Aspects of the biology, taxonomy and control of *Calystegia silvatica*

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Abstract

Calystegia silvatica or great bindweed has recently become a problematic weed in riparian zones but the information available about the control of *C. silvatica* with herbicides is limited. The current study was undertaken to gain more information about aspects of the control of *C. silvatica* with herbicides and of the biology and taxonomy of *C. silvatica*.

In Experiment 1 a range of translocated herbicides were applied to established plants in the field. Herbicides were applied in autumn and it was found that none of the herbicides applied, at the rates they were applied at, controlled 100% of the *C. silvatica*. However triclopyr/picloram/aminopyralid and 2,4-D/dicamba showed significant control of *C. silvatica* spring regrowth. Aminopyralid alone and glyphosate also showed some long term control. Both metsulfuron and clopyralid showed poor control.

The same herbicides applied in the field were also applied in autumn to young *C. silvatica* grown in pots in a glasshouse. Fluroxypyr and 2,4-D (ester) were also tested. Herbicides were applied to either the upper portion or the lower portion of the plants to determine whether it matters if only part of the plant is treated when trying to avoid spraying nearby native plants in the field. As with the field trial, no plants were totally controlled. However triclopyr/picloram/aminopyralid, aminopyralid and 2,4-D/dicamba showed good control of *C. silvatica*. Clopyralid and fluroxypyr showed poor control. The effect of fluroxypyr on *C. silvatica* was previously unknown. For most of the herbicides there was no difference in the level of control between those herbicides applied to the upper portion compared with the lower portion. Control options for *C. silvatica* are discussed.

Seeds of *C. silvatica* were found to be quite large, potentially making them vulnerable to predation. There has also been uncertainty over the amount of seeds that *C. silvatica* can produce and it was found that *C. silvatica* has, on average, one viable seed per pod. Few seeds were found in the upper soil layer beneath dense *C. silvatica* stands but all were viable. From the germination studies it was gleaned that *C. silvatica* seeds need scarification and can germinate in temperatures from 5 – 25^{o}C with few seeds emerging at 5^{o}C and rapid germination when the seeds were kept at 20 and 25^{o}C.

The plants used in the trials were identified as *Calystegia silvatica* subspecies *disjuncta* and it was found that *Calystegia silvatica* subspecies *disjuncta* is the most common form growing in the local area. A key for identifying the *Calystegia* species which could

potentially be in New Zealand was developed for this exercise, based on New Zealand and international references, and was also tested during this sampling.

KEYWORDS: *Calystegia silvatica*; great bindweed; aminopyralid; 2,4-D/dicamba; triclopyr/picloram/aminopyralid; metsulfuron; 2,4-D (ester); clopyralid; fluroxypyr; seeds; taxonomy; New Zealand.

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CHAPTER 1: General introduction

1.1 *Calystegia silvatica* and related species

1.1.1 Introduction

Calystegia silvatica is a climbing, perennial weed which is common in New Zealand but has in the past not been a weed of great significance. Recently *C. silvatica* has received more attention because it is now becoming a significant weed in riparian plantings. However because it has not historically been a significant weed, little has been studied about its biology and control. This review intends to look at the research available on its taxonomy, biology and any control methods. It will cover the taxonomy of *Calystegia silvatica* and other *Calystegia* species in New Zealand, the biology of *Calystegia silvatica* followed by brief descriptions of the biology of the closely related *Calystegia sepium* and *Convolvulus arvensis* and previous findings on control methods for these three species.

1.1.2 Taxonomy

Calystegia silvatica is known by many names including *Calystegia silvaticum*, *Calystegia silvatica*, *Calystegia sylvaticus*, great bindweed, greater bindweed or *Convolvulus* (Wilson-Davey et al. 2009). It is part of the *Calystegia* genus and a member of the *Convolvulaceae* family and is related to both the *Convolvulus* genus which includes field bindweed (*Convolvulus arvensis*) and the *Ipomoea* genus which includes kumara or sweet potato (*Ipomoea batatas*) (Sykes et al. 1988; Wilson-Davey et al. 2009). Although *Calystegia* is closely related to *Convolvulus*, the exact nature of this relationship is unknown (Stefanović et al. 2002; Wilson-Davey et al. 2009). The main differences between *Convolvulus* and *Calystegia* are differences in their ovaries, pollen grains, bracteoles, stigma lobes and corollas (Brummitt 1963; Brown et al. 2009).

The taxonomy and identification of *C. silvatica* has, in the past, been confused, overlooked or there have been differences of opinion. In some of the past studies *C. silvatica* has been ignored and not separated from *Calystegia sepium* (Brummitt 1963), or *C. silvatica* has been treated as a subspecies of *C. sepium* (Ogden 1978; Williams 2009). The situation with the identification of the *Calystegia* species is further confused by the fact that they interbreed and hybridise (Brummitt 1963; Ogden 1978; Sykes et al. 1988). Within New Zealand *C. silvatica* and *C. sepium* are regarded as two separate species (Williams 2009). However *C. silvatica* has in the past been called *Convolvulus arvensis* in New Zealand (Howell 2008) and is commonly known as just *Convolvulus*. An early New Zealand weed control book calls *C. sepium* greater bindweed (Matthews 1975). There has also been some confusion over the origin of

Calystegia sepium (Ogden 1978) and confusion remains as to whether certain subspecies are present in New Zealand. As it stands now there are four native *Calystegia* species recognised within New Zealand, (*Calystegia tuguriorum*, *Calystegia soldanella*, *Calystegia marginata* and *Calystegia sepium* subsp. *roseata*) and there is at least once introduced species, *C*. *silvatica*, both subspecies *disjuncta* and *silvatica*. *C*. *sepium* subsp. *sepium* also may be in New Zealand (Ogden 1978; Sykes et al. 1988; Wilson-Davey et al. 2009; Popay et al. 2010). *C*. *sepium* subspecies *americana* and *spectabilis* don't appear to be in New Zealand (Ogden 1978). Brief information for the five species will now be given and can be seen in the key developed as part of the thesis research (Figure 1.1). Further identification aids can be found in Brummitt (1963).

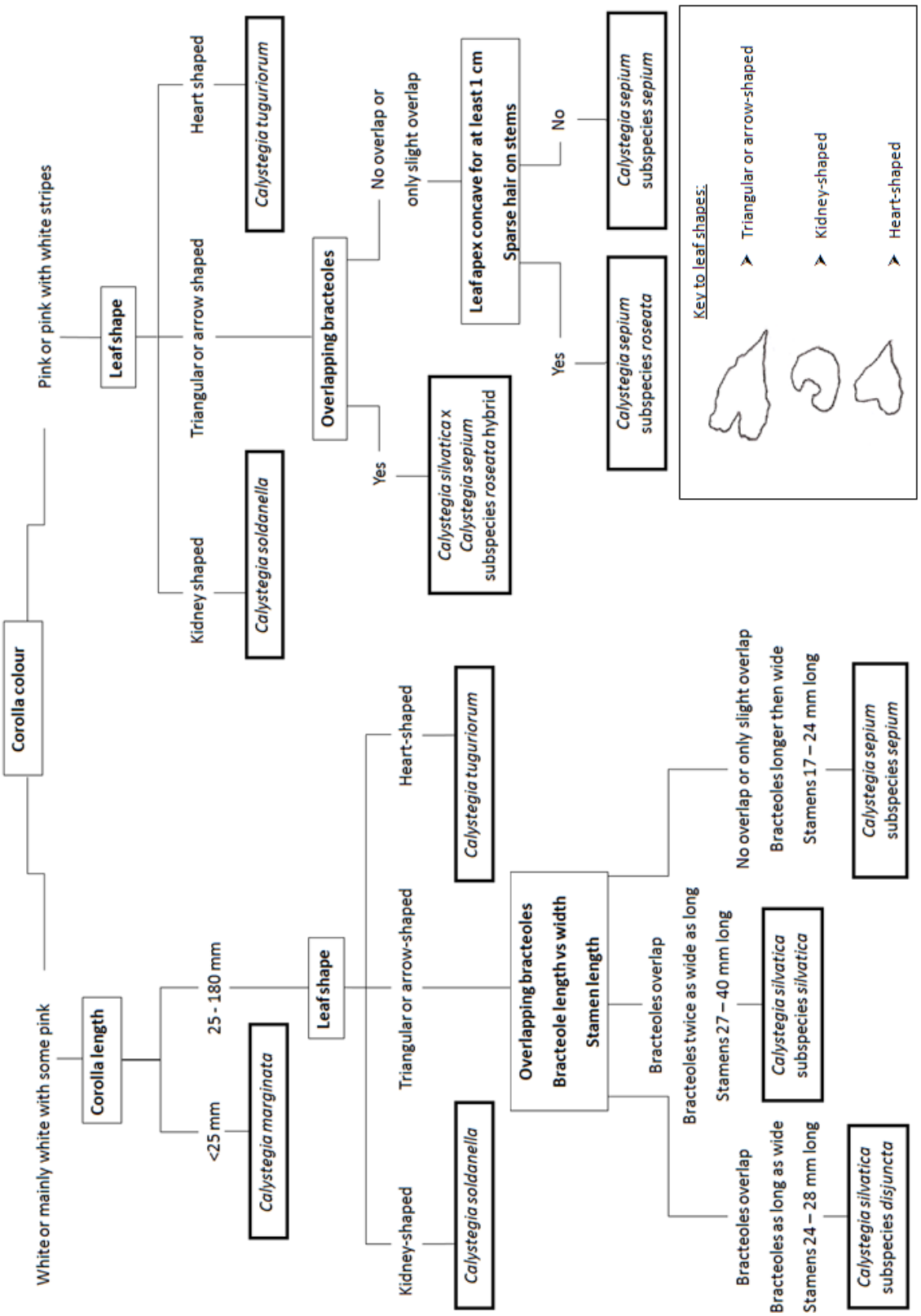

Figure 1.1: Taxonomic key for New Zealand *Calystegia* species. Information from Brummitt (1963), Ogden (1978), Sykes et al (1998), Brown et al (2009) and Popay et al (2010).

1.1.2.1 *Calystegia silvatica*

Both *C. silvatica* subspecies have large white corollas, over 50 mm in length, triangular or arrow-shaped leaves and overlapping bracteoles. These are the main differences which separate *C. silvatica* from *Calystegia soldanella*, *Calystegia tuguriorum* and *Calystegia marginata* (Brummitt 1963; Ogden 1978; Sykes et al. 1988; Popay et al. 2010). Subspecies *silvatica* has stamens between 27 mm and 40 mm long and bracteoles twice as wide as they are long. Subspecies *disjuncta* has stamens between 24 mm and 28 mm long and bracteoles as long as they are wide (Brummitt 1963; Brown et al. 2009).

1.1.2.2 *Calystegia sepium*

C. sepium subsp. *roseata* is the native *Calystegia sepium* in New Zealand and has the Maori name “pohue” (Ogden 1978). It is identified by having pink or pink with white striped flowers, non overlapping bracteoles, and triangular or arrow-shaped leaves (Brummitt 1963; Ogden 1978; Sykes et al. 1988; Popay et al. 2010). New Zealand native *C. sepium* does not exactly match *C. sepium* subsp. *roseata* from overseas, but it is identified as that here, and considered to be a native (Ogden 1978). *C. sepium* subsp. *sepium* also appears to be present in New Zealand (Ogden 1978). It has white flowers or pink flowers with white stripes, stamens 17 – 24 mm long and bracteoles that either don’t overlap or only slight overlap (Brummitt 1963; Brown et al. 2009). These features separate it from *C. silvatica*. Triangular or arrow-shaped leaves separate it from *Calystegia tuguriorum*. One way to separate out the two pink coloured *C. sepium* is to look at the leaf apexes. C. *sepium* subsp. *roseata* has attenuate or concave leaf apexes whereas *C. sepium* subsp. *sepium* does not (Brummitt 1963).

1.1.2.3 *Calystegia tuguriorum*

Calystegia tuguriorum, a New Zealand native, has either white or pink flowers. It has a 27 – 50 mm long corolla and heart shaped leaves less than 40 mm long (Brummitt 1963; Ogden 1978; Popay et al. 2010).

1.1.2.4 *Calystegia soldanella*

Calystegia soldanella, another New Zealand native, has white flowers or pink flowers with white stripes and is separated by its kidney shaped leaves. It is commonly found in sandy and coastal habitats (Brummitt 1963; Ogden 1978; Brown et al. 2009; Popay et al. 2010).

1.1.2.5 *Calystegia marginata*

Calystegia marginata, a native, has small white flowers less than 25 mm long (Ogden 1978; Sykes et al. 1988).

1.1.2.6 Hybrids

There are some specimens which have been found in earlier collections in New Zealand (Ogden 1978) and overseas (Brummitt 1963; Brown et al. 2009), which suggest hybridisation amongst the *Calystegia* genus. Plants with pink flowers with white stripes and bracteoles which overlap could be the result of hybridisation between *C. silvatica* and *C. sepium* subsp. *roseata* (Ogden 1978).

1.1.3 Biology and ecology of the common species

Calystegia silvatica, *Calystegia sepium* and *Convolvulus arvensis* are similar in growth form, lifecycle and habitat, as seen in Table 1.1. Any control strategies for one of these species may be relevant to the other species.

Table 1.1 Summary of the characteristics of *C. silvatica, C. sepium* and *C. arvensis* (Weaver & Riley 1982; Sykes et al. 1988; Parsons & Cuthbertson 2001; Williams 2009; Popay et al. 2010).

.**Weed**	***Calystegia silvatica***	***Calystegia sepium***	***Convolvulus arvensis***
Lifecycle	Perennial. Dies back in winter	Perennial. Dies back in winter	Perennial. Dies back in autumn
Flowers	White	Pink or white	Pink or white
Flowering	October - May	September - February	November - March
Seeds	Rarely	Moderate.	Variable
Seed dormancy	Unknown	10 years +	20 years +
Underground organs	Extensive rhizomes	Extensive rhizomes	Extensive rhizomes
Growth form	Sprawling and climbing, up to 3 m	Sprawling or climbing, up to 3 m	Creeping or climbing, up to 2 m
Location	Common throughout New Zealand	Common throughout New Zealand	Present in some places throughout New Zealand.
Habitat	Hedges, gardens, riversides, forest margins, pastures and waste areas	Roadsides, gardens, waste places, banks and swamps	Crops, arable land, waste places, gardens, gutters and paths

The biology and lifecycle of *C. silvatica* will now be described.

1.1.3.1 Lifecycle

C. silvatica is a perennial climbing herb. It flowers from October to May, from spring to autumn. The aerial parts of *C. silvatica* then die off in winter (Table 1.1) as the plant becomes dormant, before returning in spring (Sykes et al. 1988; Williams 2009; Popay et al. 2010). It is also known that *C. sepium* has rhizomes and root buds which are dormant during winter, before sprouting over the spring period at variable times (Willeke et al. 2012).

1.1.3.2 Flowers

C. silvatica has white, trumpet-shaped flowers (Table 1.1). They can be slightly tinged pink, are unscented and 50 – 80 mm long and 55 – 80 mm in diameter (Sykes et al. 1988; Popay et al. 2010).

1.1.3.3 Seeds

The triangular – ovoid seeds of *C. silvatica* are smooth, black and 4 – 5 mm in diameter. They are produced in round capsules with four compartments (Sykes et al. 1988; Popay et al. 2010). It is said that *C. silvatica* seeds are rarely produced (Table 1.1) (Popay et al. 2010) and are not specialised to aid their distribution. Seed dispersal is by gravity (Williams 2009).

1.1.3.4 Leaves and stems

C. silvatica has large green, arrow shaped leaves 50 – 180 mm wide and 35 – 150 mm long. These are attached by short stalks to hairless stems. These stems twine around and can be purple tinged if exposed to sunlight (Sykes et al. 1988; Popay et al. 2010). *C. sepium* is also said to have aerial shoots which can grow along the surface before growing into the soil to form new, isolated plants (Davison 1976). The shoots of *C. silvatica* may also do this.

1.1.3.5 Underground organs

The underground organs of *C. silvatica* are what make this weed difficult to control. It has rhizomes which can form large and extensive systems underground (Table 1.1) and can spread for several metres (Sykes et al. 1988; Popay et al. 2010).

1.1.3.6 Location and habitat

C. silvatica can be found throughout New Zealand and is very common. It grows in hedges, forest margins, gardens, riversides, pastures and waste areas (Sykes et al. 1988; Popay et al. 2010).

1.1.4 Riparian zones and the problems *Calystegia silvatica* causes

Recently farmers and regional councils have invested significant amounts of resources, money and time into riparian zones and plantings. Riparian zones are the areas between waterways and farmland and they are often planted with native trees and shrubs. These riparian zones and plantings can help to improve and maintain river quality by preventing erosion and by reducing nutrient flow into the water, among other things (Williams 2009; Wilson-Davey et al. 2009). The Clean Streams Accord aims to protect and improve river and stream water quality and riparian zones play an important part role with this (Ministry for the Environment 2003; Wilson-Davey et al. 2009). *C. silvatica* has been found growing in these riparian zones, where it often adversely affects the native plantings by growing over the plants, reducing their light capture and thus competing with them. The *Calystegia* plants could also harm the establishment and survival of new plantings (Wilson-Davey et al. 2009). The plants may also restrict access to the area and spread into pastures, although experience indicates that cows will eat it. Farmers often spend significant amounts of money and time on establishing these native plantings and therefore any threat to their survival is unacceptable and control is warranted. The *C. silvatica* can reinvade the area every season because of the survival capacity in its rhizomes. *C. silvatica* can also affect maize (Rahman et al. 2002) and asparagus (Rahman & Sanders 1992). Home gardeners commonly refer to *Calystegia silvatica* as "*Convolvulus*" and they have recently voted it as of the top five worst weeds in a 2012 survey conducted by the NZ Plant Conservation Network (Tapaleao 2013). *C. sepium* has also been found to affect fruit crops (Davison 1976).

1.2 Non herbicide control of *Calystegia silvatica*

Any control in riparian zones needs to be safe for the waterways, safe for the native trees which are planted and easy to implement in an often sloped area which may also be steep. Non herbicide control options for *C. silvatica* could include mowing, grazing, cultivation, flooding, hand removal, light deprivation or plastic covers, or biological control. Many of these techniques have been used in an effort to control *C. sepium* and *C. arvensis* (Parsons 1973; Selleck 1978, 1979b, a; Weaver & Riley 1982; Wang & Kok 1985; Pfirter et al. 1997; Tipping & Campobasso 1997; Guntli et al. 1999; Defago et al. 2001; Parsons & Cuthbertson 2001; Hodges 2003; Wilson-Davey et al. 2009).

However most of these techniques are impractical in riparian zones, too time consuming or may not be very successful in controlling established dense stands of *C. silvatica*. Also none of the mycoherbicides developed overseas are present and to introduce them would be expensive, and the insects studied on *C. arvensis* and *C. sepium* have not been studied on *C. silvatica* and to conduct such studies in New Zealand would be costly (Wilson-Davey et al. 2009). Because these techniques are impractical or less likely to be successful on dense established stands of *C. silvatica*, herbicide control needs to be considered.

1.3 Herbicide control

1.3.1 Timing of herbicide control

Before herbicide options are discussed it is important to note that the timing of the herbicide application is important, particularly in the case of *C. silvatica* which can have extensive underground rhizomes which need to be killed in order for control to be successful. Willeke et al (2012) found that in Germany there was a seasonal source and sink relationship and variability in rhizome and root bud sprouting in both *C. sepium* and *C. arvensis*. They found that *C. sepium* begins to sprout new shoots from rhizomes and root buds in spring and maximum shoot sprout occurred in late spring. During this sprouting period they found that the carbon and starch in the underground organs decreased and the level of starch reached its lowest point soon after maximum sprout in early summer. Following this point the levels of carbon, sugars and starch increased. In autumn there was a decrease in bud sprouting before they became dormant. Willeke et al (2012) concluded from these results that during the spring to early summer period the plant is transporting sugars from the underground organs to the stems before the compensation point is reached in early summer. After this point the plant is transporting sugars and other compounds from the stems to the underground organs. They suggested that the best time to apply herbicides would be soon after this compensation point. Applying herbicides later than this point, such as an autumn application, would ensure that the herbicides are transported to the underground organs.

It is also recommended by Weaver and Riley (1982) that herbicides should be applied to *C. arvensis* when maximum translocation is occurring, from flower budding to the full bloom stage. This could also be the time when herbicides should be applied to *C. silvatica*.

