were 0 (●), 1 (❍), 2 (▼) and 4 (▽) L/ha. Error bar represents the least significant differences (lsd) at α= 0.05 for comparisons amoung herbicide rates over time.

The white clover EWRS score from Experiment 3 indicated all treatments had an interaction (P<0.001) between herbicide rate and time. All treatments had similar EWRS scores at the first measurement on the 30th of July, with a mean of 1.0 ± 0.5. The control treatment remained at 1.0 ± 0.5 for the duration of the experiment.

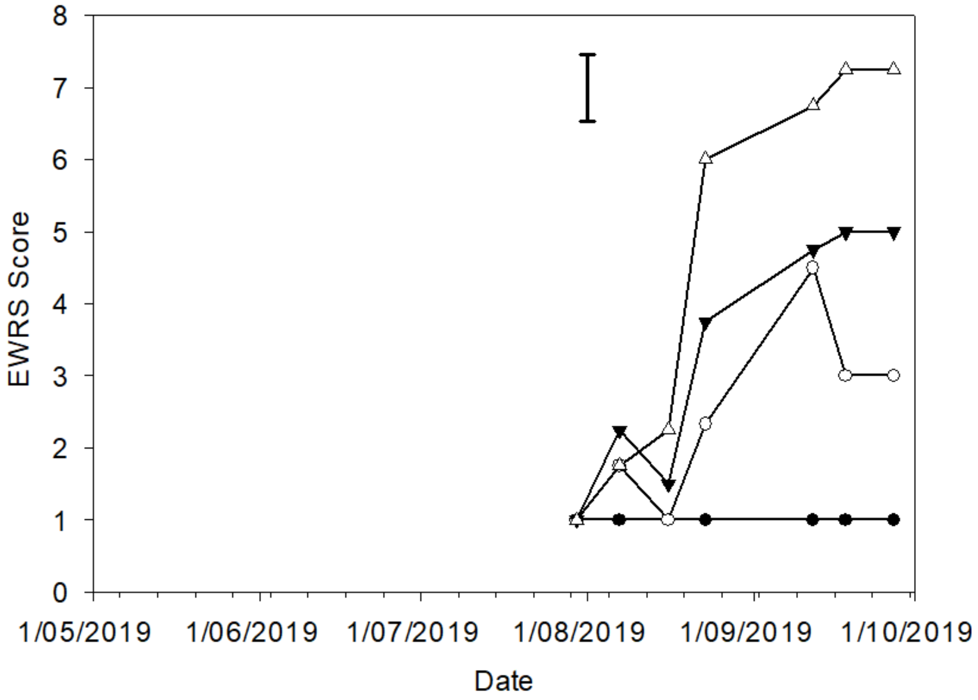

Figure 4.25 Experiment 3 European Weed Research Society (EWRS) score of white clover over time. The four glyphosate application rates were 0 (●), 1 (❍), 2 (▼) and 4 (▽) L/ha. Error bar represents the least significant differences (lsd) at α= 0.05 for comparisons amoung herbicide rates over time.

Between the 30th of July and 12th of September, the EWRS for the 1 L/ha rate increased to 4.5 ± 0.5, then decreased to 3.0 ± 0.5 from the 18th of September. The EWRS for the 2 L/ha treatment increased to 5.0 ± 0.5 on the 18th of September and remained at 5.0 ± 0.5 until the 27th of September. The 4 L/ha treatment increased to 3.8 ± 0.5 by the 23rd of August and then continued to increase to 5.0 ± 0.5 by the 27th of September.

4.3.3 Botanical composition analysis

Total dry matter (DM) harvested (September 18, 2019) ranged from a minimum of 152 kg DM/ha from 4 L/ha to 1726 kg D/ha from the control treatment (P<0.001). The mean yield of the control 1726 ± 90 kg DM/ha was higher than all other treatments. The 371 ± 90 kg DM/ha from 2 L/ha was similar to both 1 L/ha (650 ± 90 kg DM/ha) and 4 L/ha (152 ± 90 kg DM/ha).

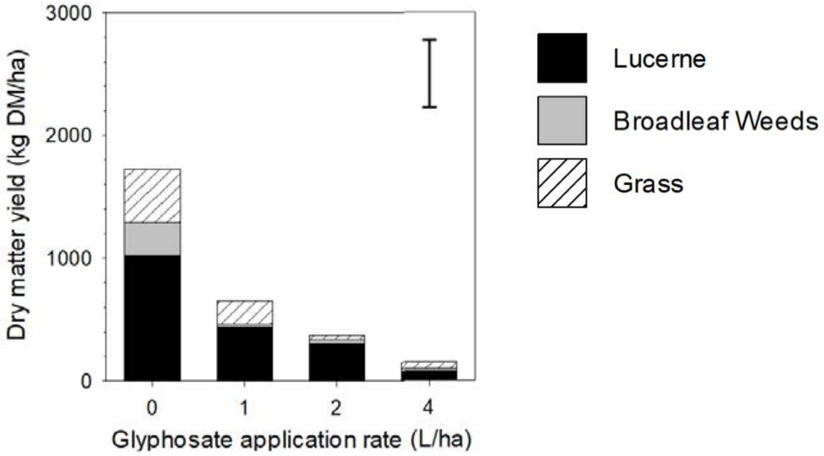

Figure 4.26 Experiment 3 botanical composition (kg DM/ha) of lucerne, broadleaf weeds and grass for each glyphosate treatment (0, 1, 2 & 4 L/ha). Error bar represents the least significant difference (lsd) for the total dry matter yield.

Lucerne contributed a minimum yield of 78 kg DM/ha from 4 L/ha and a maximum of 995 ± 75 kg DM/ha. Broadleaf weeds contributed a minimum mean yield of 20 ± 75 kg DM/ha from 4 L/ha and a maximum mean yield of 272 ± 75 kg DM/ha in the control treatments. The proportion of broadleaf weeds in 0 L/ha was 11 times greater (P<0.001) than in the herbicide treatments 23.9 kg DM/ha. Grass weeds contributed a minimum of 54 ± 47 kg DM/ha from 4 L/ha to a maximum of 459 ± 47 kg DM/ha from the control treatment. The proportion of grass weeds in the control was almost five times greater (P<0.001) than in the herbicide treatments (1 ,2, 4 L/ha). This is visually observed from Plate 4 also.