Separate trials (Davison & Bailey 1974; Banks et al. 1979; Wiese & Lavake 1986; Schoenhals et al. 1990) which looked at timing have found that the best time to apply herbicide to *C. arvensis* varies amongst the different herbicides. Glyphosate is best

applied at bloom instead of prebloom (Banks et al. 1979) or in spring. Spring is also considered the best time to apply 2, 4- D esters, (Wiese & Lavake 1986), early summer for 2,4-D amines and for dicamba autumn applications are considered best (Wiese & Lavake 1986; Schoenhals et al. 1990). For fluroxypyr late flowering herbicide applications are considered best (Macdonald et al. 1993).

1.3.2 Plant vigour

Not only is the timing of herbicide application important, but studies (Wiese & Lavake 1986; Schoenhals et al. 1990; Westra et al. 1992; Macdonald et al. 1993) have found that the vigour of *C*. *arvensis* is also important. If *C*. *arvensis* is in poor condition then the herbicide efficacy is reduced. Schoenhals et al (1990) found that as vigour increased from poor to fair, good and excellent the amount of control increased, on average, from 10 – 20%. This work shows that the vigour of *C*. *silvatica* may influence herbicide efficacy.

1.3.3 Herbicide control options

Because *C*. *silvatica* has extensive rhizomes it is desirable that any herbicide used kills both the aboveground and below ground parts, as without this *C*. *silvatica* will just regrow from the surviving root and rhizome buds and the problem would remain. Therefore it is mainly translocated herbicides, herbicides which can move throughout a plant (Rao 2000), that have shown the most effect on *C*. *silvatica* and related species in past work.

Because in the past *C*. *silvatica* has not been a weed of great significance, the control of *C*. *silvatica* with herbicides has not been studied to any great extent. One study that has been done on *C*. *silvatica* is that by Rahman and Sanders (1992). They trialled the herbicides chlorsulfuron, amitrole, clopyralid, dicamba, glyphosate, imazapyr and triclopyr on *C*. *silvatica* and *C*. *arvensis* in asparagus crops in the Waikato region of New Zealand over the growing season. The herbicides were applied in October and November and they found that all of the herbicides killed the aboveground parts of *C*. *silvatica* but regrowth occurred, with the exception of imazapyr where control continued until the next season. The control of *C*. *arvensis* showed similar results to that of *C*. *silvatica*. One possible reason why the level of control was poor, is because of the timing of the herbicide applications. There may not have been full translocation of the herbicides to the roots as the best time for maximum transport is likely to be from flower bud to flowering, similar to *C*. *arvensis* (Weaver & Riley 1982) and the herbicides were applied very early in the flowering period of *C*. *silvatica* which is from October to May (Popay et al. 2010).

One trial used 2,4,5-T on *C. silvatica* and bindweed was said to have been completely controlled (Anon, 1958). However 2,4,5-T is no longer available in New Zealand (Wilson-Davey et al. 2009), although the results are directly relevant to the control triclopyr may show, as they are similar herbicides. In an unpublished and unreplicated trial conducted in Taranaki by a spraying contractor, metsulfuron, clopyralid, aminopyralid, triclopyr and triclopyr/picloram all controlled the growth initially but regrowth occurred. The herbicides were applied during the summer period or flowering stage but the trial did not monitor the source of the regrowth or formally assess the herbicide control (Hornby 2011).

The work mentioned above appears to be the only trials on herbicide use on *C. silvatica*. Some organisations have made recommendations though. Dicamba (Upritchard 2007), 2,4-D/dicamba (McCoombs 2003; Greater Wellington Regional Council 2010; Waikato Regional Council 2012), metsulfuron (Waikato Regional Council 2012), and triclopyr/picloram (Waikato Regional Council 2012) are all herbicides recommended for use on *C. silvatica* in New Zealand. In Matthews' (1975) book, where the taxonomy is confused between *C. sepium* and *C. silvatica*, 2,3,6-TBA, dicamba and picloram/2,4-D are recommended. In the Novachem Agrichemical Manual, 2,4-D ester, 2,4-D/dicamba, dicamba, imazapyr and picloram gel are all recommended for use on *C. silvatica* or bindweeds (Young 2012), and a leaflet suggests that both 2,4-D amine and 2,4-D ester may provide control of *C. silvatica* if it has new growth (MAFF 1957).

Compared to *C. silvatica*, there have been significantly more trials on *C. sepium* as it is more widespread overseas (Wilson-Davey et al. 2009). Also, because of the taxonomic problems in the past, some of the studies on *C. sepium* could in fact have been on *C. silvatica*, especially as many fail to mention the sub species being trialled and *C. silvatica* has been considered a subspecies of *C. sepium* by some. Also, *C. sepium* and *C. silvatica* should both have similar chemical susceptibility. Herbicides which have been used on *C. sepium* include many of those listed above as well as imazethapyr (Rahman et al. 2002), MCPB (Davison 1970; Davison 1976) and nicosulfuron (Rahman et al. 2002).

It is also worthwhile looking at the susceptibility to herbicides of other closely related weeds. *C. arvensis* is closely related to *C. silvatica*, as mentioned earlier and is also a widely studied weed as it is considered to be one of the world's most important weeds (Parsons & Cuthbertson 2001). Many of the herbicides mentioned previously as having being used on *C. silvatica* and *C. sepium* have also been used on *C. arvensis*. Other herbicides which have been trialled on *C. arvensis* are MCPA, fluroxypyr, mecoprop, dichlorprop, fosamine, linuron and quinclorac (Davison & Bailey 1974; Davison 1976; Wiese & Lavake 1986; Macdonald et al. 1993; Parsons & Cuthbertson 2001).

All of the herbicides mentioned will now be given further attention and their potential use on *C. silvatica* will be examined.

1.3.4 2,4-D and other phenoxys

One of the herbicides which has been trialled and is recommended for use on *C. silvatica* is 2,4-D. The use of related phenoxys MCPA, MCPB, mecoprop and dichlorprop on *C. silvatica* wasn't found in the literature but their use on *C. sepium* and *C. arvensis* will be looked at.

1.3.4.1 2,4-D amine

In one trial by Banks et al (1979), 2,4-D amine was applied to *C. arvensis* in winter wheat at rates of 0.6, 1.1 and 2.2 kg/ha in summer and autumn when the plants were in full bloom. They found that a rate of 0.6 kg/ha applied in autumn resulted in only 20% control, one year after treatment. Increasing the rate to 1.1 kg/ha resulted in only 40% control when applied in autumn and 60% control when applied in summer. The rate of 2.2 kg/ha applied in summer also only controlled *C. arvensis* by 60% (Banks et al. 1979). A separate trial by Davison and Bailey (1974) found that the rate of 2.2 kg/ha was the most effective for 2,4-D amine on *C. arvensis* and that timing was important, as mentioned earlier, early summer applications are best. This rate is said to provide good initial control in the year of treatment (Davison & Bailey 1974; Davison 1976). A different trial, by Barlow and Hicks (1985), found that the low rate of 0.75 kg/ha almost completely killed both *C. sepium* and *C. arvensis* in the short term. Long term control was not measured. Similarly Matic and Black (1994) found a rate of 1.5 kg/ha showed good control of *C. arvensis* in the short term but long term control was reduced due to regrowth.

1.3.4.2 2,4-D ester

The amine salts of 2,4-D are often less effective than 2,4-D ester formulations (Monaco et al. 2002). In the New Zealand Novachem Manual, 2,4-D ester is recommended for use on *C. silvatica* seedlings. Schoenhals et al (1990) found that across 12 trials the butoxyethyl ester of 2,4-D (1.1 kg/ha) controlled *C. arvensis*, one year after application, by an average of 41%. During some experiments the level of control was as high as 80% and as low as 0% in other trials. This level of variation may be because of plant vigour as they found that there was no significant difference between seasons in which the 2,4-D ester was applied but they did find that there was a vigour effect, as mentioned previously (Schoenhals et al. 1990). Schoenhals et al (1990) also found that the level of control two years after the 2,4-D ester was applied was 0% on most

occasions. This is to be anticipated as any plants not fully controlled initially would be able to regrow.

A separate group of studies, by Wiese and Lavake (1986), found similar results. It was found that on average across 20 experiments 2,4-D ester at 1.1 kg/ha gave 45 - 55% control of *C. arvensis* one year after application. The level of control ranged from 0% on one occasion to as high as 97% on a different occasion. Various low volatile ester formulations were used. Two years after application the level of control ranged from 0% to 75% and averaged 31%. Similarly a trial by Matic and Black (1994) found that 2.4 kg/ha showed good control of *C. arvensis* in the short term but not long term due to regrowth.

1.3.4.3 MCPA

MCPA, a foliar applied herbicide, can be used in some crops and in pasture and is similar to 2, 4-D. Reports on its use on *C. silvatica* couldn't be found but it has been recommended as a potential option for further investigation (Wilson-Davey et al. 2009). In two trials by Davison and Bailey (1974), they found that MCPA had similar results to 2,4-D in both trials and in the second trial MCPA (2, 4 and 8 kg/ha) initially controlled 100% of the *C. arvensis* before some regrowth one year after a summer application. The amount of regrowth was significantly less than that of the untreated control and it was the rate of 2 kg/ha that showed the best results (Davison & Bailey 1974). MCPA is said to provide good control the year it is applied (Davison 1976). A trial by Matic and Black (1994) also found that MCPA (1.5 kg/ha) can show good short term control of *C. arvensis*.

1.3.4.4 MCPB

MCPB was also trialled by Davison and Bailey (1974). In the first experiment the MCPB (3.3 kg/ha) was less effective than MCPA (2.2 kg/ha). The same difference wasn't present in the second trial where MCPB, MCPA and 2,4-D all had similar results. In the second trial, where MCPB was applied in summer, *C. arvensis* was initially controlled by 100%, and one year after herbicide application there was significantly less regrowth than the untreated control (Davison & Bailey 1974). Davison (1970) also found that MCPB had some effect on *C. sepium*.

1.3.4.5 Dichlorprop

Dichlorprop is also a phenoxyalkanoic acid commonly used in non pasture areas and can control woody brush weeds (Rao 2000). It was also trialled on *C. arvensis* by Davison and Bailey (1974). Their trial showed that dichlorprop, applied at rates of 2, 4,

8 kg/ha in summer, controlled most of the *C. arvensis* within the year of control but there was regrowth one year after herbicide application, particularly when 2 kg/ha of dichlorprop was applied and the amount of regrowth was not significantly different from that of the untreated control. Dichlorprop did not show the same level of efficacy as 2,4-D, MCPA or MCPB in the trial conducted by Davison and Bailey (1974).

1.3.4.6 Mecoprop

Mecoprop, a foliar applied herbicide for use in some cereals and lawns (Young 2012), showed similar results to dichlorprop in the trial conducted by Davison and Bailey (1974). At rates of 2, 4 and 8 kg/ha mecoprop initially controlled most of the *C. arvensis,* but there was regrowth one year after application and for the lowest rate (2 kg/ha) there was no significant difference between the amount of regrowth and that of the untreated control (Davison & Bailey 1974).

Given these results it could be concluded that 2,4-D, MCPA and MCPB look to be the herbicides which could be most effective on *C. silvatica*, as opposed to dichlorprop and mecoprop which showed poor control of *C. arvensis*.

1.3.5 Pyridinecarboxylic acids

Four pyridine carboxylic acids, clopyralid, picloram, triclopyr and aminopyralid, have all been trialled on *C. silvatica* and triclopyr and picloram are recommended for use on it (Waikato Regional Council 2012). Each of these herbicides will now be examined.

1.3.5.1 Clopyralid

Clopyralid, a foliar applied herbicide, can be used in some crops, orchards, trees, turf and non clover pastures (Rao 2000; Young 2012). In the trial conducted by Rahman and Sanders (1992), clopyralid (0.3 kg/ha) was one of the herbicides trialled on *C. silvatica*. Clopyralid was applied onto *C. silvatica* in asparagus crops in New Zealand in October. After three weeks they found only 40% control of *C. silvatica* which decreased over time due to regrowth. They also concluded that clopyralid was the least effective herbicide trialled (Rahman & Sanders 1992). The level of regrowth could be because the herbicides were applied earlier than when the optimum time may occur, as mentioned previously (Section 1.3.3). In an unpublished trial (Hornby 2011), clopyralid was trialled at a the rate of 0.12 kg/100 L. Hornby (2011) found that clopyralid showed good knockdown. This unreplicated trial did not score or assess the level of knockdown nor did it investigate the source of any regrowth.

A trial by Rahman et al (2002) on *C. sepium* in maize, using clopyralid (0.3 kg/ha), found *C. sepium* was controlled by 66% two months after treatment. Long term regrowth was not measured in this trial. Rahman et al (2002) state that the clopyralid didn't provide sufficient control and showed less control than some of the other herbicides measured. These poor results could have occurred because the herbicide was applied in early spring.

Matic and Black (1994) found similar results to both these trials when twice the rate of clopyralid was applied later, in January or February in South Australia, to *C. arvensis*. This trial shows that clopyralid gave some knockback before the *C. arvensis* grew back.

1.3.5.2 Aminopyralid

Aminopyralid is a newer herbicide and can also be used in some pastures and is a foliar applied herbicide (Young 2012). In the unpublished trial by Hornby (2011) aminopyralid was applied to *C. silvatica* in February at a rate of 0.018 kg/100 L. The trial found good initial control one year after application but regrowth had occurred when the location was rechecked at two years after herbicide application. The full effects of aminopyralid on *C. silvatica* weren't studied. Because aminopyralid is a newer herbicide, reports on its use on related species is also limited.

1.3.5.3 Triclopyr

Triclopyr is another foliar applied pyridinecaryboxilic acid and can be used in pastures, waste areas and roadsides (Rao 2000). In New Zealand it is often sold in mixtures with picloram or picloram and aminopyralid (Young 2012). In the trial by Hornby (2011), triclopyr showed good initial control of *C. silvatica*. Regrowth was not formally measured. Rahman and Sanders (1992) applied1.2 kg/ha of triclopyr in spring and two weeks after treatment, *C. silvatica* was controlled by 98%. Over time it was found though that there was regrowth which may be reduced with alternative timing of herbicide application, as mentioned previously.

Lym and Humburg (1987) tested the efficacy of growth regulators and herbicides or herbicides alone on *C. arvensis*. They found that when triclopyr was used alone, at a rate of 0.38 kg/ha, *C. arvensis* in a greenhouse was controlled by only 60%. When a higher rate (1.7 kg/ha) was used in the field it provided a similar level of control, 58%, one year after application. This level of control was maintained for two years. They also found that in the glasshouse some of the growth regulators improved control but in the field the growth regulators did not improve the control obtained (Lym & Humburg 1987). Triclopyr is another herbicide which could show good knock back of *C. silvatica* given these results from trials on *C. arvensis*.

Triclopyr is also similar to 2,4,5-T. One trial used 2,4,5-T on *C. silvatica* and found there was some reduction of *C. silvatica* and bindweed was said to have been completely controlled (Anon, 1958). A trial by Davison and Bailey (1974) found that 2,4,5-T was less effective than 2,4-D, MCPA and MCPB on *C. arvensis,* and there was significant regrowth.

1.3.5.4 Picloram

Picloram can be used in non crop areas and some pastures as a spot treatment and is more residual than the other pyridinecarboxylic acids mentioned so far (Rao 2000; Young 2012). Westra et al (1992) found that when picloram (0.14 kg/ha) was applied to *C. arvensis* at different locations, the level of control averaged 56% one year after application. Doubling the rate to 0.28 kg/ha increased the control to 69% one year after application. Two years after herbicide application there was still 64% control. This could be because of the residual nature of picloram. The trial by Westra et al (1992) also found that mixtures containing picloram provided the best long term control which they attribute mostly to the picloram. When applied as a sub-surface layer in a trial by Banks et al (1979), picloram controlled *C. arvensis* for 593 days after application, although the treatment damaged the wheat.

1.3.5.5 Triclopyr/picloram

As mentioned previously, in New Zealand there are commercial mixtures of triclopyr/picloram. There is also a commercial mixture of triclopyr/picloram/aminopyralid available (Young 2012). In an unpublished trial (Hornby 2011) triclopyr/picloram showed good control one year after application. The use of this combination is also recommended for use on *C. silvatica* (Waikato Regional Council 2012).

1.3.5.6 2,4-D/picloram

The combination of 2,4-D/picloram has also been recommended for use on *C. sepium*/ greater bindweed (Matthews 1975). Trials have found that one year after application 2,4-D/picloram (0.56/0.28 kg/ha) can control *C. arvensis* by 22 – 87% (Schoenhals et al. 1990), 65% on average (Wiese & Lavake 1986) or 86% on average (Westra et al. 1992). A similar level of control (75%) can last for up to two years after application (Westra et al. 1992). This may occur due to the residual nature of picloram. Both Westra et al (1992) and Schoenhals et al (1990) found that applying 2,4-D/picloram at a lower rate of 0.56/0.14 kg/ha reduced the level of control. A higher rate of 1.12/0.28 kg/ha of 2,4-D/picloram resulted in an average level of control of 87% one year after application and 76% two years after application (Westra et al. 1992). In contrast Heering and

Peeper (1991) found that 2,4-D/picloram (1.12/0.28 kg/ha) only controlled *C. arvensis* by 17 – 25% after 48 weeks, even though there was almost complete initial control. This combination is no longer commercially available in New Zealand.

1.3.6 Sulfonylureas

1.3.6.1 Metsulfuron

Metsulfuron is a foliar applied herbicide which can be used on many woody species in waste areas and unlike the herbicides already mentioned, metsulfuron is a non hormone herbicide (Young 2012). It has been trialled on *C. silvatica* (Hornby 2011) and is recommended for use on *C. silvatica* (Waikato Regional Council 2012). In an unpublished trial by Hornby (2011), metsulfuron showed good control of *C. silvatica* but the results were not formally recorded.

In a trial by Heering and Peeper (1991) metsulfuron was applied to *C. arvensis* at three different rates, 4, 8.7 and 17.5 g/ha, in summer. Eight weeks after treatment control was 80, 96 and 98% respectively when herbicides were applied in 1987 and 58, 88 and 94% respectively when applied in 1988. Control 48 weeks after application when 4 g/ha was used was 10% in 1987, 0% in 1988. When 8.7 g/ha was applied control was 20% or 0% 48 weeks after treatment and for 17.5 g/ha metsulfuron control of *C. arvensis* was 18 or 33% 48 weeks after application (Heering & Peeper 1991). Matic and Black (1994) found that 6 g/ha knocked back *C. arvensis* but there was some regrowth. Metsulfuron +2,4-D also showed initial control but the *C. arvensis* grew back (Matic & Black 1994).

1.3.6.2 Chlorsulfuron

Chlorsulfuron is another non hormone herbicide which has been trialled on *C. silvatica* (Rahman & Sanders 1992). In the trial by Rahman and Sanders (1992), *C. silvatica* was initially controlled by 82%, three weeks after treatment, when 15 g/ha chlorsulfuron was applied in asparagus. *C. silvatica* continued to grow after treatment and by nine weeks the level of control was only 57%. In a trial by Matic and Black (1994), chlorsulfuron (19 g/ha) initially controlled only some of the *C. arvensis* when applied in January in South Australia. There was no long term control found one year after herbicide application. Chlorsulfuron/2,4-D also only controlled *C. arvensis* initially before regrowth and triasulfuron, a related herbicide, only showed a small amount of knockback (Matic & Black 1994).

1.3.6.3 Nicosulfuron

Nicosulfuron is commonly used in maize crops (Young 2012) and has been trialled on *C. sepium*. Rahman et al (2002) found that nicosulfuron (0.06 kg/ha + adjuvant) controlled *C. sepium* by 92% two months after application. A higher rate of 0.08 kg/ha + adjuvant controlled *C. sepium* by 91% two months after application. When the herbicide was applied without the adjuvant, there was less control. There was also some regrowth of *C. sepium* (Rahman et al. 2002).

1.3.7 Imidazolinones

1.3.7.1 Imazapyr

Imazapyr is a residual herbicide which can be applied to both the foliage and the soil (Rao 2000; Young 2012). It was the only herbicide in the trial by Rahman and Sanders (1992) to control *C. silvatica* by 99 - 100%. This result was achieved 5 - 6 weeks after herbicide application when a rate of 1.0 kg/ha was applied in spring. When applied at 0.25 and 0.5 kg/ha, *C. silvatica* at five weeks was controlled by 96 and 98% respectively. It was the only herbicide of those tested by Rahman and Sanders (1992) to show some long term control right through to the next asparagus growing season.