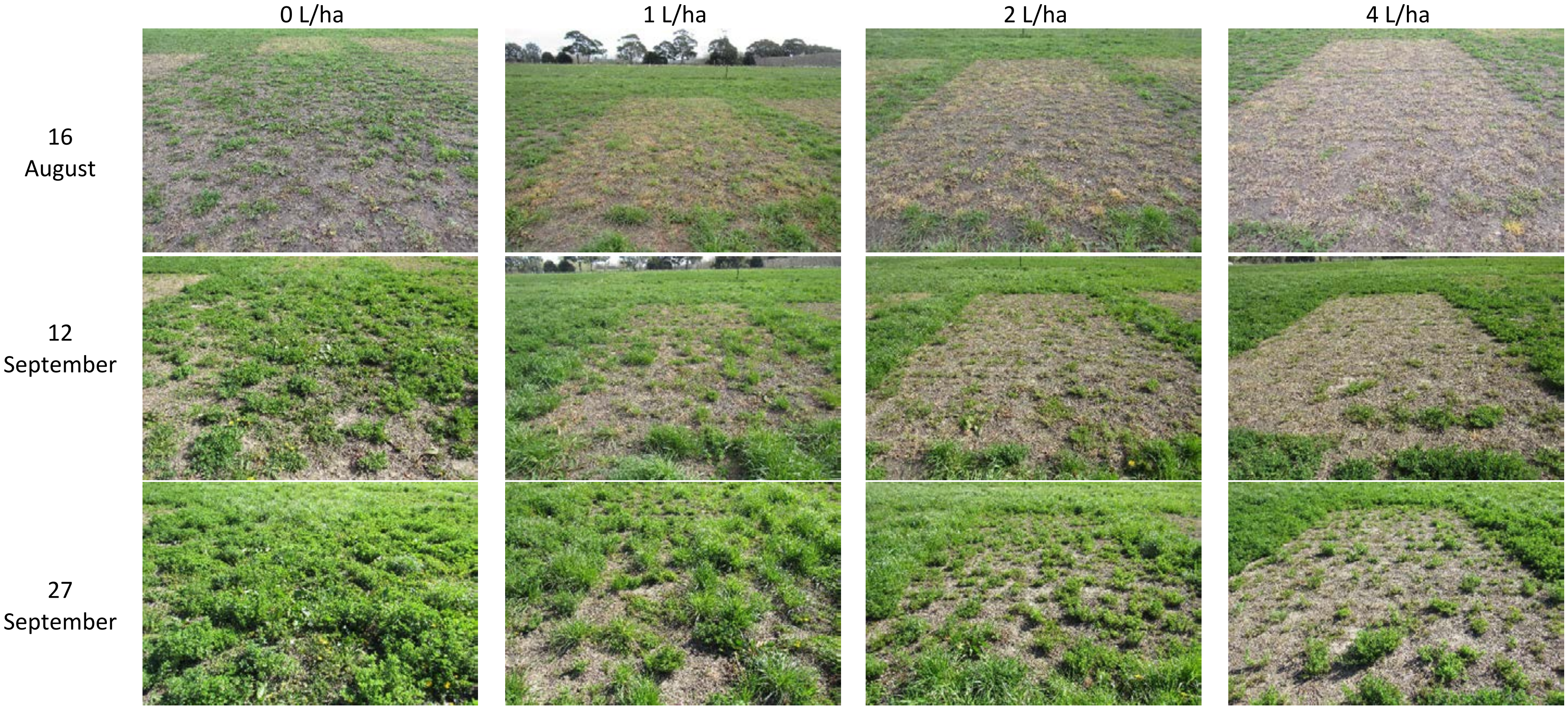

Plate 4 Experiment 3 pictures of plots 7 (0 L/ha), 2 (1 L/ha), 3 (2 L/ha) and 8 (4 L/ha) on the 16th of August, 12th of September and the 27th of September.

5 DISCUSSION

5.1 Experiment 1

The early herbicide application date (29th April) of Experiment 1 resulted in rapid mobility and effectiveness of the glyphosate, consistent with the warmer weather and actively growing plants (Tu et al., 2001). During Experiment 1 the rate of glyphosate effectiveness was visually observed to be much faster in the plots farthest away from the fence (Appendix 1), which was lined with tall Poplar trees. These trees obstructed the sunlight interception of the plots closest to them by severely blocking early to mid-day sunshine, as seen in Appendix 3. The effect of this was not observed in the statistical analysis.

Plant response to all glyphosate application rates was observed from the immediate deviation in GreenSeeker measurements on the 8th of May, 2019 (Figure 4.1). Between the 29th of April and the end of May, the mean air temperature was 10°C. At this temperature, the lucerne plants were still actively growing which consequently accelerated the rate of the effect of the herbicide. As expected, the GreenSeeker readings of the control treatment followed a uniform growth pattern throughout winter (with a mean GreenSeeker of 0.48 ± 0.03), and began increasing from the start of spring. This growth pattern supports previous studies (Moot et al., 2003) and gives an indication of the expected seasonal growth pattern of a lucerne stand. The lowest herbicide rate of 1 L/ha resulted in the least phytotoxicity damage to the lucerne, with a small decrease of GreenSeeker readings to 0.30 ± 0.03 during winter. Consequently, weed suppression was minimal from the 1 L/ha rate, with no difference between the EWRS scores of the 1 L/ha and the control treatment from all weed species (ryegrass, dandelion, dock, twitch and white clover). The proportion of broadleaf and grass weed species observed from 1 L/ha in the botanical composition (Figure 4.8) also supports this, having the greatest amount of weed species of all the herbicide treatments. The plant response to the 2 L/ha glyphosate application rate was observable from the 8th of May, with a mean GreenSeeker of 0.37 ± 0.03. The 4 L/ha plant response was even greater on the 8th of May, with a mean GreenSeeker of 0.31 ± 0.03. These two treatments had significant phytotoxicity effects on the health of all plant species present in the experimental plots. The botanical composition (Figure 4.8) indicates effective weed control in the 2 and 4 L/ha plots. However, there was also a reduction in total lucerne dry matter yield also. It can be expected from the trend observed from the GreenSeeker measurements of 2 and 4 L/ha treatments on the final record date of September 27th that means would continue to increase before they plateau at approximately 0.61 as seen in the 0 and 1 L/ha treatment means. Experiment 1 had larger “least significant differences” (lsd) values calculated for the GreenSeeker measurements, which meant there was greater variation from the seasonal growth of the lucerne than in other experiments.