When imazapyr was applied to *C. arvensis* in multiple experiments, Schoenhals et al (1990) found that when 0.56 kg/ha was used, control was above 90% one year after application on most occasions. Lower rates led to less control. It was also found that plant vigour was the important factor in determining the level of control by imazapyr, as opposed to whether it was applied in summer or autumn (Schoenhals et al. 1990). In a different trial (Heering & Peeper 1991), imazapyr applied at 0.14, 0.28 and 0.56 kg/ha controlled established *C. arvensis* by 98 – 100% eight weeks after treatment. By 48 weeks those plants treated with 0.14 and 0.28 kg/ha showed 67 – 78% and 88 – 98% control respectively. Control of *C. arvensis* that was treated with 0.56 kg/ha imazapyr remained at 99 – 100% 48 weeks after treatment. Further trials showed similar levels of control (Heering & Peeper 1991). Trials by Matic and Black (1994) also found that imazapyr showed long term control.

The results of these studies show that a high level of control of bindweeds can be obtained when imazapyr is used and these are the best results of the herbicides mentioned so far. However imazapyr may be too damaging to desirable plants to use in riparian zones.

1.3.7.2 Imazethapyr

Imazethapyr is a residual herbicide and can be used in a range of crops (Rao 2000; Young 2012). Rahman et al (2002) trialled imazethapyr in a mix with imazapyr at three different rates. They found that imazethapyr/imazapyr gave good control of *C*. *sepium* and the best long term control out of the herbicides trialled, for the short duration of the trial.

When imazethapyr was trialled on *C*. *arvensis*, Heering and Peeper (1991) found that all three different rates (0.14, 0.28. 0.56 kg/ha) controlled *C*. *arvensis* by over 89% eight weeks after herbicide application but only 0.56 kg/ha had long term control of over 78%.

Imazaquin, another imidazolinone was less effective than both imazapyr and imazethapyr, as found by Heering and Peeper (1991).

1.3.8 Dicamba and dicamba combinations

1.3.8.1 Dicamba

Another herbicide which has been trialled on *C*. *silvatica* is dicamba. Dicamba is a common herbicide in maize, wheat, some brassicas and other crops and in turf, pasture and waste areas (Rao 2000; Young 2012). When applied to *C*. *silvatica* (0.4 kg/ha) in the trials by Rahman and Sanders (1992), the initial level of control of plants up to 40 cm long was 82% after three weeks, and the control of plants up to 50 cm long was 93% after five weeks. After three to five weeks, in both trials, regrowth of *C*. *silvatica* appeared. This level of regrowth may have occurred because of the timing of herbicide application, as mentioned earlier. Control of *C*. *arvensis* was similar, 92% five weeks after treatment (Rahman & Sanders 1992).

In a trial by Rahman et al (2002) on *C*. *sepium* in maize, dicamba (0.30 kg/ha) was applied in early summer when the stems where 50 cm long. Initial control was 94% and 3 months after application, *C*. *sepium* was controlled by 96%. Long term control was not measured. The level of control is similar to that found in the trials by Rahman and Sanders (1992) on *C*. *silvatica,* and shows that dicamba is a potential herbicide for use on bindweeds. A different trial by Barlow and Hicks (1985) on *C*. *arvensis* and *C*. *sepium,* found that rates of 0.24 – 0.42 kg/ha provided almost complete control 46 days after herbicide application. Long term control wasn't measured.

Davison and Bailey (1974) found that rates of 0.25 and 0.5 kg/ha did not control *C*. *arvensis* long term and there was significant regrowth. Rates of 1.0, 2.0 and 2.2 kg/ha

performed better with very good initial control, and less regrowth than the untreated control.

Dicamba also shows good knock back of *C. arvensis* long term. Over multiple experiments Wiese and Lavake (1986) found that dicamba (1.1 kg/ha) gave good control of *C. arvensis* when applied in autumn or to healthy plants at other times during the year. On average, across the 20 studies, dicamba controlled *C. arvensis* by 57% one year after herbicide application and out of 16 studies dicamba on average controlled *C. arvensis* by 34% two years after application. The level of control was over 80% in some of the studies and these trials demonstrate that dicamba does have some long term control of *C. arvensis,* although the rate applied was twice that used on *C. silvatica* and *C. sepium* in the trials by Rahman et al (2002) and Rahman and Sanders (1992).

Sixteen trials on *C. arvensis* conducted by Schoenhals et al (1990) found that on average 1.1 kg/ha of dicamba controlled *C. arvensis* by 48% and 2.2 kg/ha controlled the plants by an average of 53% one year after the herbicides were applied in either summer or autumn. Control also reached above 80% in some of the experiments. Schoenhals et al (1990) also found that there was no control of *C. arvensis* two years after herbicide application when 2.2 kg/ha of dicamba was used. These results are similar or slightly lower than those found by Wiese and Lavake (1986).

Schoenhals et al (1990) also found that autumn applications of dicamba at 2.2 kg/ha controlled *C. arvensis* better than summer application and that there was no significant difference between summer and autumn applications of 1.1 kg/ha of dicamba. Plant vigour was found to be an important factor, as mentioned previously (Schoenhals et al. 1990).

In a different trial again, Banks et al (1979) found similar levels of control but with higher rates of dicamba. When applied at a rate of 2.2 kg/ha in autumn or summer *C. arvensis* was controlled by 60% one year after application. A higher rate of 5.6 kg/ha controlled *C. arvensis* by 90% when applied in late summer or by 80% when applied in autumn. Increasing the rate to 6.7 kg/ha increased the level of control to 100% one year after herbicide application (Banks et al. 1979). That level of control was only reached in one experiment by Schoenhals et al (1990) when 2.2 kg/ha of dicamba was used. Therefore in order for long term control *C. arvensis,* and potentially *C. silvatica,* higher rates may be needed or repeat applications with lower rates. Banks et al (1979) also found that when applied as a sub-surface layer, dicamba controlled *C. arvensis* for 593 days but damaged the wheat.

Lym and Humburg (1987) also found high levels of long term control in the field when 4 .5 kg/ha of dicamba was used. Dicamba, applied without growth regulators, controlled the *C. arvensis* by 93% three years after herbicide application. When applied with growth regulators in the field the growth regulators did not improve the control obtained (Lym & Humburg 1987)

Another herbicide, similar to dicamba, recommended for use on *C. silvatica* is 2,3,6-TBA (Matthews 1975). It is no longer available in New Zealand.

A trial by Matic and Black (1994) found that the combination of dicamba/MCPA showed initial control of *C. arvensis* but there was regrowth.

1.3.8.2 2,4-D/dicamba

Another herbicide recommended for use on *C. silvatica* is 2,4-D/dicamba (McCoombs 2003; Greater Wellington Regional Council 2010; Waikato Regional Council 2012). It can be used in some turf and waste areas, and it is said in the New Zealand Novachem Manual that this combination can control *C. silvatica* (Young 2012).

A trial by Westra et al (1992) found that when 2,4-D/dicamba (1.1/0.56 kg/ha) was applied to *C. arvensis* plants, at various locations in either summer or autumn, there was 53 – 92% control one year after herbicide application. Two years after herbicide application the level of control was only 10 – 20%. This level is to be anticipated as any plants not controlled by 100% would have grown back. Westra et al (1992) also found that there was a location effect with their results, where different locations resulted in different levels of control, and they mention that there could have been a vigour effect. They did not mention if there was a season of application effect.

1.3.8.3 Dicamba/picloram

The combination of dicamba/picloram has been trialled by both Westra et al (1992) and Wiese and Lavake (1986). Westra et al (1992) found that 0.56/0.14 kg/ha controlled *C. arvensis* by 71% on average one year after application and 31% on average two years after application. When applied with higher rate of picloram (0.56/0.28 kg/ha), there was an average of 82% control one year after application and an average of 77% control two years after application (Westra et al. 1992). This long lasting control could be because of the residual nature of picloram or because the plant was well controlled initially. In the trial by Wiese and Lavake (1986) they found that a rate of 0.28/0.28 kg/ha controlled *C. arvensis* by an average 74% one year after application.

1.3.9 Glyphosate

Glyphosate is a well known broad spectrum herbicide. It is a foliar applied, translocated herbicide that is widely used (Rao 2000; Young 2012). In the trial by Rahman and Sanders (1992), glyphosate (2.16 kg/ha) initially controlled *C. silvatica* by 64% before the level of control decreased due to regrowth. The plants may have grown back due to the timing of herbicide application, as previously mentioned. Glyphosate also has been found to reduce *C. sepium* (Rahman et al. 2002).

There have also been trials using glyphosate on *C. arvensis*. Lym and Humburg (1987) found that in a greenhouse *C. arvensis* was controlled by 60% initially when 0.38 kg/ha was applied. Wiese and Lavake (1986) found that glyphosate applied at 1.7, 3.3 and 5 kg/ha in autumn controlled *C. arvensis* by 50%, 62% and 73% one year after herbicide application. When the same rates were repeated in spring and summer there was little difference in the levels of control one year after application. From these results the authors concluded that 3.3 kg/ha was the most practical rate. The other trials conducted by Wiese and Lavake (1986) using glyphosate (3.3 kg/ha) found that when *C. arvensis* was at least 15 – 25 cm long and of good or excellent health there was an average of 71% control one year after application when the herbicide was applied in spring, summer and autumn. Two years after application the glyphosate had controlled *C. arvensis* by 53% on average across 16 studies which had different application dates (Wiese & Lavake 1986). Glyphosate is a promising herbicide for *C. silvatica* because it has shown the potential to control and knockback *C. arvensis* long term. A separate trial on *C. arvensis* by Banks et al (1979) found a similar level of control, 80 – 90% one year after herbicide application, when higher rates of glyphosate were used (4.5 and 5.6 kg/ha). This control was reached when the glyphosate was applied in late summer or early autumn to flowering plants.

Higher rates show the best results in the trials above and in other trials. Monteiro and Moreira (2004) found that the lower rate of 1.8 kg/ha poorly controlled *C. arvensis*. Davison and Bailey (1974) also showed that 1 and 2 kg/ha of glyphosate provided poor control, whereas 4 and 8 kg/ha showed good control of *C. arvensis*. Matic and Black (1994) also found that the rate of 3.24 kg/ha reduced *C. arvensis* regrowth the following season whereas lower rates, 1.08 and 2.16 kg/ha, did not.

Glyphosate has also been trialled in combination with other herbicides. In a trial by Westra et al (1992), when applied with dicamba, control of *C. arvensis* was poor, 24% one year after application. Glyphosate combined with 2,4-D also showed low control one year after, as did the combination of glyphosate, dicamba and picloram. When glyphosate was combined with both 2,4-D and picloram, control varied from 77 – 81% one year after application and 40 – 48% two years after application, depending on the

rate used (Westra et al. 1992). Matic and Black (1994) also found that 2,4-D/glyphosate showed some initial control before the *C. arvensis* grew back. Glyphosate/picloram showed better control in the trial by Wiese and Lavake (1986), with an average 70% control of *C. arvensis* one year after application.

1.3.10 Amitrole

Amitrole, another broad-spectrum herbicide which is recommended for use in waste areas, as well as in orchards, was also used on *C. silvatica* in the trial by Rahman and Sanders (1992). They found that amitrole (4.0 kg/ha) initially controlled *C. silvatica* by 63% but this level decreased over time due to regrowth. This regrowth may have occurred because the herbicide was applied in spring.

In a study in Portugal by Monteiro and Moriera (2004), a range of herbicides, including amitrole, were applied to control weeds in vineyards. One of those weeds was *C. arvensis*. The trial found that *C. arvensis* was not controlled by any of the herbicides including amitrole (3.44 kg/ha) and that the residual activity of amitrole didn't prevent the plants from growing back. Therefore amitrole may not control bindweeds as well as some of the other herbicides mentioned. However this trial applied the herbicides in late winter (Monteiro & Moreira 2004), and there would have only been new *C. arvensis* shoots emerging. Therefore the herbicides may not have been transported to the roots and rhizomes as the sugars would have been moving from the underground organs to the growing shoots.

1.3.11 Fluroxypyr

Fluroxypyr can be used in apples and cereals (Young 2012) and is suggested to be a potential option for controlling *C. silvatica* (Wilson-Davey et al. 2009). In a trial by Macdonald et al (1993) fluroxypyr was applied at 0.20, 0.30 and 0.40 kg/ha to *C. arvensis* in corn crops. They found that 0.40 kg/ha controlled *C. arvensis* the best, and control was 94% four weeks after treatment when the herbicide was applied at late flowering. Macdonald et al (1993) also found that timing was important, and that applications of fluroxypyr onto *C. arvensis* at late flowering were better than earlier applications, as previously mentioned. Matic and Black (1994) also found that fluroxypyr (0.375 and 0.750 kg/ha) initially controlled *C. arvensis* in South Australia but there was regrowth over time.

1.3.12 Linuron

Another herbicide available in New Zealand is linuron. It is used in crops including vegetable and fruit crops (Rao 2000; Young 2012). It has been suggested as one

herbicide which could be used to control *C. arvensis* in vegetable crops (Parsons & Cuthbertson 2001) and it could show some control of *C. silvatica*.

1.3.13 Fosamine and quinclorac

Quinclorac and fosamine are two other herbicides which could show some control of *C. silvatica*. Fosamine, a herbicide which can be used on woody plants and brush weeds (Rao 2000), has shown some control of *C. arvensis* when trialled (Wiese & Lavake 1986). However it isn't available in New Zealand. Quinclorac is a very persistent herbicide which can control *C. arvensis* (Rao 2000). However, like fosamine, it isn't available in New Zealand.

1.3.14 Contact herbicides

As mentioned previously, translocated herbicides would be needed to control *Calystegia silvatica*. Contact herbicides wouldn't be as successful as the herbicide would just remove the plant parts that the herbicide is applied to, and the roots would be able to grow new shoots. However one contact herbicide which is said to control bindweeds is bromoxynil (Young 2012). In one trial it was found to control *C. sepium* by 74 – 88% but there was significant regrowth (Rahman et al. 2002). It is unlikely that bromoxynil would provide better long term control of *C. silvatica* then any of the translocated herbicides mentioned. Paraquat and glufosinate, two other contact herbicides, would also probably fail to give long term control. Repeat applications of diquat can reduce *C. arvensis* but the level of control is only effective if repeat applications are made and there are no long term effects (Davison 1976).

1.3.15 Soil applied herbicides

The previous herbicides mentioned were all translocated herbicides which can be applied post emergently to the foliage of plants. Some of the herbicides mentioned can also be applied to the soil i.e. imazapyr and imazethapyr. Other soil applied translocated herbicides have also been trialled on *C. sepium* and *C. arvensis* including dichlobenil and chlorthiamid (Davison 1970). Soil applied herbicides were also trialled on *C. arvensis* seedlings in pots, including atrazine, simazine, diuron, chloroxuron, lenacil, terbacil, bromacil, chlorthiamid and dichlobenil (Davison 1976). The trial by Davison (1970) found that chlorthiamid gave better control than dichlobenil and both gave some control of *C. arvensis* and *C. sepium* for a short period. The trial by Davison (1976) also found that chlorthiamid and dichlobenil gave good soil protection for *C. arvensis* but spot sprays would still be needed. Chloroxuron and lenacil both showed poor results (Davison 1976) and bromacil doesn't control *C. arvensis* (Young 2012). In a different study (Sandford 1964) it is said that *C. arvensis* is not affected by dichlobenil,

unless the herbicide is applied under the right conditions. Another study (Spencer-Jones & Wilson 1970) found that dichlobenil resulted in suppression of *C. arvensis* when applied in spring. The other herbicide which showed control is chlorthiamid and it is not sold in New Zealand (Young 2012). Oxadiazon is another herbicide that is frequently mentioned and *C. silvatica* is said to be partially controlled or severely checked by oxadiazon (Young 2012). But with oxadiazon and the other soil applied herbicides they would probably be best suited to preventing further growth of *C. silvatica* rather than treating established plants or used in combination with foliar applied herbicides such as glyphosate.

1.3.16 Herbicide application

Another potential question could be how best to apply the herbicide. Herbicides are commonly applied by sprayer directly onto the plant or ground. But as *C. silvatica* often twines around plants it is quite possible that the plant beneath *C. silvatica* may also be affected by the herbicide. One recommendation is to apply the herbicide, dicamba in this case, by hand-painting it onto *C. silvatica* leaves or by untwining the stem and spraying it with a hand sprayer (Upritchard 2007). Another method that has been suggested is to cut and paint the stems and mulch the removed stems (McCoombs 2003; Waikato Regional Council 2012; Young 2012). Such a technique has been successful in killing weeds selectively such as old man's beard (*Clematis vitalba*) and Japanese honeysuckle (*Lonicera japonica*). The benefits of such an application method is that the herbicide can be applied in windy conditions, should decrease the possibility of wanted plants being affected and can ensure that the plant is being dosed by the herbicide. There could still be some effects on wanted plants though and only a small amount of the herbicide may reach the roots of the plants (Ward & Henzell 2004). For *C. silvatica*, where the roots are an important target for the herbicide, this is the big problem with this technique and limits its potential. Also this technique is normally only recommended for woody stem or trunk species, not for a plant with soft stems and rhizomes.

1.3.17 Use of herbicides in riparian zones

Another important consideration is the safety of the natives and the ground cover species planted in the riparian zones. Many of the herbicides mentioned have not been trialled on New Zealand natives and there is limited information available. Two trials (Harrington & Schmitz 2007; Harrington & Gregory 2009) have found that some herbicides are safe for some native species and unsafe for others. One way to reduce the risk of off target damage could be by using the techniques mentioned above, untwining the vine and spraying or applying the herbicide onto only a few leaves (Upritchard 2007).

1.3.18 Herbicide summary

From these results it can be seen that translocated foliar applied herbicides are likely to show the best results on established *C. silvatica* in riparian zones. Imazapyr has shown the best results on *C. silvatica* and *C. arvensis* out of all of the herbicides mentioned. However it is likely to be too damaging to native species planted in riparian zones. The next best herbicide, given the results, would be picloram and its combinations. Picloram showed long term results but care would also need to be taken in applying it in riparian zones because of its residual nature. Other herbicides which showed particularly promising results in the trials on *C. silvatica*, *C. sepium* and *C. arvensis* are glyphosate and dicamba. Both herbicides showed long lasting control when higher rates were used. Those herbicides which showed some control or knock back of the species long term include 2,4-D both amine and ester, MCPA, MCPB, imazethapyr, imazaquin, fluroxypyr, aminopyralid, triclopyr, triclopyr/picloram, 2,4-D/dicamba and nicosulfuron, and both 2,4-D and 2,4-D/dicamba are recommended for use on *C. silvatica*. These herbicides could have some control of *C. silvatica*. Some of the herbicides also have shown less efficacy including clopyralid, metsulfuron, chlorsulfuron, triasulfuron, amitrole and some of the combinations. Dichlorprop and mecoprop were also less effective than 2,4-D, MCPA and MCPB.

1.4 Conclusion

There has been much confusion and uncertainty about the taxonomy of *C. silvatica*. One subspecies of *C. silvatica* has been identified as growing at Massey University and it is not known how widespread this subspecies is. Much of the research available has also only been done on *C. sepium* and *C. arvensis* and it is not known if that information is directly relevant to *C. silvatica* even though they are related and similar. Some of the studies may also have applied the herbicides at an ineffective time or have not formally assessed long term control. This leads to uncertainty over which herbicide may be the best for control of *C. silvatica* in riparian zones and it is difficult to make control recommendations. There is also some uncertainty over the role seeds play in the spread of *C. silvatica* and where they are a source for new growth if control is successful.

1.5 Research Objectives

- To determine the effects of a range of translocated herbicides on established *C. silvatica* in the field

- To determine the effects of a range of translocated herbicides applied to just a part of *C. silvatica*

- To discover whether applying herbicides to the basal leaves provides better control of *C. silvatica* than herbicide application to the top portion

- To determine how frequently viable seeds are formed within *C. silvatica* flowers

- To determine how best to break the dormancy of *C. silvatica* seed

- To find how much viable seed is present in the soil under dense infestations of *C. silvatica*

- To discover the identity of the *Calystegia* plants which commonly grow in local areas

CHAPTER 2: Field studies of herbicide applications to established *Calystegia silvatica*

2.1 Introduction

Calystegia silvatica can affect riparian plantings by growing over the native plants, reducing their access to light and competing with them. It can also affect the establishment of new plantings. When farmers commit resources, time and money to establishing riparian zones, the problems caused by *Calystegia silvatica* are unacceptable (Wilson-Davey et al. 2009).

Previous research on the use of herbicides on *Calystegia silvatica* has been minimal and the research conducted has been done on either young shoots or the results have not been formally assessed (MAFF, 1957; Rahman & Sanders 1992; Hornby 2011). The trial reported in this chapter aimed to improve our knowledge on how to control this weed by assessing the effectiveness of a range of translocated herbicides on established *C. silvatica* plants in the field.

On a Massey University farm in Palmerston North there is a significant infestation of established *C. silvatica* plants growing near the Turitea Stream. Some of these plants were very large and extensive whereas other plants were smaller. These plants were isolated from each other and able to be used in this trial however there were only limited numbers of suitable plants available. Because of this only a few herbicides are able to be trialled.

For this trial some of the more promising options were selected for further assessment from the list of herbicides discussed in Chapter 1. Glyphosate and aminopyralid have both been previously trialled on *C. silvatica* (Rahman & Sanders 1992; Hornby 2011). Picloram has shown potential on *C. arvensis* (Banks et al. 1979; Westra et al. 1992) and has been used in combination with triclopyr on *C. silvatica* before (Hornby 2011). Picloram is available in New Zealand in a formulation with aminopyralid and triclopyr. Clopyralid showed either poor results in previous trials on *C. silvatica* (Rahman & Sanders 1992), *C. sepium* (Rahman et al. 2002) or *C. arvensis* (Matic & Black 1994) or, according to Hornby (2011), clopyralid "showed good signs of knockback" (p.2). Metsulfuron has also showed poor results on *C. arvensis* (Heering & Peeper 1991; Matic & Black 1994). However they have also been included in the trial reported below as clopyralid may be one of the safer herbicides to use around riparian plantings and metsulfuron has been recommended for use on *C. silvatica* (Waikato Regional Council 2012). Another herbicide also recommended for use on *C. silvatica* is 2,4-D/dicamba

(McCoombs 2003; Greater Wellington Regional Council 2010; Waikato Regional Council 2012).

The objective of this experiment was:

- To determine the effects of a range of translocated herbicides on established *C. silvatica* in the field

2.2 Materials and methods

2.2.1 Location

The trial was located in ungrazed areas near the Turitea Stream and adjacent to Paddocks 64 and 65 of the Massey University No 1 Dairy Farm on Poultry Farm Road 2 km south of Palmerston North.

2.2.2 Plot information

The species of bindweed in the plots had white corollas ranging from 55 – 79 mm in length and stamen lengths ranged from 24 – 31 mm. The bracteoles on each flower overlapped and the width of each bracteole was equal, or almost equal, to the length of the bracteole. Leaf lengths of those collected were over 56 mm long. Therefore, using the taxonomic information summarised in Figure 1.1 of Chapter 1, the plants that were treated in this field trial were identified as *Calystegia silvatica* subsp. *disjuncta*.

The plots were set up around the established *C. silvatica* plants which were separate and isolated from other plants in the area used. Some of the plants were growing over trees or shrubs, some over fallen logs and some growing across the ground. Bamboo stakes were put in to mark the four corners of each area to be treated. The area within each of the plots was measured and the ground covered by the *C. silvatica* plants within the plots was estimated. The size of the plants ranged from 1.2 m^2 to 29.9 m^2 (Plates 2.1 and 2.2). The plots were grouped in blocks based on the size of the plants. Block 1 plants covered areas ranging from 13.7 m^2 to 29.9 m^2, the area covered by blocked two plants ranged from 7.3 m^2 to 13.0 m^2. Block 3 plants covered areas ranging from 3.1 m^2 to 7.2 m^2 and Block 4 plants covered areas ranging from 1.2 m^2 to 2.5 m^2. The treatments were then randomly assigned within each block, giving a randomised complete block design. There were four replicates for each treatment, with a total of 28 plots used for this trial.

The other plants in each plot included *Salix* spp. (willow), *Juglans* spp. (walnut), *Sambucus nigra* (elder), *Solanum aviculare* (poroporo), *Coprosma* spp. and *Melicytus ramiflorus* (mahoe). Grasses within the plots included *Lolium perenne* (perennial ryegrass), *Arrhenatherum elatius* (tall oat grass), *Holcus lanatus* (Yorkshire fog), *Dactylis glomerata* (cocksfoot) and *Phalaris arundinacea* (reed canary grass). Other weeds present in the plots included *Conium maculatum* (hemlock), *Tradescantia fluminensis* (wandering Jew), *Stachys silvatica* (hedge woundwort), *Ranunculus repens* (creeping buttercup), *Galium aparine* (cleavers) and *Clematis vitalba* (old man's beard).

Plate 2.1: Coloured bamboo pegs mark out the corners of Plot 24 which was one of the smallest plants in the trial. The *C. silvatica* was growing over a *Melicytus ramiflorus* tree.

Plate 2.2: Established *C. silvatica* growing over *Sambucus nigra*, one of the largest plants in the trial.

2.2.3 Herbicide treatment and application

The seven treatments used in this trial included the herbicides in Table 2.1 and an untreated control.

Table 2.1: Summary of the herbicides and the rates used.

Active ingredient	**Trade name**	**Formulation**	**g ai/100 litre**
2,4-D/dicamba	Banvine	amine salts	240 +120
aminopyralid	Tordon Max	tri-isopropylamine salt	18
clopyralid	Versatill	amine salt	90
glyphosate	Roundup 360 Pro	isopropylamine salt	540
metsulfuron	Answer	methyl ester	15
triclopyr/picloram/ aminopyralid	Tordon Brushkiller XT	butoxyethyl ester + amine salt + amine salt	90 + 30 + 2.4

The herbicides were applied using a 15 litre solo backpack sprayer on the 16 April 2012 between 9.00 am and 3.00 pm. All of the herbicides had Done That dye added to them at a rate of 1ml/litre to check how much had been sprayed. Only 90% of each plant in the herbicide treatment was sprayed with herbicide. The other 10% was unsprayed to simulate how *C. silvatica* in riparian strips would need to have parts left unsprayed to avoid herbicide application to newly planted natives, and usually it was the upper 10%

which was left unsprayed. The weather was overcast, and humid, with one brief period of light drizzle occurring during spraying. There was no rain 24 hours after herbicide application. There was little to no wind and the maximum temperature reached during spraying was 18^{o}C.

Other weather data for the months after herbicide application is given in Table 2.2. The weather information is from a weather station within one kilometre of the trial area.

Table 2.2: Summary of temperatures and rainfall for the trial period April to December 2012. Data is from Grasslands AgResearch weather station, close to the field sites.

Month	**Average max temp (^{o}C)**	**Average min temp (^{o}C)**	**Average soil temp at 10 cm (^{o}C)**	**Total rain (mm)**
April	19.5	8.4	13.7	28.2
May	15.0	3.9	9.2	62.2
June	12.8	3.4	7.4	93.4
July	13.1	4.2	7.0	109.6
August	14.4	5.6	8.9	98.2
September	15.2	5.7	9.9	58.8
October	16.1	7.1	12.0	64.6
November	17.6	7.8	14.5	20.2
December	22.3	12.5	18.4	100.2

2.2.4 Assessment methods

Each week after herbicide application a score was given to each plot, ranging from 0 if the *C. silvatica* plant was dead to 10 if the plant was healthy and all the leaves green. A score of 9 was given if 10% of the leaves were brown, yellow or affected and a score of 8 if 10 – 20% of the leaves affected. A score of 5 was given if the leaves were 50% green/healthy and 50% affected. A score of 2 was given if the only 10 – 20% of the leaves were green and healthy and the rest affected and a score of 1 if there were less than 10% healthy leaves. The plots were scored weekly from one week after herbicide application (23 April 2012) till 11 weeks after herbicide application (2 July 2012). Notes were made about the health of the untreated 10% portion of each plant at each initial assessment.

Notes on the health of the trees and shrubs, underneath and near the treated *Calystegia silvatica* plants were recorded on 28 May 2012.

New *Calystegia silvatica* shoots were noticed in and around the plots on 22 August 2012. On 29 August 2012 (19 weeks after herbicide application) a count of all shoots was made. By germinating seeds in the lab, it was possible to determine what seedlings of *Calystegia silvatica* look like. Some were found in surrounding areas (Plate 2.3) and each treatment plot was searched, while recording shoot regrowth, to see if they contained any. Shoots from all of the rhizomes and root buds were also counted and the number of shoots over 10 cm recorded. The number of shoots was counted weekly until 22 weeks (19 September 2012) after herbicide application.

Once the *C. silvatica* plants present in plots had started to grow across the ground and up other plants, coverage assessments were made. These cover assessments were made on 10 October 2012 (25 weeks after treatment), 24 October 2012 (27 weeks), 7 November 2012 (29 weeks) and the final coverage assessment was made on 5 December 2012, 33 weeks after herbicide application. To determine the coverage of *C. silvatica* the percentage of plants covering the ground was estimated by eye. If *C. silvatica* covered the entire area 100% was recorded. If there were no plants present 0% was recorded.

Plate 2.3: The distinctive cotyledons of a *C. silvatica* seedling (centre) that established amongst *Tradescantia fluminensis* near one of the trial plots.

2.2.5 Problems that affected the trial area

The paddock next to the plots was frequently grazed. On 30 April 2012 cows broke through the fence and affected several of the plots, especially one of the untreated control plots. As a result of the damage it was excluded from the rest of the scoring, but was included in the new growth and coverage measurements as the plants grew back after the winter period. Cows also breached the fence on 13 August 2012 but didn't cause any notable damage.

On 17 July 2012 it was found that several plots were slightly flooded by the swollen Turitea Stream. The plots were not fully flooded and the pegs remained once the stream receded. One 2,4-D/dicamba plot was badly damaged on 17 July 2012 (Plate 2.4) when part of the hillside, on which the plot was located, was involved in a slip and the entire plot disappeared. This plot was excluded from analysis after that date.

Plate 2.4: Landslip covering a 2,4-D/dicamba plot on 17 July 12.

2.2.6 Statistical analysis

The scores for one untreated control were excluded following grazing and it was treated as a missing plot until the plant grew back in spring. The 2,4-D/dicamba plot affected by a landslip was treated as a missing plot after 17 July 2012. SAS was used to perform an analysis of variance of the data collected and least significant differences between means were calculated if treatments were shown to have a significant effect

(P<0.05) on the scores or shoot numbers. To determine the cover of *C. silvatica* the coverage percentage data was multiplied by the plot size and compared to the original plant coverage. The percent difference between the original plant coverage and the post treatment plant coverage was calculated and the data subjected to an analysis of variance.

2.3 Results

2.3.1 Initial treatment effects

Two weeks after herbicide application the health of the plants within the treated plots was significantly less than the health of the untreated control plants as seen in Table 2.3. It can be seen in Plate 2.6 that 2,4-D/dicamba had affected *C. silvatica* and the plant was less healthy than the plant shown in Plate 2.5, taken before herbicide application. Triclopyr/picloram/aminopyralid affected *C. silvatica* more rapidly than the other herbicides. After four weeks the untreated plants began dying back for winter. For the other treatments the effects of the herbicides generally resulted in the plants dying back more rapidly, with the exception of the metsulfuron plants which were not significantly different to the untreated control plants (Table 2.3). The health of the plants in the untreated control plots worsened between the 30 April 2012 and 18 June 2012. By nine weeks after application many of the aboveground parts of plants in the herbicide treatments were dead or close to death, as were the plants in the untreated control (Table 2.3). There were no significant block effects detected in the analysis of this data, suggesting larger plants died back as fast as the smaller treated plants.

Table 2.3: Scores (0 = dead, 10 = healthy) for each herbicide at 2, 5 and 9 weeks after treatment (WAT) with herbicide.

		30/04/12	**14/05/12**	**18/06/12**
Treatment	**Rate (g ai/100 litres)**	**2 WAT**	**4 WAT**	**9 WAT**
2,4-D/dicamba	240 +120	4.3	2.5	0.0
aminopyralid	18	4.3	3.0	0.0
clopyralid	90	4.5	2.3	0.5
glyphosate	540	3.5	1.5	0.0
metsulfuron	15	5.5	3.8	0.5
triclopyr/picloram/ aminopyralid	30 + 90 + 2.4	2.5	2.0	0.0
untreated	-	7.0	5.3	0.5
LSD (P<0.05)		1.4	2.1	NS[1]

[1]NS = not significant (P<0.05)

For most of the herbicide treatments the unsprayed portion of each plant showed signs of treatment effects at the same or similar time as the rest of the plant. The two exceptions were the 2,4-D/dicamba and clopyralid treatments where the untreated portions displayed treatment effects later than the rest of the plant. Triclopyr/picloram/aminopyralid killed the unsprayed portion of the plant more rapidly than any of the other treatments. Clopyralid killed the unsprayed portion of the plant slower than any of the other herbicides but the unsprayed portion was dead two weeks before the aboveground parts of the plants within the untreated control were all dead.

2.3.2 Health of non-target plants

Most of the non-target plants, trees and shrubs appeared unaffected by any of the herbicide treatments six weeks (28 May 2012) after herbicide application. A few willow leaves in one aminopyralid plot showed some effect and a few branches of gorse in one triclopyr/picloram/aminopyralid plot showed some browning. In the case of the gorse there was probably less care taken in an endeavour to avoid spraying the shrub underneath.

2.3.3 *C. silvatica* regrowth in spring

On 29 August 2012, 19 weeks after herbicide treatment, most of the plots had some regrowth from *C. silvatica* rhizomes and root buds and by 22 weeks there were significant differences between the treatments (Table 2.4). After 22 weeks the number of *C. silvatica* shoots from rhizomes per m^2 was highest in the metsulfuron plots. There were also high numbers of shoots in the clopyralid plots and both treatments were not significantly different from the untreated control at 22 weeks after herbicide application. All other treatments had significantly less regrowth with 2,4-D/dicamba and triclopyr/picloram/aminopyralid having the lowest number of shoots per m^2 at 22 weeks. These results were not affected by the original size of the plants before herbicide application as there was no significant block effect.

None of the plots had any seedlings emerge within them. The shoots that did emerge in the plots in spring were all from rhizomes or root buds.

Table 2.4: Number of shoots from rhizomes or root buds per m^2 for each herbicide at 19 and 22 weeks after treatment with herbicide (WAT).

Treatment	Rate (g ai/100 litres)	29/08/12	19/09/12
		19 WAT	22 WAT
2,4-D/dicamba	240 +120	0.0	0.6
aminopyralid	18	0.1	1.0
clopyralid	90	1.0	3.8
glyphosate	540	0.5	2.7
metsulfuron	15	1.5	5.3
triclopyr/picloram/aminopyralid	30 + 90 + 2.4	0.1	0.3
untreated	-	0.8	3.5
LSD (P<0.05)		NS[1]	3.4

[1]NS = not significant (P<0.05)

The number of shoots over 10 cm was recorded at 19 and 22 weeks and the results can be seen in Table 2.5. At 19 weeks no treatment had significantly more or less shoots over 10 cm than the untreated control, but the metsulfuron treatment did have significantly more shoots over 10 cm then the 2,4-D/dicamba and triclopyr/picloram/aminopyralid treatments. At 22 weeks the 2,4-D/dicamba and triclopyr/picloram/aminopyralid treatments still had only a small number of shoots over 10 cm in length, though this wasn't significantly different from the untreated control. The metsulfuron treatments had a large number of shoots over 10 cm, though again this was not significantly different from the untreated controls.

Table 2.5: The number of shoots over 10cm for each herbicide at 19 and 22 weeks after treatment with herbicide (WAT).

Treatment	Rate (g ai/100 litres)	29/08/12	19/09/12
		19 WAT	22 WAT
2,4-D/dicamba	240 +120	0.3	5.0
aminopyralid	18	1.3	16.8
clopyralid	90	5.0	26.5
glyphosate	540	2.3	13.8
metsulfuron	15	12.0	70.5
triclopyr/picloram/aminopyralid	30 + 90 + 2.4	1.0	6.8
untreated	-	4.0	35.5
LSD (P<0.05)		10.8	35.8

2.3.4 Final coverage of *C. silvatica*

When the initial coverage of the *C. silvatica* prior to herbicide treatment was compared to the coverage of plants at 25 weeks after herbicide application (Table 2.6), there were significant differences between the untreated control and the 2,4-D/dicamba, triclopyr/picloram/aminopyralid and aminopyralid treatments. All three treatments had plants which covered significantly less area than the original plants before herbicide treatment. By 29 weeks the plants had grown and there was no longer any significant difference, although both the 2,4-D/dicamba and triclopyr/picloram/aminopyralid plants were less than 25% of their original size, compared with 85% in the untreated controls.

When the initial coverage of *C. silvatica* prior to herbicide application was compared to the coverage of plants at 33 weeks, there was again no significant difference between the treatments and the untreated control (Table 2.6). This was because of the large amount of variability between the plots in each treatment. For 2,4-D/dicamba all three of the plots had plants that covered less ground than the original *C. silvatica* plants (22.7, 33.0 (Plate 2.7) and 84.0%). Triclopyr/picloram/aminopyralid plots also had plants cover less area than the plants originally did (43.0, 51.2, 65.4, and 94.8%). The plots treated with aminopyralid also had three plants cover less area (8.6, 69.2, 82.6 %), but also had one plot where the plant covered almost twice the same area as the plant that originally grew in that plot (192.7%). Clopyralid and glyphosate plots also had three plots which covered less than 100% of the plant area, with 56.9, 59.3 and 65.8% for clopyralid and 37.9, 42.8 and 95.1% for glyphosate. Each treatment also had one plot which covered a significantly larger area than the original plant did prior to herbicide application. Clopyralid had one *C. silvatica* plant (Plate 2.9) cover an area of 260.6% of the original area (Plate 2.8) and glyphosate had one plant which covered 188.3% of the original area. Metsulfuron was the only treatment in which all four of the plots had coverage that was similar to or more than the original plant coverage, 90.0, 95.2, 129.4 and 155.7%. These results show the variability for each treatment and this variability was not due to the original size of the plants as there was no significant block effect within the analysis of variance and the plots were blocked on the size of the plants (Section 2.2.2).

Table 2.6: The coverage of plots by *C. silvatica* stems, expressed as a percentage of the cover present immediately prior to spraying, for each treatment, 25, 29 and 33 weeks after treatment with herbicide.

Treatment	Rate (g ai/100 litres)	10/10/12 25 WAT	7/11/12 29 WAT	5/12/12 33 WAT
2,4-D/dicamba	240 +120	4.6	17.1	46.6
aminopyralid	18	10.2	36.8	88.3
clopyralid	90	18.1	66.6	110.7
glyphosate	540	17.6	48.2	91.0
metsulfuron	15	41.0	48.9	117.6
triclopyr/picloram/ aminopyralid	30 + 90 + 2.4	6.1	24.5	63.6
untreated	-	40.8	84.8	136.7
LSD (P<0.05)		27.0	NS[1]	NS[1]

[1]NS = not significant (P<0.05)

Plate 2.5: *C. silvatica* before herbicide application of 2,4-D/dicamba.

Plate 2.6: *C. silvatica* almost two weeks after the herbicide application of 2,4-D/dicamba. The leaves of the *C.* silvatica are yellowing and sick.

Plate 2.7: *C. silvatica* 33 weeks after the herbicide application of 2,4-D/dicamba. The large vines covering the tree have not grown back in spring.

Plate 2.8: A *C. silvatica* plant before application of clopyralid.

Plate 2.9: The *C. silvatica* plant 33 weeks after application of clopyralid. The vine has grown back to cover an area more than the original area the plant covered (Plate 2.8).

2.4 Discussion

2.4.1 Initial herbicide control

All of the herbicides decreased the health of the *C. silvatica* plants after herbicide application, particularly triclopyr/picloram/aminopyralid. An unpublished report by Hornby (2011) found that the same combination without aminopyralid, just triclopyr/picloram, also initially controlled *C. silvatica* in the field. The same trial also found that aminopyralid and metsulfuron provided some initial control of *C. silvatica*. The current trial found similar results and that glyphosate also showed some initial control of *C. silvatica*. Rahman and Sanders (1992) also found glyphosate provided some initial control of *C. silvatica* but that clopyralid showed only 40% control of *C. silvatica* three weeks after treatment. The current field trial found better results although clopyralid was slower to kill the unsprayed portion of the plants. Hornby (2011) also found that clopyralid showed knockback of *C. silvatica*.