The lucerne plants from Experiment 1 suffered severe phytotoxicity damage probably caused by the extended period of time between the plots being topped (April 4th) and the early application of herbicide 25 days later (Plate 2). Monk et al., (2016) recommended a period of 7-10 days between grazing and herbicide application of a lucerne stand in late June/early July, and similarly Moot et al., (2003) advises spraying 7-14 days post hard grazing in June or early July. The early application date of Experiment 1 meant the plants experienced temperatures warm enough to support active regrowth of the lucerne stand, and their shoot growth rates which decrease as temperatures cool (Moot et al., 2003). Lucerne shoot growth rate is higher in spring than autumn at the same temperatures (Moot et al., 2003), but the early autumn shoot growth rates in Experiment 1 were still higher in Experiments 2 and 3 (Figure 3.1). The average mean temperature between topping and spraying for Experiment 1 was 11°C, which increased the accumulation of thermal time (sum of daily mean temperature). The actively growing lucerne, combined with the extended period of time between topping and spraying of 25 days, allowed for the lucerne stand to regrow substantial herbage by the time of the glyphosate application. According to calculations by Brown et al., (2000), approximately 20 kg DM/ha/d would have been produced from the stand during this time. To support this, calculations using a linear regression estimated the lucerne shoot dry matter between herbage removal (topping) to herbicide application, using the shoot dry matter (DM) yield against calculated accumulated thermal time. This was done using a base temperature of 5°C (Thiebeau et al., 2011).

The calculated thermal time between the herbage removal and the spray date for Experiment 1 was 320°Cd, meaning an estimated lucerne regrowth of 974 kg DM/ha/°Cd was achieved. Moot et al., (2003) states that the phyllochron (thermal time interval between successive nodes) of the “Kaituna” lucerne variety in winter was constant at 37 ± 5 °Cd. After topping, the light interception of lucerne is reduced due to the reduction in dry matter. Therefore, a lag phase of dry matter production occurs between the herbage removal and the regrowth of a lucerne stand. Fick et al., (1988) quantified this ‘lag phase’ as 200°Cd for a lucerne stand. Using the calculated accumulated thermal time between herbage removal and herbicide application of 320°Cd, minus the 200°Cd of lag phase, then dividing by the 37 ± 5 °Cd it takes to grow a leaf, approximately 3 new leaves were grown on the lucerne plants prior to the application of herbicide. As glyphosate is a non-selective herbicide, the greater proportion of plant surface area available for herbicide penetration, the greater the effect of the herbicide. This meant the lucerne recorded decreased total dry matter yield (kg DM/ha) for all herbicide treatments compared with the control treatment (Figure 4.8). This lead to greater bare ground exposure due to the stand thinning out, and therefore enabled the invasion of spring annuals at the start of September.

The ryegrass plants in Experiment 1 were successfully suppressed under the higher glyphosate treatment rates of 2 and 4 L/ha, with a mean EWRS score of 8.90 ± 0.40. This is classified as commercially unacceptable for the target crop by the EWRS score (Figure 4.3). Total ryegrass yields were reduced to 40 and 81 kg DM/ha in the 2 and 4 L/ha treatments respectively (Figure 4.8), from the 1335 kg DM/ha recorded in the control plots. The 1 L/ha treatment rate was not effective at reducing ryegrass health, observed from (Figure 4.3) as there was no difference between the control and 1 L/ha rate (Figure 4.3) with an EWRS mean score of 1.5 ± 0.4. The 1 L/ha treatment did however reduce total ryegrass yields, from 1335 kg DM/ha in the control to 589 kg DM/ha under 1 L/ha of glyphosate.

The target broadleaf perennial weed species examined in Experiment 1, dock and dandelion, were both suppressed by the 2 and 4 L/ha treatments until the 23rd of August. These two plant species have long tap roots, therefore are susceptible to penetration by glyphosate. In comparison, twitch has a complex root system that complicates the efficiency of glyphosate. During this time, the average EWRS score for dock was 4.1 ± 0.5, which was classified as having no effect on yield by the EWRS score (Table 3). The EWRS score for both dock and dandelion returned to 1.0 ± 0.5 by the 12th of September. It was observed throughout the experiment that the increase in EWRS scores from the dock plants observed were from existing plants. However, the improvement noted of dandelion EWRS scores for September (Figure 4.4) was observed from the growth of new plants, not from the survival of existing plants. The recovery of the dock plants and growth of new dandelion plants was not substantial enough to increase the overall broadleaf weed yield (Figure 4.8). While the EWRS score of both dandelion and dock for 1 L/ha was not different from the control treatment at any point during the experiment, the broadleaf weed yield decreased by 30% from the 1 L/ha treatment (Figure 4.8). Therefore, it can be concluded that while dandelion and dock were classified as healthy plants by the EWRS score for all herbicide treatments at the end of Experiment 1, the glyphosate application reduced yields of these broadleaf weeds.