The field trial presented here may have found better initial control because the herbicides were applied in autumn, during or after full bloom, as the plants were senescing whereas Rahman and Sanders (1992) applied herbicides in spring. Hornby (2011) applied herbicides at a similar time to the current field trial, in autumn, but did not formally assess the full effects of the herbicides (Chapter 1). The herbicides were applied at this time because this is when the herbicides are most likely to be translocated to the underground organs of *C. silvatica*. It also means that the aboveground parts of the plants were already dying off before winter dormancy. Therefore the herbicide effects may be initially better than that found in other trials. Because the plants were dying off before winter also means that the initial measurements in this trial only give an indication to how the aboveground parts are affected by the herbicides and is not the best measurement of long term control.

2.4.2 Health of non target plants

The health of most of the other plants appeared to suffer no ill effect from the herbicide treatments and this may have been because of the careful spray technique used, as well as the low water rates, which targeted only the vines as much as possible and thus little herbicide made contact with non-target plants during application. The implications of this for treating *C. silvatica* in riparian plantings will be discussed further in Chapter 6.

2.4.3 *C. silvatica* spring regrowth

The results in this trial show that, while all of the plants had some regrowth in spring, indicating that not all of the belowground parts were killed, the triclopyr/picloram/aminopyralid and 2,4-D/dicamba treatments had the least amount of regrowth and the least amount of shoots over 10 cm at 19 and 22 weeks. Hornby (2011) also found that triclopyr/picloram showed long term control of *C. silvatica*. In the current trial on *C. silvatica*, 2,4-D/dicamba also showed good control. This is different from what Westra et al (1992) found with *C. arvensis* and this could be because of species differences. In the current trial metsulfuron showed poor results and a similar amount of shoots as the untreated control emerged. These results are similar to those found on *C. arvensis*, where there was good initial control or knockback but regrowth soon occurred (Heering & Peeper 1991; Matic & Black 1994). The field trial present here also showed that clopyralid showed the same amount of regrowth as the untreated control. Rahman and Sanders (1992) also found that *C. silvatica* plants treated with clopyralid grew back and the clopyralid was ineffective. Clopyralid has also been found to not provide long term control of both *C. sepium* (Rahman et al. 2002) and *C. arvensis* (Matic & Black 1994). Aminopyralid and glyphosate showed intermediary results. Aminopyralid has not been extensively studied on *C. silvatica* or related species and glyphosate has showed varying results, with some regrowth, depending on the rate and species, as discussed in Chapter 1, Section 1.3.9.

Counting the shoots that had grown in spring was the best measurement taken in this trial as it was not influenced by winter dieback, and gave an indication as to the herbicide effects on belowground parts, something that the initial scoring system was unable to measure.

The shoots that emerged in the plots were all from rhizomes or root buds. No new seedlings emerged. This could have been because of the extensive ground cover present in most of the plots which may have prevented seed germination. Or perhaps there are few seeds in the soil because seeds are rarely produced (Popay et al. 2010) or because of predation. This will be further investigated in Chapter 4.

2.4.4 Final plant cover

At 25 weeks the area that the *C. silvatica* plants covered in each plot was estimated and plants in both the 2,4-D/dicamba and triclopyr/picloram/aminopyralid treatments had covered far less area than the original plants, which shows that these treatments affected both the belowground parts of the plants as well as the aboveground parts.

By 29 weeks there was no significant difference but both triclopyr/picloram/aminopyralid and 2,4-D/dicamba still showed long term effects.

The area that the *C silvatica* plants covered in each treatment was also estimated at 33 weeks after herbicide application. The results showed a lot of variability and although there was no significant difference between the treatments a lot of useful information could be derived. All of the plants in the triclopyr/picloram/aminopyralid and 2,4-D/dicamba treatments still covered less area than the original plants and this shows that both treatments have long lasting effects. Glyphosate and aminopyralid also showed some long term control. Most of the plants in the clopyralid treatments were also less than the original plants which shows that even though there was significant regrowth initially some of the clopyralid plants did not grow back to their original size which indicates that clopyralid may have had an effect on some of the below ground parts of *C. silvatica*. Metsulfuron did not show any long term control.

The final coverage results showed that if *C. silvatica* is not 100% controlled initially then any regrowth which emerges can continue to grow and reinvade the site, even if there were only just a few shoots which survived the winter. Therefore if a few shoots did emerge in spring, after herbicide application, then a second herbicide application would be needed in order to ensure that the plants don't re-establish. This will be further discussed in Chapter 6.

This final coverage assessment may have been affected by other plants in the trial area. The plants chosen for the field trial were in ungrazed waste areas where there were numerous *C. silvatica* plants, some of which were growing near those selected as plots. These plants were not treated with herbicides and there is a chance that some of these plants may have sent up shoots in the plot area. This may help explain the variability in the results obtained after 33 weeks. The variability was not due to the size of plants as larger plants appeared no more difficult to control than smaller plants. Some of the regrowth may also have been influenced by other species in the plots. A few plots in particular had large populations of tall hemlock as the *C. silvatica* was growing back in spring. This shading may have reduced the amount of regrowth and the *C. silvatica* may not have been able to recover and climb towards better light conditions.

Another factor which could have influenced the results is the age or generation of the original plants. It was not known how long each of those plants had been growing in that area and some of the plants could have been growing there for many seasons and had well established extensive rhizome and root systems. Some of the plants may have only emerged the first time in the previous spring. This could have impacted how well the herbicides affected the below ground parts of the *C. silvatica*. Other biological

factors such as the insect damage on some of the plants could also have influenced the results.

2.4.5 Summary

This trial aimed to determine the effects of a range of translocated herbicides on established *C. silvatica* in the field. It was found that none of the herbicides tested totally controlled both the aboveground and belowground parts of *C. silvatica*. Both triclopyr/picloram/aminopyralid and 2,4-D/dicamba showed the most control, but would require a second application of herbicide, which was not trialled in this field experiment. Aminopyralid, glyphosate and clopyralid provided intermediary effects and metsulfuron showed poor control. Further implications will be discussed in Chapter 6.

CHAPTER 3: Glasshouse studies of herbicide applications to *Calystegia silvatica*

3.1 Introduction

Because by nature field trials can produce variable results, the decision was made to assess a range of translocated herbicides on *C. silvatica* in a more controlled environment of a glasshouse which would give a more accurate index of what herbicide works best. The glasshouse trial was run concurrently to the field trial.

All of the same herbicides used in the field trial, namely glyphosate, clopyralid, metsulfuron, aminopyralid, triclopyr/picloram/aminopyralid and 2,4-D/dicamba, were trialled again in the glasshouse trial along with 2,4-D and fluroxypyr. These two herbicides were added to the analysis because there were plants available for extra treatments and because 2,4-D has been recommended for use on *C. silvatica* (MAFF 1957; Young 2012) and fluroxypyr is considered to be a herbicide with the potential to control *C. silvatica* (Wilson-Davey et al. 2009).

Another factor to be considered when applying herbicides in riparian zones is how to apply the herbicides in a way which would avoid as much off target damage as possible while still controlling *C. silvatica*. If it isn't possible to apply herbicide to the whole plant without risking damage to the natives then the herbicides could be applied between the native plantings or around them. Whether this would still provide good control is one aspect considered here.

If the herbicides could only be applied to part of the plant then the question that arises is which part of the plant would show the best control if herbicides were only applied to that part. The hypothesis that was also developed and trialled in this glasshouse trial was that applying herbicides to the basal leaves of the vines could control *C. silvatica* better than herbicide applications to the tips and upper leaves of the plants. This is because of the source sink relationship of plants which means that the basal leaves are likely to send the sugars to the closest sink which is the roots and rhizomes where the herbicides need to reach in order for long lasting control. The upper portion of the plants may still be sending sugars to the closest sink, the tips, which means that the herbicides would be unlikely to translocate to the underground organs, which would reduce the level of control. If this hypothesis is proven correct this could mean that just the basal leaves of the vines would need to have herbicide application in order to control the plant, which would reduce off target effects.

The objectives for this experiment were:

- To determine the effects of a range of translocated herbicides applied to just a part of *C. silvatica*

- To discover whether applying herbicides to the basal leaves provides better control of *C. silvatica* than herbicide application to the top portion

3.2 Materials and methods

3.2.1 Location

The trial was located in a glasshouse and a shadehouse at the Massey University Plant Growth Unit located on Batchelar Road 2 km south of Palmerston North.

3.2.2 Pots and potting mix

Daltons base mix made up of *Pinus radiata* bark with calcium ammonium nitrate (fines A grade) 50%, fibre 30%, pacific pumice (7 mm) 20%) and serpentine super (1kg/M3) (www.daltons.co.nz), was used to fill 100 planter bags (100 x 100 x 200 mm or PB 3). Dolomite (150g), short term fertiliser (100g) and long term fertiliser (200g) were mixed into to the potting mix before it was used. The short term fertiliser used was Woodace 14-6-11.6 ((N) 14.0%, (P) 6.0%, (K) 11.6%, (Mg) 1.0%, (S) 4.0%, (Fe) 1.0%, (Mn) 0.5% and (C) 2.0%) and the long term fertiliser used was Woodace 18-2.2-8.3 ((N) 18.0%, (P) 2.2%, (K) 8.3%, (Mg) 0.3%, (S) 1.0%, (Fe) 0.3%, (Mn) 0.2% and (Zn) 0.2%), further information can be found in (Lebanon Seaboard Coporation 2010; Lebanon Turf 2013).

3.2.3 Rhizome collection

An area behind the Plant Growth Unit, where large amounts of *Calystegia silvatica* plants were growing amongst *Casuarina spp*. and *Agapanthus praecox*, was the source of the rhizomes used in this trial. To ensure correct identification of the *Calystegia silvatica*, 10 flower and some leaves were collected from random locations in the area. The length of the corollas ranged from 64 – 69 mm and all of the flowers were white. The bracteoles overlapped and the width of the bracteoles was almost equal to the length of the bracteoles. Stamen lengths ranged from 27 - 28mm. Based on the key (Figure 1.1) the species identified was *Calystegia silvatica* subsp. *disjuncta*.

On 17 February 2012 100 rhizomes were dug from the ground beneath the *C. silvatica* plants and kept moist between sheets of wet paper. The rhizomes selected each had fibrous roots and purple buds (Plate 3.1) and ranged in length between 15 and 55 cm.

The rhizomes were then planted later the same day, one per pot, and buried 2cm – 3cm below the soil surface. The pots were numbered and placed in a temperature regulated glasshouse. On 7 March 2012, rhizomes from the same source were planted into four pots from which no plants had successfully established.

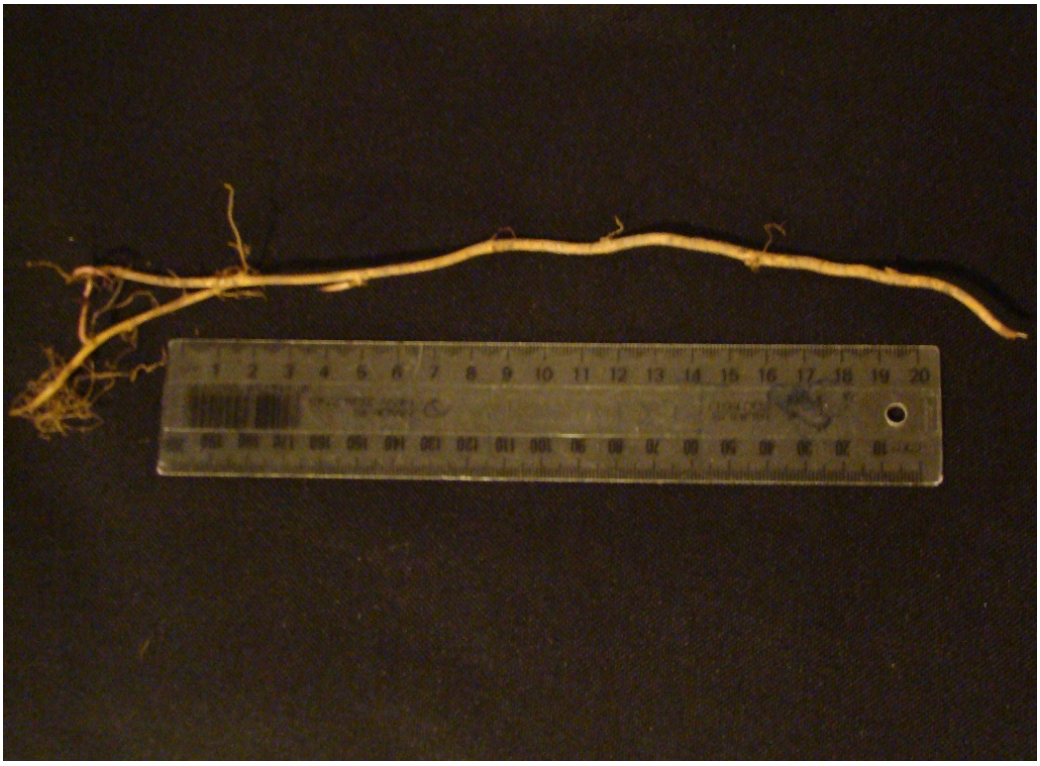

Plate 3.1: An example of a rhizome segment planted in each pot.

3.2.4 Plant care

All of the pots were watered immediately after the rhizomes were planted and were regularly hand watered until drip fed irrigation was set up on 29 February 2012. The plants were then watered for brief periods daily, morning and evening.

String was tied to overhead wires, which were 2 m above the ground, and attached to the pots (Plate 3.2). The vines produced by plants were then trained to wind up these strings. To ensure the vines remain on the string, they were tied with twist ties. The vines were trained on a regular basis until herbicide application.

To ensure consistency amongst the pots, the plants were trimmed on 29 and 30 March 2012, 3 and 4 April 2012, 14, 17 and 18 April 2012. All of the shoots of the plant were cut except for six, the main shoots and some of the lateral shoots, for the plants which had more than six shoots. If the shoots reached the overhead wires then the tips of them were also trimmed to prevent the vines from tangling with other plants.

Insect damage was found on many of the plants on 5 March 2012. Slug bait (Slugout, active ingredient 18g/kg metaldehyde in the form of granular bait) was laid on 7 March 2012. Yellow sticky insect traps were also hung around the glasshouse. Caterpillars were found on 9 March 2012. Acephate pesticide was applied on 14 March 2012 at a rate of 25g/100 litres. White flies were found (19 March 2012), as were aphids (28 March 2012). Two pots were treated with acephate (29 March 2012) because of the aphids. The entire glasshouse was sprayed with acephate on 4 April 2012 at a rate of 25g/100 litres. Aphids were still present on the plants right through the rest of the trial until the vines were cut down. Caterpillars which left windowing of the leaves, webbing and frass were found on 19 April 2012 and remained on many of the plants until the vines were cut (Plate 3.3).

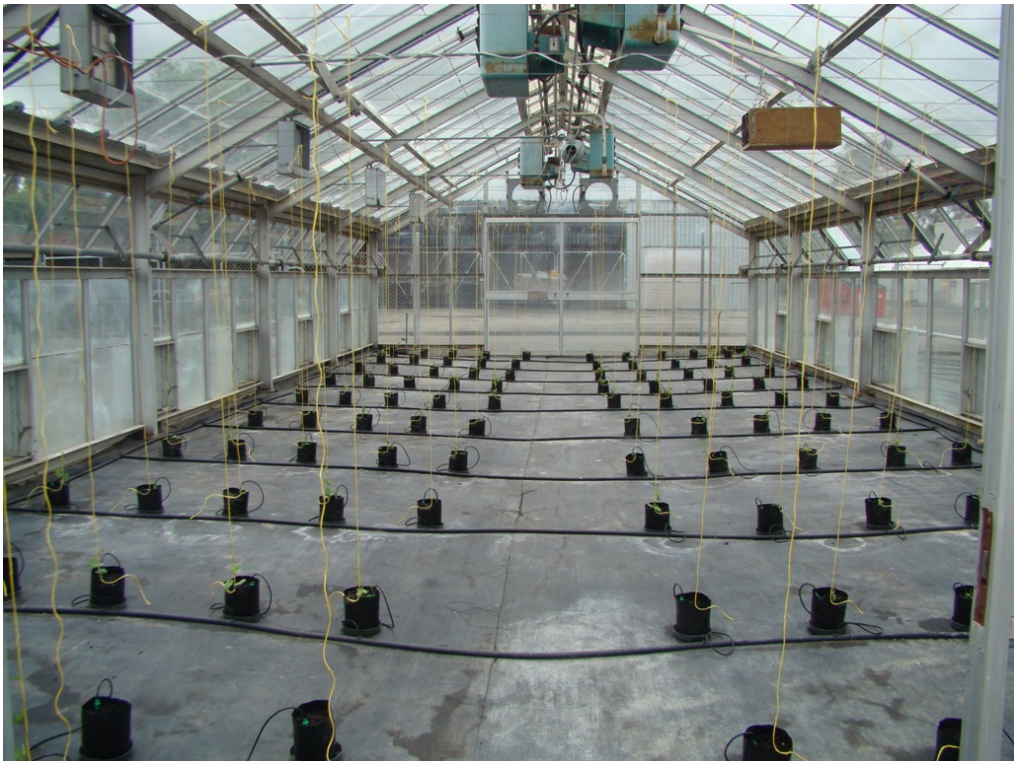

Plate 3.2: Pots set up with tied string and the irrigation system.

Plate 3.3: A caterpillar found on a *C. silvatica* leaf, with webbing, windowing and frass, tentatively identified by Professor Qiao Wang as being a looper caterpillar (Geometridae).

3.2.5 Plant health

Many of the plants wilted at some stage before herbicide application, perhaps due to the irrigation not being correct, or perhaps a root problem. Some plants recovered from the wilt whereas other plants had all shoots die off. Many of the plants in the trial also had some yellow leaves and senescence before herbicide application Because of the varying degrees of health of the plants, a score of four was given if the plants were healthy from the moment they emerged, a score of three if there was only slight wilt, two if there was significant senescence, one if there had been significant wilt and a zero if all above ground parts were dead. These scores were used in the ranking.

The leaves on all plants in the glasshouse were counted (19 April 2012). All of the leaves which were full, developed and unfolded were counted but those which were still unformed were not. Yellow leaves were counted, but partially brown or dead leaves were not.

The plants were then ranked and allocated to treatments within a randomised complete block design based on how many leaves they had, the health of the plant (scores) and how many shoots had reached over two metres.

3.2.6 Treatments

The treatments used in this trial consisted of each herbicide listed in Table 3.1, which were applied to the upper parts of the plants for the first eight treatments and applied to the lower half of plants for the next eight treatments. There were also two control treatments which received no herbicide. There were a total of 18 treatments with four replicates for each treatment.

As the leaves of *C. silvatica* were quite waxy, and applied water beaded and fell off the leaves, 1.0 ml of Boost penetrant was added to each herbicide mixture. This enabled the herbicides to stick better to the leaves and minimised any dripping.

Table 3.1: Summary of herbicides and rates used.

Active ingredient	**Trade name**	**Formulation**	**g ai/100 Litres**
2,4-D	Pasture Kleen	ethyhexyl ester	780
2,4-D/dicamba	Banvine	amine salts	240 + 120
aminopyralid	T-Max	tri-isopropylamine salt	18
clopyralid	Versatill	amine salt	90
fluroxypyr	Starane 200	methylheptyl ester	100
glyphosate	Roundup 360 Pro	isopropylamine salt	540
metsulfuron-methyl	Answer	methyl ester	15
triclopyr/picloram/ aminopyralid	Tordon Brushkiller XT	butoxyethyl ester + amine salt + amine salt	90 + 30 + 2.4

3.2.7 Herbicide application

Each of the 72 plants in the trial had a tie positioned halfway down each plant, with half the leaf mass above the tie in the upper area and half the leaf mass in the lower area. The herbicides were then applied to either the upper section or the lower section.

The herbicides were applied in the afternoon of 21 April 2012 to the leaves with a small paintbrush. The herbicides were made up in one litre lots with 1.0 ml of Boost penetrant. From this, 1.0 ml was measured out, and applied to all of the leaves, both the upper and lower surfaces, and the stems of either the upper or lower portion, depending on the treatment. A new paintbrush was used for each herbicide.

3.2.8 Methods of assessment

The pots were scored one week after herbicide application (28 April 2012). They were then scored weekly until 10 weeks (30 June 2012) after herbicide application.