The inconsistency of the presence of twitch and white clover throughout the plots of Experiment 1 made it difficult to observe their response to the glyphosate applications. The white clover was only analysed part way through Experiment 1 on the 1st of July, which limited data analysis. However, the white clover was not effectively removed by the glyphosate. Slight reduction in yield was observed from the 2 and 4 L/ha treatments (Figure 4.7) of the white clover between mid-July and mid-August. However, this was only temporary, and all plants improved by September. Twitch was affected by the 4 L/ha of glyphosate, with a mean EWRS score of 6.2 classifying it as commercially unacceptable. The EWRS score for 4 L/ha returned back to 2.0 by the end of the experiment however, with mild phytotoxicity symptoms. Throughout the duration of the experiment, it was observed that under the 4 L/ha treatment, the twitch grew back in larger patches after the glyphosate application. This meant that new twitch shoots appeared outside the original area that the twitch had occupied prior to herbicide application. In comparison, the twitch observed under the lower herbicide application rates such as 1 and 2 L/ha did not. Due to the underground root network of rhizomes, twitch is known for being persistent and difficult to remove. Glyphosate is typically recommended as a suitable herbicide for eliminating twitch as it is a translocatable herbicide, and is therefore able to target the plant from the roots (Espeby et al., 2014). However, glyphosate has only been proven efficient at removing twitch if each plant has produced 3-4 leaves (Espeby et al., 2014). As Experiment 1 was topped prior to herbicide application, it is likely that the twitch sugars were moving out of the rhizomes, to establish above-ground dry matter. Therefore, movement towards the roots of the plant where the rhizomes would have been reduced, which reduced the overall effectiveness of the herbicide. The 4 L/ha herbicide treatment in Experiment 1 successfully suppressed all other plant species in the experiment, including lucerne. The twitch had reduced competition for resources needed for growth such as light and water, and was therefore able to grow back in patches larger than before the glyphosate was applied. Consequently, the 4 L/ha glyphosate rate actually increased the conditions for twitch to thrive in. In comparison, twitch was not affected by the 1 or 2 L/ha rates, with EWRS scores indicating mild symptoms. However, due to the increased growth of the vertically dominant plant species such as ryegrass and lucerne, the sunlight interception of the twitch was reduced towards the end of the experiment under the 1 L/ha and control treatment. As a result, it was observed visually that the twitch became overgrown in these plots. It is unknown if the twitch would grow back after grazing of the control and 1 L/ha plots.

Other plant species such as shepherds purse (*Capsella bursa-pastoris)* and bitter cress (*Cardamine hirsuta)* became abundant in the herbicide treatment plots after application. Shepherds purse can grow all year round, however is most abundant in winter due to reduced competition from other plant species. This was observed from Experiment 1, whereby the winter dormancy of lucerne and inactivity from the other weed species suppressed by the herbicide application, which increased the abundance of shepherds purse. Bitter cress does not germinate under competition pressure such as shading. This may be why it was not observed in the control treatments for Experiment 1. Several germinations of both these plant species can occur within a year. Therefore, the germination and opportunistic establishment of these weeds was made possible due to the decreased competition from other plant species and by not being susceptible to the timing effects of the glyphosate application. A different herbicide application may have been effective to control them.

For Experiment 1, the most effective herbicide treatment for Experiment 1 was the 2 L/ha rate. The visual representation of the plots on the final week of measurements (Plate 2) suggested that the control and 1 L/ha treatments appeared to have more canopy cover. However, the botanical analysis (Figure 4.8) indicated that for the greatest amount of lucerne cover and minimal weed presence, the 2 L/ha glyphosate target rate was the most suitable. This would be optimal for a cut and carry or lucerne silage proposition.

5.2 Experiment 2

The herbicide application date (June 6th) of Experiment 2 showed that the effect of the glyphosate was delayed, and was not detected by the GreenSeeker measurements until the 26th of June. Compared with Experiment 1, the time between the herbage removal (31st of May) and herbicide application (June 6th) was 6 days, which is consistent with the time frame previously mentioned by Monk et al., (2016) and Moot et al., (2003) of between 7-14 days. The average air temperature was 5°C for those 6 days, making the calculated thermal time for the 6 days 42°C. Due to the lag phase of 200°C, this meant that no herbage matter grew back between the time of herbage removal and the date of application. This reduced the phytotoxicity effect of the glyphosate on the lucerne. The maximum EWRS score of lucerne from Experiment 2 of 5.0 ± 0.5 supports this compared with the maximum EWRS score of lucerne in Experiment 1 of 7.8 ± 0.4. The botanical composition (Figure 4.18) also supports this, with lucerne yields of 860 kg DM/ha from 1 L/ha, 724 kg DM/ha from 2 L/ha and 822 kg DM/ha from 4 L/ha.

The delayed time of herbicide effectiveness was also due to the seasonal growth stage of the lucerne in the stand at the start of June. Moot et al., (2003) reported that shoot growth in autumn is reduced as the lucerne plant partitions it’s energy into restoring root reserves, in preparation for spring growth. Therefore, at the start of winter the rate of canopy expansion is reduced, as demonstrated from the GreenSeeker response of the control treatment in Experiment 2 (Figure 4.11). The later date of herbage removal for Experiment 2, combined with the herbicide application, removed the competition weeds that would otherwise reduce the amount of light interception for the lucerne. The first leaves produced, as lucerne regrows from herbage removal, are small and therefore the initial light interception and lucerne dry matter increases are decreased (Moot et al., 2003). By suppressing the weed competition, the light interception of the lucerne can reach critical levels earlier (Brown et al., 2003). Lucerne growth rates then become linear, related to temperature and water supply. The water supply for Experiment 2 totaled 231 mm, and combined with optimum sunlight, meant lucerne growth lost to phytotoxicity effects was minimal. In Experiment 2, all herbicide treatments achieved similar lucerne yields of approximately 801 kg DM/ha. This was less than the proportion of lucerne in the control treatment of 1077 kg DM/ha. This indicated that the lucerne did not take advantage of the spare space created by the herbicide treatments. This can be expected of an 8 year old stand. It would be assumed that a younger stand would take advantage of the increased space available, as observed from Moot et al., (2003) and Mills et al., (2008), resulting in increased canopy closure and increased total lucerne yield (kg DM/ha).