A score was given to each pot, ranging from 0 if the plant was dead or 10 if the plant was healthy and all the leaves green. A score of 9 was given if 10% of the leaves were brown, yellow or affected and a score of 8 if 10 – 20% of the leaves were affected. A score of 5 was given if the leaves were 50% green/healthy and 50% affected. A score of 2 was given if the only 10 – 20% of the leaves were green and healthy and the rest affected and a score of 1 if there were less than 10% healthy leaves or just a green vine remaining. The whole plant was considered, not just the treated portion.

At each scoring the amount of leaves affected by herbicide, or those that were yellowing, were noted and the percentage of affected leaves within the upper portion then the lower portion were recorded. As it was not possible to determine whether leaves were yellow due to senescence or the effects of herbicide, they were bulked together for this assessment.

On 3 July 2012 all of the 100 pots had all of their aboveground parts cut, whether dead or alive, though most plants had died back before winter by then. The pots were then moved from the glasshouse into an unheated shadehouse. The pots in the shadehouse were then checked on a weekly basis after the transfer to determine whether any new growth in the pots occurred. The first growth was noted on 5 October 2012, and from this date onward the shadehouse was checked on a twice weekly basis and the number of shoots that had emerged in each pot was counted and recorded.

Once quite a few shoots had emerged and some shoots were over 30 cm long, the shoots were counted, had their length measured and were cut at ground level with scissors, and the dry weight of the shoots per pot determined by drying them in a 75^{o}C oven. This first harvest occurred on 6 - 7 December 2012 (32 weeks after treatment). Following this harvest the number of new shoots that emerged were also counted. Once a number of shoots again reached over 30 cm there was a second harvest where shoot number, total shoot length and shoot dry weight was obtained on 8 January 2012 (37 weeks after treatment). Because many of the shoots appeared mottled or unhealthy, the health of those shoots was also scored with 0 if there were no shoots, a score of 1 or 2 if the shoots were severely mottled, disfigured or sick and a score of 5 if they were completely healthy. On 9 January 2012 the roots and rhizomes were removed from the pots and the potting mix washed off. The roots/rhizomes of each pot were divided into three parts: healthy rhizome, healthy fibrous roots and rotten,

necrotic or unhealthy rhizomes or roots. The dry weight of each of these parts for each pot was then determined by drying them in a 75^{o}C oven and weighing them.

3.2.9 Glasshouse and shadehouse temperatures

The temperatures (Table 3.2 and 3.3) were recorded using a data logger which recorded the temperature every ten minutes.

Table 3.2: Summary of glasshouse temperatures during trial period.

Month	**Average temp (^{o}C)**	**Average max temp (^{o}C)**	**Average min temp (^{o}C)**
22 - 29 February	20.0	25.9	16.3
March	19.8	25.1	16.3
April	18.7	23.6	15.0
May	15.0	20.0	10.6
1 - 27 June	14.0	19.5	10.1

Table 3.3: Summary of shadehouse temperatures during trial period.

Month	**Average temp (^{o}C)**	**Average max temp (^{o}C)**	**Average min temp (^{o}C)**
3 - 31 July	8.6	14.1	4.6
August	9.8	16.6	5.7
September	10.1	15.9	6.0
October	12.5	18.7	7.7
November	13.7	20.7	8.7
December	17.9	24.7	13.0
1 – 9 January	17.7	24.7	11.8

3.2.10 Statistical analysis

An analysis of variance was performed on all that had been collected within SAS using a complete block design with a factorial comparison of upper and lower applications. As there was often a significant interaction between the upper and lower treatments for at least one of the herbicides, then the least significant differences were calculated using a randomised complete block design, to separate out the upper and lower effects for each herbicide.

3.3 Results

3.3.1 Initial effects, two weeks after treatment

The initial scores, two weeks after treatment, are shown in Figure 3.1. The two untreated controls were not completely healthy, which would have been a score of 10, because of the senescence and dead leaves on the plants, as they died off before winter (Plate 3.4).

It can also be seen that the upper and lower treatments of 2,4-D, 2,4-D/dicamba, metsulfuron and triclopyr/picloram/aminopyralid and the upper treatment of fluroxypyr all have plants which are significantly affected and less healthy than the plants that were untreated. All other treatments, such as the lower clopyralid treatment (Plate 3.6), were not significantly affected when compared to the untreated controls.

There was also a difference between the upper and lower treatments of fluroxypyr and the upper and lower treatments of 2,4-D/dicamba. In both cases the upper treatments had significantly less healthy plants than their respective lower treatments.

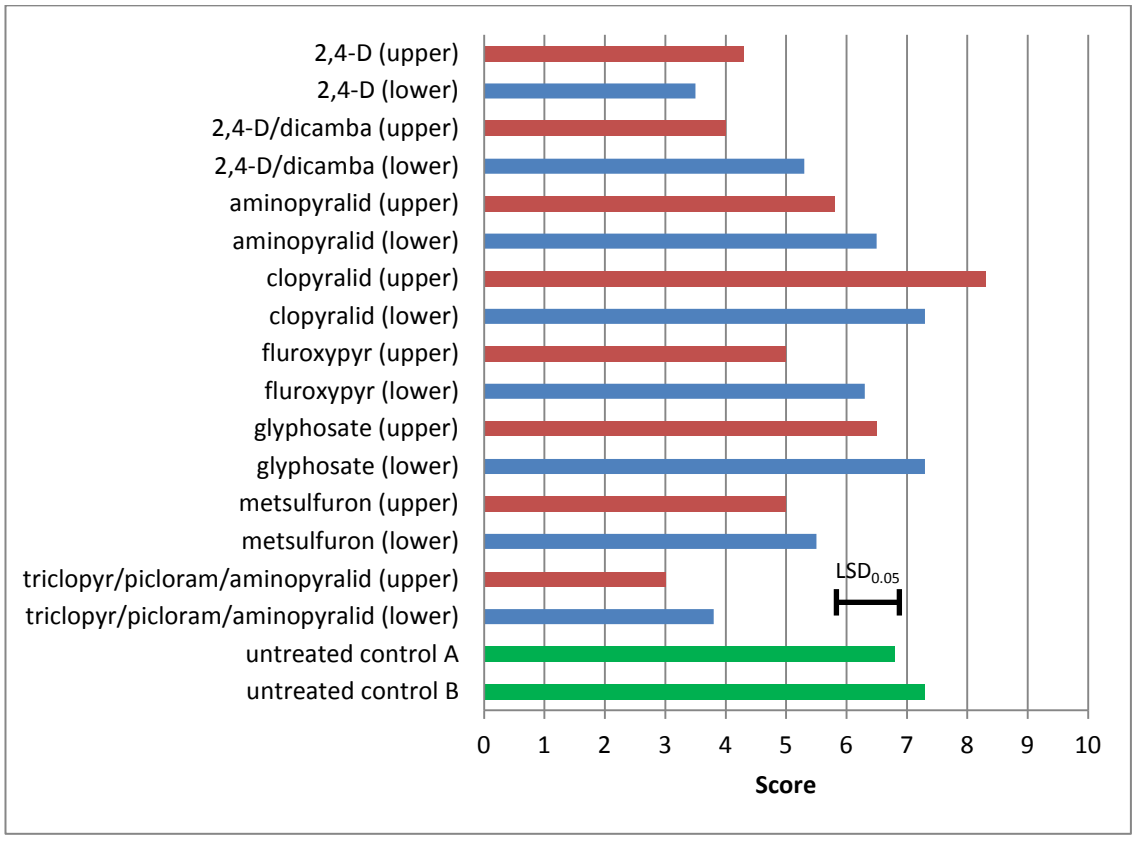

Figure 3.1: Scores (0 = dead, 10 = healthy) for each herbicide treatment at two weeks after herbicide application (5 May 2012).

The amount of leaves affected for each portion of each plant was also measured two weeks after herbicide application and the means are presented in Table 3.4. Both untreated controls had some leaves affected due to senescence. Metsulfuron, 2,4-D, 2,4-D/dicamba and triclopyr/picloram/aminopyralid all quickly affected the entire plant, regardless of which section the herbicide was applied to. Triclopyr/picloram/aminopyralid was one of the treatments in which the whole plant was discoloured, and therefore showed some effect, but was not yet dead or close to death and thus assigned a score of around 4 (Plate 3.5). The fluroxypyr and aminopyralid treatments only showed localised effects.

Table 3.4: The upper and lower percentage of leaves and vines affected for each herbicide treatment at two weeks after herbicide application (5 May 2012). Affected leaves were either senescing, dead, necrotic or severely discoloured. The numbers in grey indicate where herbicide was applied.

Treatment	**Rate (g ai/100 litres)**	**Upper % affected**	**Lower % affected**
2,4-D (upper)	780	100.0	85.0
2,4-D (lower)	780	100.0	100.0
2,4-D/dicamba (upper)	240 +120	100.0	87.5
2,4-D/dicamba (lower)	240 +120	82.5	100.0
aminopyralid (upper)	18	85.0	42.5
aminopyralid (lower)	18	42.5	100.0
clopyralid (upper)	90	17.5	20.0
clopyralid (lower)	90	20.0	32.5
fluroxypyr (upper)	100	100.0	62.5
fluroxypyr (lower)	100	55.0	100.0
glyphosate (upper)	540	36.3	57.5
glyphosate (lower)	540	20.0	45.0
metsulfuron (upper)	15	82.5	93.8
metsulfuron (lower)	15	72.5	87.5
triclopyr/picloram/ aminopyralid (upper)	30 + 90 + 2.4	100.0	100.0
triclopyr/picloram/ aminopyralid (lower)	30 + 90 + 2.4	100.0	100.0
untreated control A	-	25.0	27.5
untreated control B	-	25.0	45.0
LSD (P<0.05)		17.6	25.8

Plate 3.4: One of the untreated controls, taken on 1st May 2012, two weeks after herbicide application to those plants that were treated. This plant was given a score of 8 on the 5th May 2012.

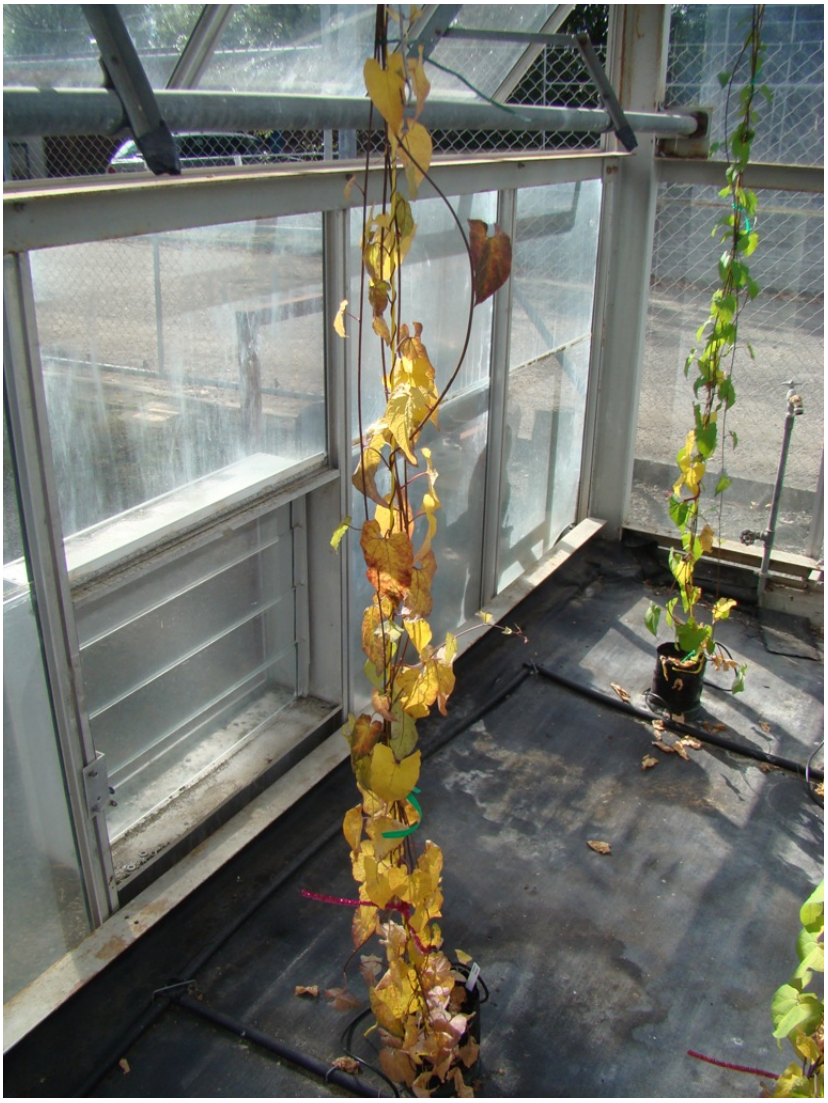

Plate 3.5: The lower triclopyr/picloram/aminopyralid treatment, one of most affected initially, taken on 1st May 2012, two weeks after herbicide application. This plant was given a score of 4 on the 5th May 2012 and 100% of the leaves in both the upper and lower portions showed some effect.

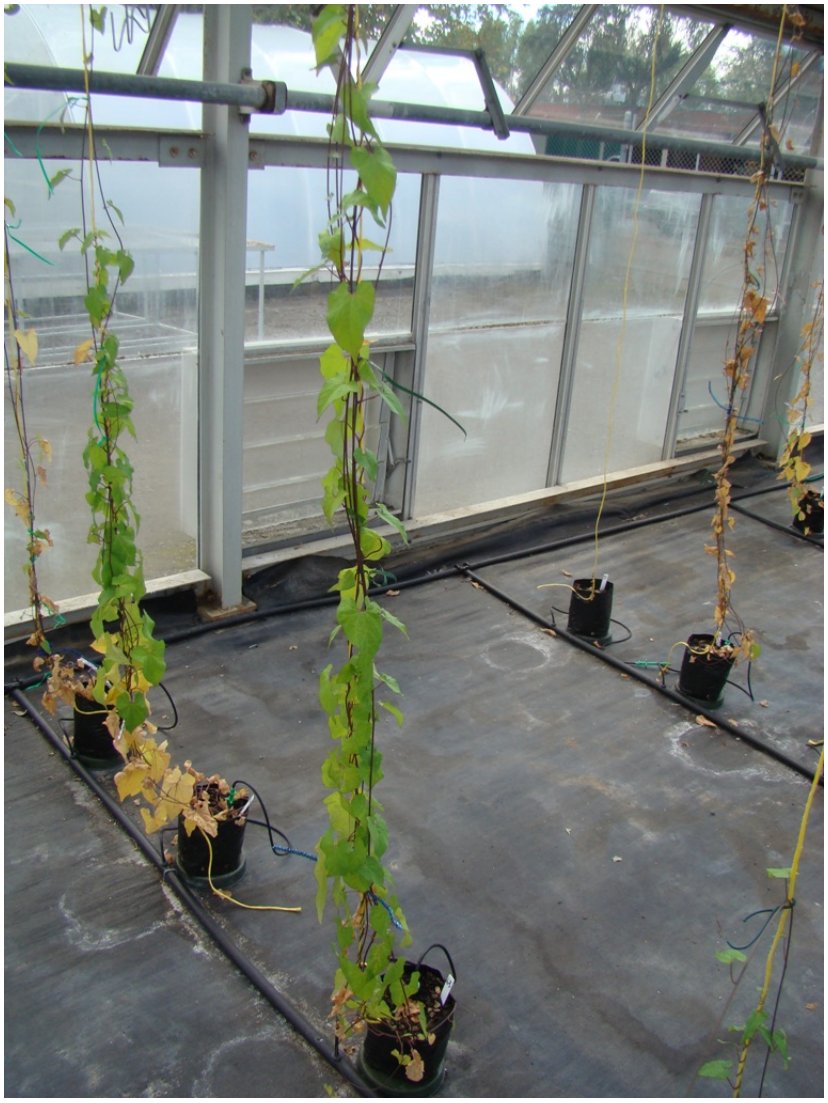

Plate 3.6: Lower clopyralid treatment, one of the treatments least affected two weeks after herbicide application (1 May 2012).

3.3.2 Initial effects, four weeks after treatment

The plants that were untreated were even less healthy at four weeks as the aboveground parts of the *C. silvatica* plants died back before winter dormancy (Figure 3.2). As a result the herbicide effects of the upper and lower treatments of aminopyralid, clopyralid and glyphosate could not be separated out from the normal winter die-back and whereas the lower treatment if 2,4-D/dicamba was significantly less healthier than the untreated controls at 2 weeks, after 4 weeks the same initial herbicide effect could not be separated from winter die-back as the results were not significantly different from the untreated controls at 4 weeks. Those treatments that did have significantly less healthy plants than the untreated controls were the upper and lower treatments of 2,4-D, fluroxypyr, triclopyr/picloram/aminopyralid and metsulfuron and the upper treatment of 2,4-D/dicamba. There were still significant differences between the upper and lower treatments of 2,4-D/dicamba at 4 weeks.

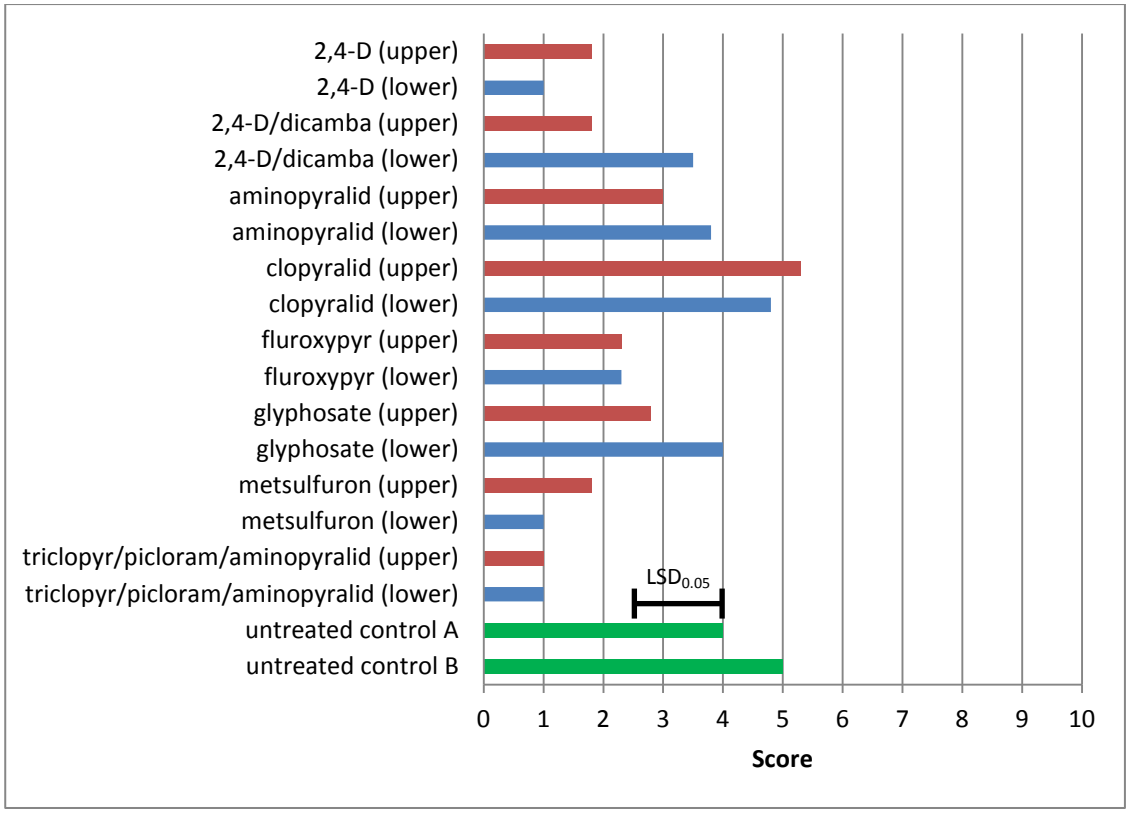

Figure 3.2: Scores (0 = dead, 10 = healthy) for each herbicide treatment at four weeks after herbicide application (19 May 2012).

After 4 weeks both untreated controls had more leaves affected due to senescence (Table 3.5). Almost all the leaves and plants were also affected, to some degree, in the metsulfuron, 2,4-D, fluroxypyr and triclopyr/picloram/aminopyralid treatments, regardless of which section the herbicide was applied to. For some of the treatments, particularly 2,4-D/dicamba, the percentage of leaves and vines affected decreased as those leaves initially counted as affected or dead fell off the vines, leaving only those which may be healthier and this decreased the total amount of the portion affected. The upper treatment of glyphosate showed a higher percentage of affected leaves on the untreated portion rather than the treated portion as those leaves in the lower half were more likely to show the effects of the plants dying off before winter and this effect was not able to be fully separated from the herbicide affects. The aminopyralid treatments still only show localised herbicide effects.