The ryegrass in Experiment 2 was successfully suppressed under all herbicide application rates with EWRS scores of between 5.0 and 8.0 ± 0.9, deeming the species commercially unacceptable for July and August. However, the EWRS scores of all herbicide treatments decreased back down to 1.0 or 2.0 ± 0.9 by September (Figure 4.13). Of note was the large least significant difference (lsd) observed from the EWRS score (Figure 4.13), indicating high variation amoung treatment means. Visual observation throughout the duration of Experiment 2 observed that the EWRS scores from September were from new ryegrass plants coming through, not from the failed elimination of the original plants at the beginning of the experiment. The botanical composition (Figure 4.18) supports this which showed decreased grass species yield from all herbicide treatments, with the most prominent from the 4 L/ha treatment with only 16 kg DM/ha of grass, the least of all the treatments. The grass yield from the control treatment for Experiment 2 was not as high (523 kg DM/ha) as observed in Experiment 1 (1335 kg DM/ha). This is likely due to the longer period of time for plant regrowth that Experiment 1 had post herbicide application, compared to Experiment 2. This trend is emphasised further in Experiment 3 (Figure 4.26), when the time for plant regrowth after herbicide application was even less between 24th of July and the 27th of September.

The glyphosate application of Experiment 2 was successful at suppressing the broadleaf weed species, such as dock and dandelion, however did not remove all of the plants or reach peak EWRS scores like other weed species. All herbicide treatments were not different from each other for both dandelion and dock. Dandelion had a peak EWRS score of 5.3 ± 0.9 on the 16th of August, and dock had a peak EWRS score of 7.3 ± 0.6 also on the 16th of August. The most effective treatment rate for the removal of the broadleaf weeds was 2 L/ha which yielded 54 kg DM/ha of broadleaf weeds. According to Abbas et al., (2017) docks require large amounts of glyphosate for the herbicide to successfully kill the weed. The best date to apply herbicide to eliminate docks is in spring, when the plant is actively growing (Abbas et al., 2017). Therefore, greater dock response and consequent herbicide effectiveness may have occurred if the glyphosate had been applied in spring.

As observed from Experiment 1, the twitch plant growth was highly variable amoung treatment plots. Twitch was suppressed more in Experiment 2 than in Experiment 1. All herbicide treatments had an effect on the twitch, with a mean EWRS score of 8.5 ± 0.07, which is commercially unacceptable and shows good control. The 1 and 4 L/ha treatment means returned to lower EWRS scores by the end of the experiment indicating mild plant phytotoxicity symptoms. However, the 2 L/ha EWRS treatment remained at 8.0 ± 0.07 till the end of the experiment. This variation in herbicide efficacy could be due to the timing of the glyphosate application for Experiment 2. More consistency from the herbicide performance may have occurred if applied when all plants are actively growing. Glyphosate application to control twitch may be less effective due to the plant dormancy throughout winter. However, if the glyphosate were applied in spring as recommended (Espeby et al., 2014), this would have phytotoxicity effects for the lucerne, and desired outcomes for the stand would not be achieved. To eliminate the twitch without causing phytotoxicity harm to the lucerne, a hard graze in early Spring (September) followed by glyphosate application should give sufficient results. The hard graze would remove any green leaf that the glyphosate could contact on the lucerne and then translocate to the roots, therefore reducing the phytotoxicity effect on the lucerne. The glyphosate application would target the twitch, by which time the lucerne would have re-established it’s canopy. Consequently, the future sunlight interception of the twitch would be eliminated, resulting in permanent removal of the twitch.

The white clover present in Experiment 2 was not effectively removed by the glyphosate. Similar to Experiment 1, the growth of the white clover was reduced by the 2 and 4 L/ha target application rates with an EWRS score of 5.8 ± 0.3 by mid-August. However, all treatment EWRS scores reduced down to 1.5 ± 0.3 by the end of the experiment indicating that the white cover was stunted but not entirely eliminated from the stand. The visual abundance of white clover in Experiment 2 appeared greater at the start of August, before the effects of the glyphosate were observed by the EWRS scores of the plant. When the white clover EWRS scores returned to healthy plant classification by September, the abundance of the plant was reduced. This observation could be due to the increased competition presented by the lucerne in spring, reducing the light available to the white clover. This would have slowed dry matter yield increases of the white clover in early spring, as the lucerne out-competes the white clover with vertical growth.

The most effective glyphosate application rate for Experiment 2 was 2 L/ha. Optimal weed suppression and lucerne growth were achieved from both 2 and 4 L/ha treatments, observed from the botanical composition analysis (Figure 4.18). The recommended application rate of glyphosate on lucerne is between 2 and 3 L a.i./ha (Agpro, 2019). Due to the variation of glyphosate products applied to each experiment, the amount of active ingredient applied with the 2 L/ha treatment in Experiment 2 was 1.4 L a.i./ha, and 2.3 L a.i./ha for the 4 L/ha treatments. Therefore, for optimum winter weed control in lucerne an application rate of between 2-3 L a.i./ha would provide sufficient results.

5.3 Experiment 3

Experiment 3 had the least amount of time between herbage removal and glyphosate application, which decreased the likelihood of phytotoxicity effects on the resident lucerne. The calculated thermal time for Experiment 3 between the herbage removal and spray date was 2.55°C, meaning no lucerne regrowth would have occurred between the removal of herbage and spray application. This was considerably lower than the accumulated dry matter regrowth of the previous two experiments as the time between topping and spraying was only a day. However, due to the application of herbicide in late winter, the lucerne stand began spring growth after application, as observed from the control treatment (Figure 4.19) Similar to Experiment 1, Experiment 3 observed accelerated herbicide effects due to the active growth of the lucerne. Evidence of this is observed from the botanical composition (Figure 4.26) whereby the yield of lucerne was significantly reduced with increasing active ingredient concentration. The later herbicide application date for Experiment 3 (24th July) increased the possibility of climatic influence on plant response. The initial mean GreenSeeker measurement of all treatments, including the control, decreased from 0.51 ± 0.03 on the 24th of July to 0.23 ± 0.03 by the 30th of July occurred. This was likely due to several frosts between the 23rd of July and the 8th of August, with minimum temperatures down to -2.72˚C. Moot et al., (2003) concluded that lucerne stands develop nodes through the winter period and should be spelled until spring to ensure nodes are not removed by grazing. It seems likely that the late July application date was too late to avoid damage to developing buds in Experiment 3. As the stand in Experiment 3 was not spelled, due to topping on the 23rd of July, this mimicked the effect of grazing. This caused delayed regrowth and reduced dry matter production of all plant species in all plots, as observed from figure (Figure 4.19). Consequently, the effects of this delayed growth were observed until the 27th of September (Plate 4), whereby Experiments 1 and 2 had a minimum regrowth of 812 kg DM/ha and 929 kg DM/ha from the 4 L/ha treatments, which would have been suitable dry matter production for grazing at the start of lambing. In contrast, minimum herbage regrowth from 4 L/ha of Experiment 3 was 152 kg DM/ha. The maximum herbage produced from Experiment 3 under 1 L/ha herbicide treatment (650 kg DM/ha) was still less than the minimum produced from Experiments 1 and 2.

The plant yield of the lucerne and all target weed species (ryegrass, dandelion, dock, twitch and white clover) was significantly reduced from all herbicide treatments in Experiment 3. The EWRS scores of all plants displayed similar response trends, whereby the severity of the plant phytotoxicity effects were most prominent by late August. At that time, the 4 L/ha treatments had the highest EWRS score of between 7-9.0 for all plant species, deeming all commercially unacceptable. By mid-September this phytotoxicity had reduced in the lucerne and ryegrass, where it plateaued at a mean EWRS score of 7.0 ± 0.6 for 4 L/ha. For the dandelion, dock, twitch and white clover the EWRS scores for the 4 L/ha treatment increased in September, where the treatment means plateaued again at a mean of 8.0 ± 0.6 for 4 L/ha. The EWRS treatment means of all plant species for 1 and 2 L/ha plateaued in September at 5.0 ± 0.6 for 2 L/ha, 2.0 ± 0.6 for 1 L/ha. Unlike Experiments 1 and 2, treatment means for the herbicide treatments did not decrease for the EWRS scores or increase for the GreenSeeker measurements, indicating that plant regrowth had not occurred by the end of the experiment. This could be due to the reduced amount of time for species recovery as observed in Experiments 1 and 2, as the duration of Experiment 3 was only 2 months. However, the more efficient response could also be due to the application date being closer to spring. As mentioned previously, spring is the best time to target twitch and docks as they are actively growing (Abbas et al., 2017). Therefore, the later application may have influenced the efficiency of the glyphosate. Further measurements taken later in spring could have confirmed this.

5.4 Conclusions

1. From Experiment 1 it was concluded that the rate of 2 L/ha of glyphosate was proven most effective at suppressing perennial weeds. However, lucerne suffered phytotoxicity effects due to the delay in herbicide application.

2. From Experiment 2 the most effective glyphosate application rate was 2 L/ha, whereby optimum balance was achieved of perennial weed suppression and minimised phytotoxicity effects to the lucerne.

3. From Experiment 3 all herbicide rates significantly reduced the total lucerne yield due to phytotoxicity damage. The late application delayed spring grow, making the application of glyphosate inadequate from this experiment.

6 GENERAL DISCUSSION AND CONCLUSIONS

Objective 1 of this research was to determine the optimum date and rate for the use of glyphosate in older lucerne stands. This was confirmed to be 2-3 L a.i./ha of glyphosate applied at the start of winter (early June). This was to account for the variation of glyphosate formulations used and consequent rates of active ingredient. Table 2 indicated the amount of active ingredient applied in the target rate of 2 L/ha was 1.4 L a.i./ha, and 2.3 L a.i./ha in the target rate of 4 L/ha. Optimal results were observed from the target glyphosate rates of 2 and 4 L/ha treatments, therefore from 1.4 and 2.3 L a.i./ha. At these rates, from Experiment 2 which the glyphosate was applied on the 6th of June, successful suppression of dandelion, ryegrass, twitch, dock and clover was observed. The suppressive effects of the glyphosate were only temporary for the twitch, dock and clover however, with mean EWRS scores returning to healthy plant classification. This was due to either new plant growth or the recovery of existing resident weeds, which contributed low dry matter yields to the botanical composition (Figure 4.18). Broadleaf weed content was reduced from approximately 27% to 6% under the 2 L/ha treatment and 10% under the 4 L/ha treatment. Grass weeds were reduced from 24% to 11% under the 2 L/ha treatment and <2% under 4 L/ha. The application date of June 6th, combined with the herbicide rate of 2 L a.i./ha, provided optimum weed suppression without severe phytotoxic effects to the lucerne and provided optimum date and rate for the use of glyphosate in older lucerne stands. Therefore Objective 1 was met.

Objective 2 of this research was to quantify the phytotoxicity effect of glyphosate on lucerne. Lucerne yield at the time of herbicide application and the timing of herbicide application influenced the phytotoxic effect of glyphosate on lucerne. Experiment 1 had an extended period of time between herbage removal and herbicide application which allowed the regrowth of approximately 3 new leaves. Consequently, Experiment 1 had the highest EWRS score for lucerne of all the experiments, of 7.8 ± 0.39, which reduced total lucerne yield (Figure 4.8). Therefore, no lucerne regrowth should be allowed before herbicide application. The timing of herbicide application, in relation to the seasonal growth of lucerne, observed least phytotoxic effect when applied in winter when the lucerne was dormant. If the herbicide were applied to actively growing lucerne, this would increase the translocation of the chemical, increasing it’s efficiency. Objective 2 was met by concluding that the phytotoxicity effects on lucerne can be reduced through removal of herbage immediately before spraying and by applying the herbicide in winter when the lucerne is dormant.

The third objective was to quantify the effect of glyphosate on the target weed species. All experiments observed successful perennial weed suppression of dandelion, dock, twitch, clover, and ryegrass under the 2 and 4 L/ha target application rates. Ryegrass was successfully suppressed to EWRS scores of 8-9 in all three experiments under the 2 and 4 L/ha glyphosate application rates, and a mean EWRS score of 4 in Experiment 2 and 3. Dandelion was suppressed in all experiments, with a mean EWRS score of 4 for 2 and 4 L/ha treatments. The suppression of dock, twitch and white clover was temporary however. The efficacy of the glyphosate on the target perennial weed species in this experiment was influenced by the timing of herbicide application in relation to the individual plant species seasonal growth stage. Removal of dock and twitch could be achieved with a glyphosate application in spring, however glyphosate is proven ineffective at removing white clover.