Table 3.5: The upper and lower percentage of leaves and vines affected for each herbicide treatment at four weeks after herbicide application (19 May 2012). Affected leaves were either senescing, dead, necrotic or severely discoloured. The numbers in grey indicate where herbicide was applied.

Treatment	Rate (g ai/100 litres)	Upper % affected	Lower % affected
2,4-D (upper)	780	100.0	85.0
2,4-D (lower)	780	100.0	100.0
2,4-D/dicamba (upper)	240 +120	97.5	87.5
2,4-D/dicamba (lower)	240 +120	65.0	100.0
aminopyralid (upper)	18	87.5	62.5
aminopyralid (lower)	18	50.0	100.0
clopyralid (upper)	90	60.0	45.0
clopyralid (lower)	90	52.5	80.0
fluroxypyr (upper)	100	97.5	75.0
fluroxypyr (lower)	100	87.5	100.0
glyphosate (upper)	540	75.0	95.0
glyphosate (lower)	540	60.0	95.0
metsulfuron (upper)	15	100.0	92.5
metsulfuron (lower)	15	100.0	100.0
triclopyr/picloram/ aminopyralid (upper)	30 + 90 + 2.4	100.0	100.0
triclopyr/picloram/ aminopyralid (lower)	30 + 90 + 2.4	100.0	100.0
untreated control A	-	52.5	47.5
untreated control B	-	42.5	77.5
LSD (P<0.05)		20.8	20.8

3.3.3 Eight weeks after treatment, 16 June 2012

After eight weeks the aboveground parts of the plants in the untreated controls were near death (Figure 3.3) and as a result even less herbicide effects could be separated out from normal winter die-back. Only the lower treatments of metsulfuron and the upper and lower treatments of triclopyr/picloram/aminopyralid had significantly less healthy plants than the untreated control plants. One of the other significant differences is between the health of the plants in the upper and lower treatments of fluroxypyr where the upper treatment had significantly healthier plants than the plants treated on the lower portion with fluroxypyr.

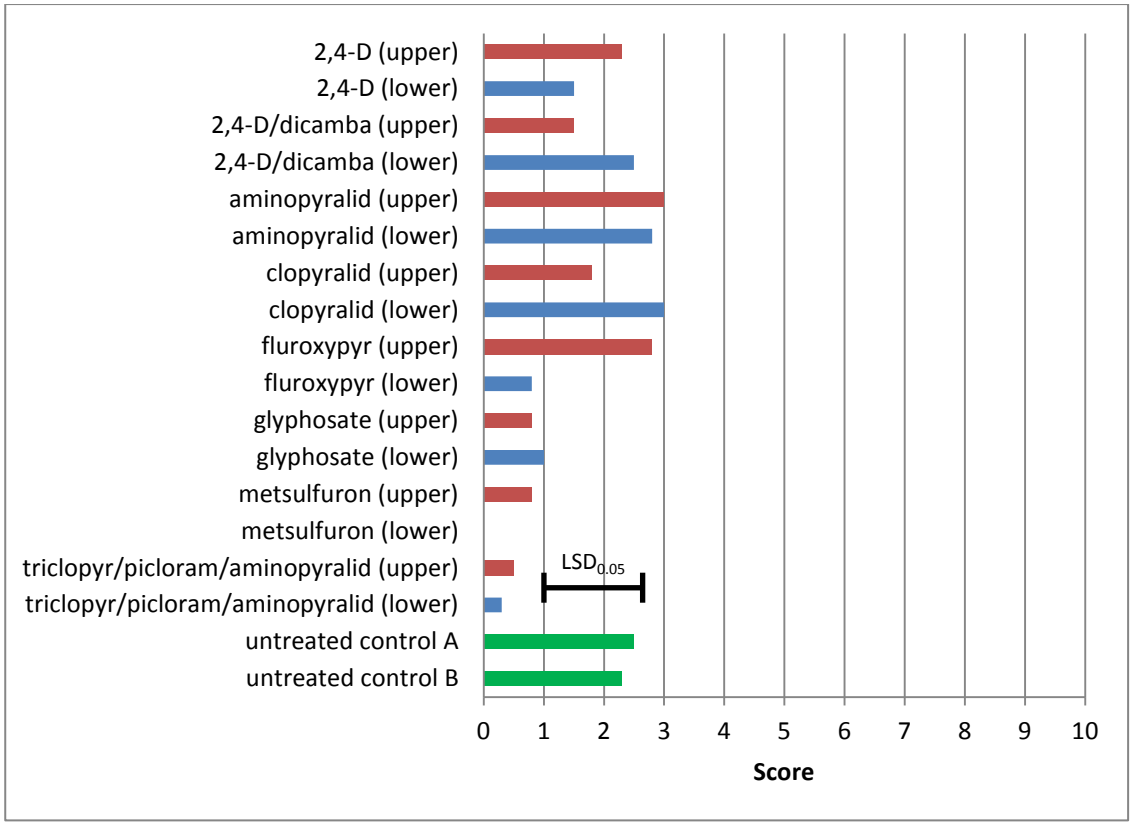

Figure 3.3: Scores (0 = dead, 10 = healthy) for each herbicide treatment at eight weeks after herbicide application (16 June 2012).

By eight weeks most of the leaves and vines were affected (Table 3.6) as all of the plants, including the untreated controls, were dead or near death (Figure 3.3) because the plants were dying back before dormancy or because of the herbicide treatments. Only the aminopyralid (upper) and clopyralid (lower) treatments showed localised effects where the untreated portion of those plants were significantly less affected and healthier than the untreated controls (Table 3.6).

Table 3.6: The upper and lower percentage of leaves and vines affected for each herbicide treatment at eight weeks after herbicide application (16 June 2012). Affected leaves were either senescing, dead, necrotic or severely discoloured. The numbers in grey indicate where herbicide was applied.

Treatment	Rate (g ai/100 litres)	Upper % affected	Lower % affected
2,4-D (upper)	780	100.0	67.5
2,4-D (lower)	780	100.0	77.5
2,4-D/dicamba (upper)	240 +120	97.5	85.0
2,4-D/dicamba (lower)	240 +120	70.0	100.0
aminopyralid (upper)	18	97.5	52.5
aminopyralid (lower)	18	70.0	100.0
clopyralid (upper)	90	97.5	80.0
clopyralid (lower)	90	52.5	92.5
fluroxypyr (upper)	100	100.0	70.0
fluroxypyr (lower)	100	95.0	100.0
glyphosate (upper)	540	95.0	100.0
glyphosate (lower)	540	97.5	97.5
metsulfuron (upper)	15	100.0	97.5
metsulfuron (lower)	15	100.0	100.0
triclopyr/picloram/ aminopyralid (upper)	30 + 90 + 2.4	100.0	100.0
triclopyr/picloram/ aminopyralid (lower)	30 + 90 + 2.4	100.0	100.0
untreated control A	-	80.0	75.0
untreated control B	-	85.0	87.5
LSD (P<0.05)		17.6	21.7

3.3.4 Regrowth at 24 and 28 weeks after treatment

By 24 weeks (10 October 2012) a lot more shoots had started to emerge in the metsulfuron treated pots than in any other treatment or in the untreated pots (Table 3.7). The metsulfuron upper treatment also had more shoots emerge than the metsulfuron lower treatment.

After 28 weeks (7 November 2012) there were even more emerged shoots in the metsulfuron treated pots and still significantly more shoots than the untreated controls which had now started to emerge. The 2,4-D lower treatment also had significantly more shorts emerge than the untreated controls. Many of the other treatments had no shoots emerge at all by this stage, or very few, although this was not significantly different from the untreated controls.

Table 3.7: Average number of emerged shoots per pot for each herbicide at 24 and 28 weeks after treatment (WAT) with herbicide application.

Treatment	Rate (g ai/100 litres)	10/10/12	7/11/12
		24 WAT	28 WAT
2,4-D (upper)	780	0.0	1.5
2,4-D (lower)	780	0.3	2.3
2,4-D/dicamba (upper)	240 +120	0.0	0.3
2,4-D/dicamba (lower)	240 +120	0.0	0.0
aminopyralid (upper)	18	0.0	0.0
aminopyralid (lower)	18	0.0	0.0
clopyralid (upper)	90	0.0	0.5
clopyralid (lower)	90	0.0	0.8
fluroxypyr (upper)	100	0.0	0.3
fluroxypyr (lower)	100	0.0	0.3
glyphosate (upper)	540	0.0	0.0
glyphosate (lower)	540	0.0	0.3
metsulfuron (upper)	15	1.3	2.5
metsulfuron (lower)	15	0.5	1.8
triclopyr/picloram/ aminopyralid (upper)	30 + 90 + 2.4	0.0	0.3
triclopyr/picloram/ aminopyralid (lower)	30 + 90 + 2.4	0.0	0.0
untreated control A	-	0.0	1.0
untreated control B	-	0.0	0.3
LSD (P<0.05)		0.4	0.8

3.3.5 Shoot harvest at 32 weeks and shoot and root harvest 37 weeks after herbicide application

After 32 weeks there were still several pots which had no shoots emerge. No shoots had emerged in any of the 2,4-D/dicamba (lower) treated pots and most of the pots treated with 2,4-D/dicamba (upper), aminopyralid (upper and lower) and triclopyr/picloram/aminopyralid had no emerged shoots. One pot treated with glyphosate (upper) also had no new shoots.

The 2,4-D, clopyralid, fluroxypyr and metsulfuron treated pots showed a similar amount of regrowth to pots that were untreated (Table 3.8). Those pots that had glyphosate applied to the lower portion of the plant had significantly more shoots emerge than the untreated controls and significantly more shoots than the glyphosate (upper) treated pots. The grams of dry weight per pot per treatment showed a similar

result, with many of the treatments having the same amount of shoots emerge as the untreated controls.

The 2,4-D upper and lower treatments also had longer shoots than the untreated controls. All other treatments were not significantly different from the untreated controls, even those treatments which had few or no shoots emerge. This would be because of the variability in the results.

Table 3.8: Average number of emerged shoots, grams of dry weight per pot and shoot total length for each herbicide at 32 weeks after herbicide application.

Treatment	Rate (g ai/100 litres)	Emerged shoots	gDW/pot	Shoot total length (cm)
2,4-D (upper)	780	5.5	0.73	73.0
2,4-D (lower)	780	6.8	0.93	86.9
2,4-D/dicamba (upper)	240 +120	0.3	0.03	1.1
2,4-D/dicamba (lower)	240 +120	0.0	0.00	0.0
aminopyralid (upper)	18	0.3	0.00	1.0
aminopyralid (lower)	18	0.5	0.03	3.6
clopyralid (upper)	90	2.8	0.43	30.1
clopyralid (lower)	90	3.8	0.35	20.4
fluroxypyr (upper)	100	5.5	0.58	55.1
fluroxypyr (lower)	100	3.3	0.33	24.0
glyphosate (upper)	540	1.3	0.03	4.0
glyphosate (lower)	540	8.0	0.45	43.5
metsulfuron (upper)	15	5.3	0.73	47.0
metsulfuron (lower)	15	3.3	0.58	38.3
triclopyr/picloram/ aminopyralid (upper)	30 + 90 + 2.4	1.5	0.20	19.1
triclopyr/picloram/ aminopyralid (lower)	30 + 90 + 2.4	0.8	0.00	5.9
untreated control A	-	5.0	0.63	41.0
untreated control B	-	4.0	0.53	33.2
LSD (P<0.05)		2.0	0.32	35.4

After 37 weeks most of the pots had new shoots within them except for a few in the upper and lower 2,4-D/dicamba, aminopyralid and triclopyr/picloram/aminopyralid treatments. One pot treated with glyphosate (upper) also remained empty. The results of the second harvest (Table 3.9) also showed that there were less shoots in the 2,4-D/dicamba, aminopyralid and triclopyr/picloram/aminopyralid upper and lower treatments than in the untreated controls. They also weighed less and were not as long as the untreated control shoots. The other treatments showed similar results to

those found in the first harvest (Table 3.8) and were not significantly different from the untreated controls with most of the measurements. Both the lower glyphosate and the upper fluroxypyr treatments showed particularly poor results.

By the second harvest many shoots appeared mottled or sick looking and thus the health of those shoots was also scored (Table 3.9). Those shoots in the 2,4-D/dicamba (lower) and aminopyralid (upper and lower) treatments were severely mottled or sick and they were significantly sicker than those shoots in the untreated controls.

In many of the measurements (Table 3.9) there were also significant differences between the upper and lower glyphosate treatments and the lower glyphosate treatment had the most regrowth out of all of the treatments.

Table 3.9: Average number of emerged shoots, shoot health scores (0 = no shoots, 5 = healthy), grams of dry weight per pot and shoot total length for each herbicide at 37 weeks (8 January 2013) after herbicide application.

Treatment	**Rate (g ai/100 litres)**	**Emerged shoots**	**Health of shoots**	**gDW /pot**	**Shoot total length (cm)**
2,4-D (upper)	780	7.0	3.8	0.54	70.9
2,4-D (lower)	780	5.8	4.0	0.54	67.9
2,4-D/dicamba (upper)	240 +120	0.5	1.8	0.03	8.1
2,4-D/dicamba (lower)	240 +120	1.0	1.0	0.05	16.4
aminopyralid (upper)	18	0.8	1.3	0.06	5.9
aminopyralid (lower)	18	1.5	1.0	0.15	17.4
clopyralid (upper)	90	4.5	3.0	0.60	58.6
clopyralid (lower)	90	5.0	3.5	0.78	105.6
fluroxypyr (upper)	100	8.8	4.3	0.94	115.3
fluroxypyr (lower)	100	6.0	3.0	0.77	93.9
glyphosate (upper)	540	4.5	2.0	0.40	65.5
glyphosate (lower)	540	9.8	3.3	1.27	127.5
metsulfuron (upper)	15	5.5	4.0	0.64	63.6
metsulfuron (lower)	15	4.8	3.3	0.43	37.0
triclopyr/picloram/ aminopyralid (upper)	30 + 90 + 2.4	2.0	2.3	0.24	28.9
triclopyr/picloram/ aminopyralid (lower)	30 + 90 + 2.4	0.5	2.3	0.01	2.4
untreated control A	-	5.3	3.3	0.74	76.6
untreated control B	-	4.8	3.5	1.04	116.9
LSD (P<0.05)		2.7	1.8	0.39	45.6

The upper clopyralid treatment had a higher total root /rhizome weight than the untreated control (Table 3.10). The total underground organ weight for the upper and lower treatments of 2,4-D, 2,4-D/dicamba, metsulfuron and triclopyr/picloram/aminopyralid were lower than the weights found for the untreated controls. Those same treatments also had a significantly lower amount of healthy rhizomes than the untreated controls. When the fibrous roots are compared only the upper clopyralid treatment is significantly different from the untreated controls and it had a higher amount of fibrous roots. Only the lower 2,4-D/dicamba treatment and the upper triclopyr/picloram/aminopyralid treatment (Plate 3.7) had a higher amount of rotten parts than the untreated controls (Plate 3.8). All other treatments were not significantly different from the untreated controls.

Table 3.10: The quantity (g DW/pot) of healthy rhizome, fibrous roots and necrotic tissue found within pots 37 weeks after herbicide application (9 January 2013).

Treatment	**Healthy rhizomes (g)**	**Fibrous roots (g)**	**Rotten parts (g)**	**Total (g)**
2,4-D (upper)	5.09	0.51	0.06	5.66
2,4-D (lower)	4.25	1.39	0.34	5.99
2,4-D/dicamba (upper)	1.49	0.37	0.39	2.25
2,4-D/dicamba (lower)	3.25	1.33	0.65	5.22
aminopyralid (upper)	9.67	1.81	0.04	11.52
aminopyralid (lower)	6.48	1.58	0.11	8.16
clopyralid (upper)	15.21	2.55	0.11	17.88
clopyralid (lower)	9.33	0.94	0.13	10.40
fluroxypyr (upper)	8.83	0.92	0.21	9.96
fluroxypyr (lower)	7.43	2.12	0.18	9.72
glyphosate (upper)	8.34	1.30	0.17	9.81
glyphosate (lower)	12.95	2.01	0.06	15.01
metsulfuron (upper)	4.64	0.58	0.39	5.61
metsulfuron (lower)	2.71	0.41	0.53	3.64
triclopyr/picloram/ aminopyralid (upper)	2.30	1.34	0.69	4.33
triclopyr/picloram/ aminopyralid (lower)	0.18	1.52	0.56	2.26
untreated control A	10.69	1.20	0.24	12.13
untreated control B	10.80	1.12	0.25	12.18
LSD (P<0.05)	4.52	1.14	0.36	5.26

Plate 3.7: The healthy rhizome and the rotten parts of a plant that received the upper treatment of triclopyr/picloram/aminopyralid. No healthy fibrous material was present.

Plate 3.8: Healthy rhizomes, healthy fibrous roots and the rotten parts of an untreated control.

3.4 Discussion

3.4.1 Initial herbicide effects

Like the field trial (Chapter 2), the herbicides were applied when the plants were beginning to senesce as this was thought to be the best time to apply the herbicides as the plant was most likely to be transporting sugars to the underground organs as the leaves were senescing. Thus the herbicides would also likely be translocated to the underground organs. This senescence and the dying off of the aboveground parts is also the reason why these initial results do not show the full effects of the herbicides as the normal death of the aboveground parts of the plant would affect the health of the plant and not be able to be fully separated from the herbicide effects when scoring. Furthermore the initial measurements are only able to measure the aboveground effects of the herbicides and not whether the herbicides have affected the belowground organs, which for *C. silvatica* is the most important part to target as

that is where regrowth is likely to occur from. However some information about the initial effects of the herbicides on *C. silvatica* was obtained.

Triclopyr/picloram/aminopyralid was the fastest and most effective herbicide applied to affect the health of *C. silvatica* initially. Whether applied to the upper portion or lower portion the entire plant was 100% affected two weeks after herbicide application. The same results were found in the field trial (Chapter 2).

Metsulfuron and 2,4-D were the next best herbicides to affect *C. silvatica* initially and the treated and untreated portions were quickly affected. Metsulfuron also initially affected the plants in the field trial (Chapter 2), although it appears as though the plants were affected faster in the glasshouse than in the field. Hornby (2011) found that *C. silvatica* was affected by metsulfuron initially and Heering & Peeper (1991) found metsulfuron affected *C. arvensis* by 80 – 96% eight weeks after treatment, depending on the rate used. The trial presented in this chapter found that metsulfuron had initially affected *C. silvatica* by a similar amount eight weeks after herbicide application. Unlike metsulfuron, 2,4-D was not trialled in the field trial (Chapter 2) due to limited plots available.

Fluroxypyr and 2,4-D/dicamba also significantly affected the *C. silvatica* plants initially, including the untreated portions. When applied to established plants in the field (Chapter 2), 2,4-D/dicamba was one of the most effective herbicides although the untreated portion was affected slower than the rest of the plant. In the current trial 2,4-D/dicamba affected the untreated portion quicker. This could be because the plants were smaller than those in the field or because a set dose was applied to all of the leaves in the treated portion with the addition of surfactant.

In this trial glyphosate showed slower effects than in the field trial (Chapter 2). This could be because those plants in the glasshouse only had herbicide applied to 50% of the plant, whereas those plants in the field had herbicide applied to 90% of the plant, and those plants in the glasshouse only had 1 ml of herbicide applied, which may not have been a fatal dose. Rahman and Sanders (1992) also found that glyphosate only provided some initial control of *C. silvatica* and Matic and Black (1994) only found some knockback initially in a trial on *C. arvensis*.

The two remaining treatments, aminopyralid and clopyralid, both showed poor initial results. Aminopyralid did not affect the *C. silvatica* plants as significantly as the other treatments, and only showed localised herbicide effects on the leaves and vines. Those plants treated with aminopyralid in the field (Chapter 2) showed better and faster results than those found in the glasshouse. Again this is likely due to the fact that the herbicide was applied to 90% of the plant in the field and only 50% in the glasshouse. Clopyralid also showed poor results initially and the plants were often as healthy as

those in the untreated controls. The field trial (Chapter 2) showed better results and where the health of the plants was affected faster. This difference could be because the glasshouse plants only had herbicide applied to half the plant. Other trials have also found that clopyralid shows only some knockback of *C. silvatica* (Rahman & Sanders 1992), *C. sepium* (Rahman et al. 2002) and *C. arvensis* (Matic & Black 1994).