Based on meeting the Objectives 1, 2 and 3, to meet the aim of this research the potential to utilise glyphosate was evaluated, which involved financial justification and practical analysis of the herbicide application from each experiment. Financially, glyphosate compares cheaper than paraquat and atrazine formulations. Depending on the target weed species and the glyphosate formulation, recommended application rates differ.

Table 4.0 Cost price analysis of existing lucerne winter weed control methods in New Zealand. All prices were sourced from AgPro (2019).

Product	Recommended Application Rate (AgPro)	Market Cost/Litre ($)	Dilution (water)	Total Cost ($)/ha
Synergy Glyphosate 360	2.0 L/ha	5.73	200 L	11.50
Paraquat	2.0 L/ha	9.40	250 L	18.80
Atrazine	2.0 L/ha	7.74	250 L	15.50
Paraquat + Atrazine	1.5L/ha (Atrazine) + 2.0 L/ha (Paraquat)	17.74	250 L	39.80

The most expensive form of winter weed control in lucerne is paraquat and atrazine combined, which totals $39.80 per hectare. The price of glyphosate per hectare was only 28% of the price of paraquat and atrazine, at $11.50 per hectare. Therefore, the use of glyphosate for winter weed control in lucerne is a more economically viable solution.

The practical analysis of the use of glyphosate was evaluated for all three experiments. Experiment 1 observed phytotoxicity effects to the lucerne plant that decreased the total lucerne yield (kg DM/ha). The high glyphosate application rates (2 and 4 L/ha) suppressed the abundance of ryegrass, dock, dandelion and twitch, and young growth of these plants post herbicide application contributed minimal dry matter yield. Due to the age of the stand, the suppression of these weeds increased the proportion of bare ground, accelerating the abundance of spring annuals such as shepherds purse and bitter cress in late September. Review of Experiment 1 questions the suitability of applying glyphosate, if it had to be applied at the end of April. Depending on the intended use of an individual lucerne stand, the ryegrass component of the botanical composition analysis (Figure 4.8) could be utilised to a farmer’s advantage. If an aged lucerne stand is intended to be sold for silage or baleage, then the purity of the stand is vital and all weed species must be eliminated. In the case of Experiment 1, the 2 L/ha target rate would be the most satisfactory. However, if the aged stand was to be used for grazing purposes, the control treatment was justifiably more suitable than applying any glyphosate treatment. Under the control treatment 1335 kg DM/ha of grass and 747 kg DM/ha of lucerne was produced (Figure 4.8). Excluding the broadleaf weed proportion of the stands yield, as livestock consumption is unlikely, this provides 2,082 kg DM/ha of feed suitable for grazing. In comparison, the 2 L/ha treatment (Figure 4.8) had a grass yield of 40 kg DM/ha and the lucerne provided 676 kg DM/ha, giving a total 716 kg DM/ha of stock feed. Lucerne yield was reduced in the 2 L/ha treatment, compared with the control, due to phytotoxicity damage. The control provided 1,366 kg DM/ha more stock feed than the 2 L/ha treatment, composed of 1,295 kg DM/ha of grass and 31 kg DM/ha of lucerne. Moot et al., (2016) quantified lamb live weight gains on lucerne monocultures in spring as 334 g/lamb/day. Grace et al., (2018) quantified the lamb liveweight gains of ryegrass alone to be 222g/day. Therefore, while a farmer would favour the proportion of lucerne over ryegrass for greater liveweight gains, this proves satisfactory liveweight gains can be achieved. As well as this, a farmer would remove the cost of the herbicide and consequently the cost to apply it.

If a farmer were to leave the aged stand as the control treatment, the proportion of existing broadleaf weed species (555 kg DM/ha) would likely increase by the following season as the lucerne stand thins out due to age and grazing selection pressure (Box, 2014). The ryegrass yield would also likely increase further. In the case of Experiment 1, if a farmer did choose to leave the stand as the control treatment indicated, the following winter the stand would probably have to be reevaluated. Options for the stand include applying glyphosate to remove the competition from weeds, then direct drilling lucerne back into the existing stand to try replenish the lucerne. Problems with autotoxicity from allelopathy may be encountered however, whereby the resident stand emits a toxin that restricts the germination and growth of the new lucerne stand. The older the lucerne stand the greater the concentration of toxin, and therefore in this example the chance of germination inhibition is high. Another option would be to start again with a new lucerne stand. This would guarantee a greater strike rate than the first option.

Experiment 2 was the closest of all three experiments to the recommended herbicide application rate and date specified by Moot et al., (2003). In winter, herbicide application timing is key to ensuring sufficient lucerne yields match livestock feed demands in spring. This was achieved by ensuring crop regrowth was as early and vigorous as possible (Moot et al., 2003). Experiment 2 observed the least amount of phytotoxic damage to the lucerne stand, while also reducing the weed population. The balance of both objectives for winter weed control is essential to ensuring sufficient lucerne growth for subsequent years. Given the age of the lucerne stand where the Experiments took place, the consideration of subsequent years is unlikely due to the declining quality of the stand. Due to the increased proportion of bare ground exposed from the application of glyphosate, spring annuals invaded the stand in early September. To target weed species such as these, atrazine could be applied in conjunction with the glyphosate. This could eliminate the spring competition that threatens the total dry matter yield of the lucerne. Roux et al., 2014 concluded atrazine and glyphosate combined was successful as a winter herbicide, with reduced weed content from >40% in the unsprayed plots to <1% in the sprayed plots. It was reported by Roux et al., (2014) that lucerne yields increased due to decreased weed content. Similar results were also achieved by Moot et al., (2003) and Mills et al., (2008) who applied mixtures of paraquat and atrazine. The lucerne stands in these sources were 3 and 5 years old respectively. Although lucerne yield increases were not observed from any of these three experiments, this could be due to the age of the stand. Potential increases in lucerne yield could be achieved from glyphosate and atrazine if applied to a younger stand.

Experiment 3 was applied too late in winter that herbage regrowth was not achieved before optimal grazing time in spring. Glyphosate delayed lucerne bud regrowth, meaning lucerne regrowth was not optimal by the start of spring, observed from Plate 4. For a Canterbury lucerne crop, peak feed demand must match ewe and lamb demand in early spring, when lambs are at foot in early September. Given the more prominent effects of global warming, and the increasing weather variability, this is now becoming earlier (SOURCE). If a farmer was left with a lucerne stand in late July that had been invaded with perennial weeds such as Experiment 3, the application of glyphosate may not be justifiable. While the glyphosate was successful at reducing the weed content under all herbicide treatments, the justification of application is questioned by the consequential reduction in lucerne yield also. In the case of Experiment 3 (Figure 4.26), all herbicide treatments reduced the total lucerne yield, with the 4 L/ha treatment producing a lucerne yield of 77 kg/DM/ha. Combined with the late lucerne regrowth in early September, Experiment 3 does not provide sufficient results to validate the application of glyphosate.

6.1 Conclusions

1. The spray date and rate for the most desirable winter weed control in an aged lucerne stand is at the beginning of winter (start of June), between 2-3 L a.i./ha.

2. Phytotoxicity effects of the glyphosate on lucerne can be reduced by an initial hard graze before herbicide application in early winter to reduce the amount of green leaf exposed to the effects of the herbicide while the plant is dormant.

3. Successful suppression of perennial weed species such as dandelion and ryegrass is achievable in an aged lucerne stand with higher application rates of glyphosate (2 and 4 L a.i./ha). Suppression of other weeds including docks, twitch and clover was achieved, although only temporarily.

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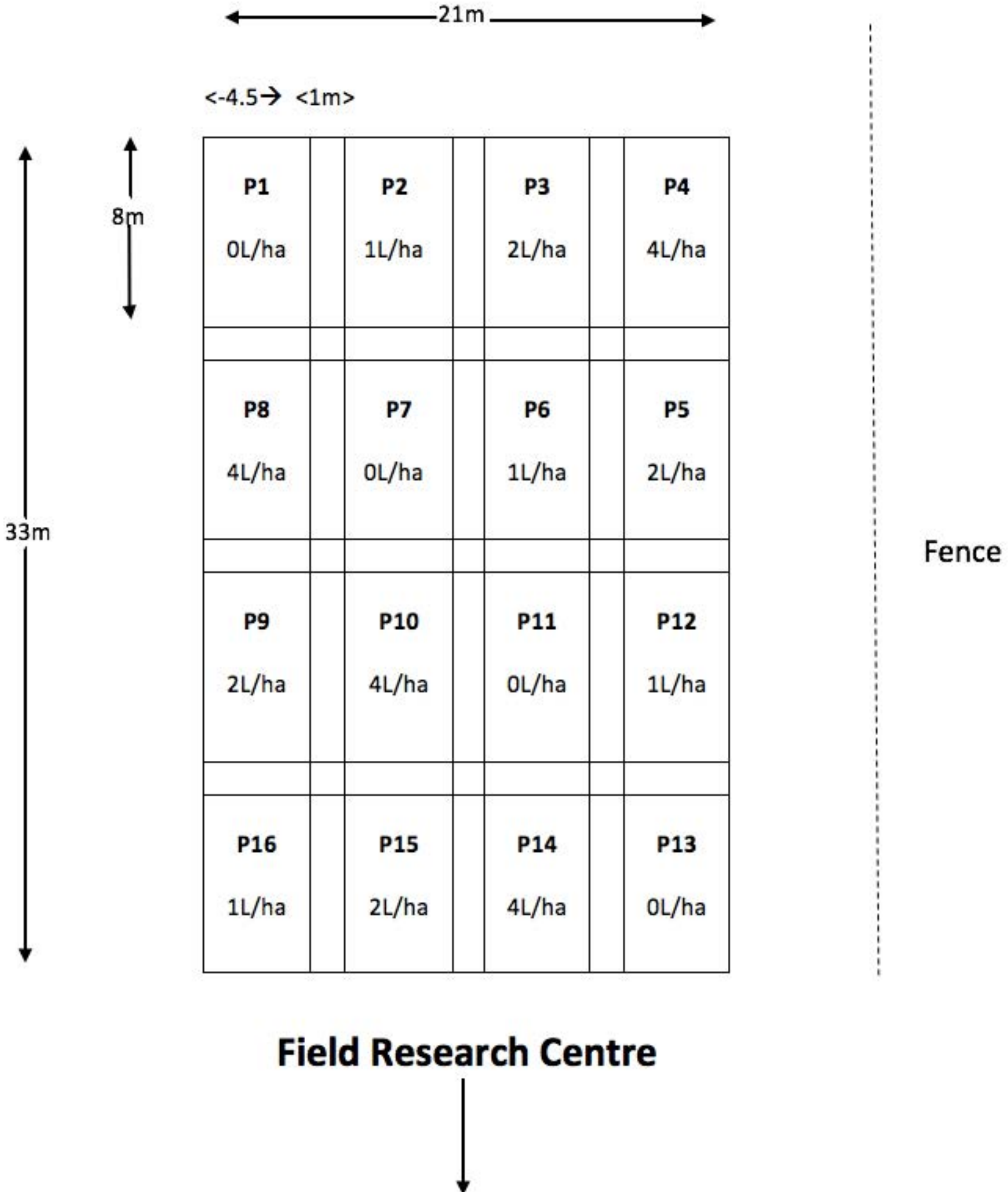
APPENDICES



Appendix 1 Experiment design of all three experiments.

Appendix 2 Drone picture taken on the 21st of August 2019 of Experiment 1, 2 and 3 (right to left).

Appendix 3 Drone picture taken on the 17th of October, 2019 of Experiment 1, 2 and 3 (in order from right to left).

Appendix 4 Picture of Experiment 1, facing East towards the Field Research Centre, indicating the shading effect the trees along the fence line had on the experiment plots. Picture was taken the 6th of May, 2019.