3.4.2 *C. silvatica* spring regrowth

The pots were moved to the shadehouse after the initial measurements. While the pots were in the shadehouse new shoots did not emerge until 5 October 2012, a considerable period of time after shoots had emerged in the field (22 August 2012), as seen in Chapter 2. This could be because those plants in the shadehouse were smaller and thus had less reserves or because they were in damper conditions with less exposure to full sunshine than those plants in the field. In the shadehouse the pots were also more exposed to colder temperatures than they were when they were in the glasshouse. This could have delayed new shoot growth. The shoot growth may also have been delayed because the plants were grown quite late and their normal growth pattern may have been disrupted because of this. There were also few shoots in the untreated control 24 and 28 weeks after herbicide application and as a result few treatments were significantly different from the untreated control.

Shoots emerged sooner in the metsulfuron treatments than in any other treatment. The same was found in the field (Chapter 2) where more shoots had emerged in the metsulfuron treatments by 22 weeks than any other treatment. Heering and Peeper (1991) and Matic and Black (1994) found that *C. arvensis* also grew back after treatment with metsulfuron. Metsulfuron may have stimulated the shoots to regrow early, but further investigation would be required to confirm this. After 28 weeks, the pots in which 2,4-D was applied to the lower portion of *C. silvatica*, also had more shoots emerge than the untreated controls. Other trials which used 2,4-D found that while it can provide good initial control of *C. arvensis* there is significant regrowth in most cases (Wiese & Lavake 1986; Schoenhals et al. 1990; Matic & Black 1994).

Because the regrowth occurred later in the season, the first two initial regrowth measurements do not show the whole picture about which treatments gave long term control of *C. silvatica*. Those measurements taken at 32 and 37 weeks however show a more accurate picture of the long lasting effects of the herbicides on *C. silvatica* as the shoots had emerged in most cases and accurate measurements were able to be taken and at 37 weeks the roots and rhizomes were able to be destructively sampled to show the effects the herbicides had on the roots and rhizome.

After 32 and 37 weeks had passed several pots treated with 2,4-D/dicamba, aminopyralid and triclopyr/picloram/aminopyralid had no new shoots emerge. These

three herbicides therefore showed long lasting control of *C. silvatica* and were the three best treatments out of those tested in the current trial. These results were better than what was found in the field trial (Chapter 2) where although there was some long term control, by 33 weeks these three treatments had several plants regrow. This difference is probably because those plants in the field would have had more extensive root systems than those smaller plants in the glasshouse. However these results do show that these three herbicides show potential for the control of *C. silvatica* and should be investigated further.

These results are similar to what has been found on *C. silvatica* in a separate trial. Hornby (2011) found that triclopyr/picloram and aminopyralid showed long term control of *C. silvatica*.

The other herbicides didn't show the same level of control and for the most part the upper and lower treatments of the herbicides showed similar results to the untreated controls and thus there was little control of the *C. silvatica* and its below ground parts. Previous trials with 2,4-D showed only partial control of *C. arvensis* long term as there was regrowth (Wiese & Lavake 1986; Schoenhals et al. 1990; Matic & Black 1994). The current trial found similar results, despite the species differences. *C. arvensis* also grew back after applications of fluroxypyr (Matic & Black 1994), as did the *C. silvatica* in the current trial. Neither 2,4-D nor fluroxypyr were trialled in the field due to limited available plants. Clopyralid, glyphosate and metsulfuron were trialled in the field (Chapter 2) and all three herbicides showed similar efficacy on *C. silvatica* in the field as in the glasshouse. Trials using metsulfuron, clopyralid and glyphosate also found that *C. arvensis* (Wiese & Lavake 1986; Heering & Peeper 1991; Matic & Black 1994), *C. sepium* (Rahman et al. 2002) and *C. silvatica* (Rahman & Sanders 1992; Hornby 2011) usually grew back after herbicide treatment.

3.4.3 The underground organs in spring

In order for control to be successful it is the underground organs of *C. silvatica* which need to be affected. The original rhizomes planted into the pots in the beginning of the experiment weren't weighed or identical, however the results do still show clear distinctions between the amount of rhizomes, rot and fibrous content of each treatment. Both the triclopyr/picloram/aminopyralid and 2,4-D/dicamba treatments had smaller rhizomes than the untreated controls. As did the 2,4-D and metsulfuron treatments. This could be either because the rhizomes were affected by the herbicides or because the amount of rhizome was less because of the effort required to put up new shoots in spring. In the case of the triclopyr/picloram/aminopyralid and 2,4-D/dicamba treatments the rhizomes were likely to have been affected by the herbicides as so few shoots grew in spring and therefore they were unlikely to be reduced from regrowth. The 2,4-D and metsulfuron treatments both grew a significant

number of shoots by the time of the 37 week harvest and these shoots were cut, forcing the plant to send up new shoots. This would have resulted in such small rhizomes as the plant was continuously putting up new shoots but was unable to send sugars back to the rhizomes as the shoots were too small and were harvested. If either 2,4-D or metsulfuron were applied to *C. silvatica* in autumn and there was regrowth in spring, then the rhizomes could be quite exhausted and small, which may have occurred in the current trial, and a second herbicide application, before the rhizomes are able to recover, could fully control *C. silvatica*.

The amount of rot or necrotic parts of the underground organs was also measured and the results showed that all of the treatments including the untreated controls had some rotten or necrotic parts. This could be because conditions were too damp, a pathogen was present, or because parts of the rhizomes and roots are normally affected and exhausted after new shoots have emerged. Further investigation would be needed to confirm this.

Only the treatment where 2,4-D/dicamba was applied to the lower portion and the treatment where triclopyr/picloram/aminopyralid was applied to the upper portion was there significantly more rotten parts than either of the untreated controls. Both herbicides could have affected the underground organs and caused this damage, but further investigation would be needed in order to confirm this. No trial could be found which looked at the below ground effects of these herbicides on *C. silvatica* to compare these results to.

3.4.4 Comparison between upper and lower treatments

When each of the different measurements were compared there were some differences on some occasions, between the treatments applied to the upper portion and the treatments applied to the lower portion of the *C. silvatica* plants, for a particular herbicide. However most of these differences were sporadic and not consistent. The hypothesis was that, at the time of herbicide application, only the basal leaves may be translocating sugars to the nearest sink which would be the rhizomes and roots, whereas the upper portion would still be translocating sugars to the growing tips, the closest sink. However this appears to have not been the case. Spring or summer applications could show that herbicide applications to the basal leaves are best, as the upper portions of the plants would still be translocating sugars to the tips of the plants.

There was only one herbicide in which there were consistent differences between the upper and lower treatments. When glyphosate was applied to the lower portion of the plant significantly more shoots emerged than when the glyphosate was applied to the upper portion of the plant. There was also a significant difference in the total amount

of underground organs and rhizomes and it could be concluded that treatment of glyphosate to the upper portion of *C. silvatica* showed better control in this case than applications of glyphosate to the lower portions of the plants. Why this occurred is not easily explained. This effect is possibly because when the herbicides were applied to the basal leaves, where many leaves were already were senescing and yellowing, the leaves dropped off quickly and therefore less herbicide reached the roots and rhizomes, whereas the upper portions of the plants had less yellow leaves and therefore more herbicide may have been absorbed. However this same effect was not found for any of the other herbicides.

3.4.5 Summary

The glasshouse trial was completed to give a more accurate picture of the effects of a range of translocated herbicides on *C. silvatica*, which is not influenced by other factors such as the size of the plants, the unknown amount of established roots and rhizomes, competition from other plants and weather conditions such as frosts, as they were in the field. Those plants in the current trial were all of a similar size and had a similar amount of rhizomes and root systems potted. There was unavoidable insect damage to both plants in the field and glasshouse, however those plants in the glasshouse had less damage.

This trial showed that no herbicide gave 100% complete control of *C. silvatica*, however 2,4-D/dicamba, triclopyr/picloram/aminopyralid and aminopyralid did show some long lasting control. The other herbicides did not. Similar results were found in the field trial (Chapter 2). The efficacy of these herbicides could be improved if the herbicides were applied at a higher rate or if there was a second follow-up herbicide application. This will be further discussed in Chapter 6.

Application to either the basal leaves or the upper portion was also measured to test whether herbicide could be applied to just a portion of the plant, not the whole plant, and still show the same level of efficacy, and to test which portion is best, to encourage minimal herbicide application so as to avoid as much off target herbicide damage as possible if the herbicides were to be used in riparian zones. The results did not show, for these herbicides, that applying to the basal leaves provided better control. There were a few significant differences between the upper and lower treatments in some of the results but not much of an overall difference, except for glyphosate where it was application to the upper portion that showed better control than herbicide application to the basal leaves.

The use of these herbicides in riparian zones and the implications of these results will be further discussed in Chapter 6.

CHAPTER 4: Aspects of *Calystegia silvatica* seed biology

4.1 Introduction

Calystegia silvatica seeds may need to be scarified before germination can occur. Brummitt (1963) found that more *C. silvatica* seeds, which had a small piece of testa removed, germinated in potting compost than those with no testa removed manually. Both *Convolvulus arvensis* and *Calystegia sepium* are known to produce seed (Weaver & Riley 1982; Parsons & Cuthbertson 2001; Williams 2009; Popay et al. 2010) which have a hard seed coat that needs to be broken by mechanical means or with acids (Steinbauer & Grigsby 1959; Weaver & Riley 1982; Parsons & Cuthbertson 2001). *Calystegia tuguriorum* also needs to have its impermeable seed coat broken for germination to occur (Burrows 1996). *C. arvensis* seeds are also said to germinate at a wide range of temperatures from 5^{o}C to 40^{o}C, with the best germination occurring when temperatures alternated between 35^{o}C and 20^{o}C (Brown & Porter 1942).

There is limited information available about *Calystegia silvatica* seeds and the conditions under which they can germinate. These experiments seek to address this gap in the knowledge by assessing the viability and germination conditions of *C. silvatica* seeds. The three objectives here are:

- To determine how frequently viable seeds are formed within *C. silvatica* flowers
- To determine how best to break the dormancy of *C. silvatica* seed
- To find how much viable seed is present in the soil under dense infestations of *C. silvatica*

4.2 Sampling of *Calystegia silvatica* seeds from established plants

4.2.1 Materials and methods

The seeds were sourced in June 2012 from *Calystegia silvatica* plants growing over trees, shrubs and the ground in a waste area next to the Turitea Stream on Poultry Farm Road 2 km south of Palmerston North.

Flower heads and leaves were taken from the plants that were used to source the seeds. Each of the flowers and leaves was measured and the taxonomic key (Figure 1.1) used to determine the species and subspecies. The flowers sampled from the plants had 68 – 75 mm long, white corollas. Stamen length ranged from 28 – 31 mm long. The bracteoles of the flowers overlapped and the width of those bracteoles were of equal or almost equal size of the length of the bracteoles. The leaves measured over 55 mm and were triangular or arrow shaped. The key (Figure 1.1) identifies this species as *Calystegia silvatica* sub sp. *disjuncta*, the same as that trialled in both the field and glasshouse trials (Chapters 2 and 3).

Any seed capsules collected that did not appear to be full (Plate 4.1) or were open (Plate 4.2) were discarded. Each of the unopened seed capsules that appeared to be full (Plate 4.2) was opened and the seeds counted and assessed as being healthy, shrivelled or very shrivelled (Plate 4.3), underdeveloped, insect damaged or had evidence of fungi. Healthy seeds were black, full, not soft and without holes or major cuts. Brown seeds that were of a similar size, not soft and without holes or major cuts were also counted as healthy seeds, although they may be less mature than the black seeds (Brummitt 1963). Shrivelled and very shrivelled seeds were identified as not being full, smaller and were wrinkly. A seed was considered to be insect damaged if the seeds had holes, were hollow or had evidence of insect activity (frass and webbing). Seeds were categorised as being affected by fungi if they were covered in pink or white fungal material. Those seeds identified as underdeveloped were generally brown and soft to the touch. A total of 550 full, unopened seed capsules were assessed and the percentage of seeds in each category was calculated. The seeds were also weighed and the 1000 seed weight calculated.

Plate 4.1: *C. silvatica* seed heads from established plants that were avoided while collecting seeds as they were shown to be empty of seed when dissecting initially.

Plate 4.2: *C. silvatica* seed heads from an established plant. The far left seed capsule was open and therefore discarded. The other four seed capsules were full and whole and therefore sampled.

Plate 4.3: A range of *C. silvatica* seeds. The seed on the far left is very shrivelled and the seed next to it is shrivelled. The centre seed has fungi present and the two seeds on the right are considered to be healthy, normal seeds.

4.2.2 Results

There was an average of 2.3 seeds per full capsule. Of the total amount of seeds, 64.4 % of them were healthy, 6.2 % were underdeveloped, 5.2 % were shrivelled and 10.5 % very shrivelled. There were also 6.1 % of seeds with evidence of insect activity and 7.6 % seeds with evidence of fungal material.

The 1000 seed weight for the seeds was measured as 43.4 grams.

During collection it was also observed that bumble bees and honey bees visit *Calystegia silvatica* flowers.

4.2.3 Discussion

The sampling of seeds from established *C. silvatica* shows that *C. silvatica* plants can produce a number of seeds, an average of 2.3 per capsule. Whether this was an exceptional year or normal seed production cannot be said because the plants where only sampled in one season.

Out of thefull seed heads that were sampled it can be seen that some appeared healthy and therefore it is possible that each *C. silvatica* flower could put 1.5 healthy seeds on average into the soil beneath the plants.

A good portion also appeared unhealthy through development problems and the rest of the seeds had the presence of another organism. Although the example here does not show that a high percentage of seeds were affected by insects it does indicate that there is some predation of *C. silvatica* seeds.

The seeds of *C. silvatica* are large and bigger than *Convolvulus arvensis*, which has a 1000-seed weight of 9.8 g and *Solanum nigrum*, or black nightshade, which has a 1000-seed weight of 0.8 g (Benvenuti 2007). *C. silvatica* seeds are also bigger than those of *Ulex europaeus* (gorse) which has a 1000-seed weight of 6.2 g (Harrington et al. 2011). *C. silvatica* seeds have a similar weight to *Calystegia soldenella* seeds which have a 1000-seed weight of 37.9 g (Yang et al. 2012).

It was also observed during sampling that honey bees and bumble bees visit the *Calystegia* flowers and it is likely that these are the two main insects which pollinate *C. silvatica* in New Zealand. Other insects could include flies or wasps (Brummitt 1963).

4.3 The germination conditions of *Calystegia silvatica* seed

4.3.1 Materials and methods

The seeds identified as healthy and normal while sampling (Section 4.2), were used to test the germination conditions for *C. silvatica* seed

All of the seeds used in the germination tests were dusted with thiram before use. This was because untreated seeds are often put into thiram to give the seeds the best chance against fungi, and to prevent fungi being a factor in the germination tests.

The treatments were tested in a randomized complete block design and were blocked on the four dates the seeds were harvested. The three treatments were

- scarified and prechilled
- scarified and non prechilled
- non-scarified, prechilled

Some seeds need to be scarified as part of the process to break dormancy. To test whether *Calystegia silvatica* seeds need to be scarified before germination four replicates of 25 seeds were scarified. The edge of the seed, away from the embryo,

was pierced with the scalpel before being kept at 25^{o}C along with four replicates of 25 seeds without scarification. This technique was the same technique used by Brummitt (1963). Some seeds also need to be chilled as part of the process to break dormancy. Both the scarified and non-scarified seeds were kept in moistened anchor seed germination paper and prechilled for seven days at 5^{o}C before being kept at 25^{o}C. To test whether *Calystegia silvatica* seeds do need to be chilled before germination four replicates of 25 scarified seeds were also kept at 25^{o}C, without being prechilled.

Anchor seed germination paper (45.5 x 15.0 cm) was moistened with water (Plate 4.4) and used in all of the germination tests. The seeds were put in between three sheets of the paper (two on the bottom and one on the top) and the paper rolled up into a roll before being placed in the incubators. Anchor germination paper was used on the advice of the head analyst for Seed Tech Services, Massey University.

The seeds were then assessed 7, 15, 22, and 27 days after incubation and the number of normal germinated seeds, abnormal seedlings, dead seeds, hard seeds and fresh ungerminated seeds counted.

Seeds were said to have germinated normally when the emerged seedling (hypocotyl + primary root) was over 0.5 - 1 cm long and healthy, a similar minimum standard to that used by Steinbauer and Grigsby (1959). Abnormal seeds were those which had emerged but were disfigured, or unhealthy. Dead seeds were those in which water entered the seed but nothing germinated and the seed case was empty and/or soft. Fresh ungerminated seeds were those in which water had entered the seed and the seed remained healthy, but no germination occurred. The seeds had swelled in size and were soft to the touch. Hard seeds were those which were impermeable to water and no germination occurred. The seeds remained hard.

The second experiment was to determine the preferred temperature for the germination of scarified *Calystegia silvatica* seeds and three replicates of 25 seeds were kept at 5^{o}C, 10^{o}C, 15^{o}C, 20^{o}C and 25^{o}C in a completely randomised design. The seeds were then assessed 7, 14, 21 and 28 days after incubation and the number of normal germinated seeds, abnormal seedlings, dead seeds, hard seeds and fresh ungerminated seeds were counted.

Plate 4.4: Germinated seedlings on moist anchor germination paper used for germination.

SAS was used to perform an analysis of variance of the data collected and least significant differences between means were calculated if treatments were shown to have a significant effect ($P<0.05$) on the percentage of normal, abnormal, dead, fresh ungerminated or hard *C. silvatica* seeds.

4.3.2 Results

Most of the scarified seeds germinated (Table 4.1) within 7 days, with the rest of the seeds which germinated, germinating by 15 days after they were incubated. The non-scarified seeds had some normal seeds germinated seven days after incubation with some occurring later, 22 and 27 days after incubation. There were significant differences between the two treatments in the amount of normal seeds which germinated and the amount of dead, fresh ungerminated and hard seeds that were present (Table 4.1).

Table 4.1: Percentage of normal and abnormal seedlings and dead, fresh ungerminated or hard, scarified or non-scarified *C. silvatica* seeds.

Treatment	Normal seedlings	Abnormal seedlings	Dead	Fresh ungerminated	Hard
non-scarified	14.0	0.0	10.0	4.0	72.0
scarified	71.0	1.0	28.0	0.0	0.0
LSD (P<0.05)	10.9	NS[1]	14.0	3.3	9.8

[1]NS = not significant (P<0.05)

There were no differences in the results between the prechilled and non chilled seed germination (Table 4.2). Both prechilled and non chilled seeds germinated by 15 days with most of the germination occurring within7 days.

Table 4.2: Percentage of normal and abnormal seedlings and dead, fresh ungerminated or hard, prechilled or non chilled *C. silvatica* seeds.

Treatment	Normal seedlings	Abnormal seedlings	Dead	Fresh ungerminated	Hard
Prechilled	71.0	1.0	28.0	0.0	0.0
Non chilled	69.0	7.0	24.0	0.0	0.0
LSD (P<0.05)	NS[1]	NS	NS	NS	NS

[1]NS = not significant (P<0.05)

Between the 5^{o}C and the other temperatures there were significant differences in the amount of germinated, dead and fresh ungerminated seeds (Table 4.3). Those seeds kept at 5^{o}C had fewer normal seeds germinate and less dead seeds counted. More fresh ungerminated seeds were found in those seed replicates kept at 5^{o}C.

Table 4.3: Percentage of normal and abnormal seedlings, and dead, fresh ungerminated or hard *C. silvatica* seeds across five different temperatures after 28 days.

Treatment	Normal seedlings	Abnormal seedlings	Dead	Fresh ungerminated	Hard
5^{o}C	16.0	0.0	10.7	73.7	0.0
10^{o}C	86.7	0.0	13.3	0.0	0.0
15^{o}C	82.7	0.0	17.3	0.0	0.0
20^{o}C	80.0	0.0	20.0	0.0	0.0
25^{o}C	80.0	0.0	20.0	0.0	0.0
LSD (P<0.05)	9.7	NS[1]	7.1	5.1	NS

[1]NS = not significant (P<0.05)

There were also differences in the timing of the germination of the normal seeds and the size of the seedlings when they germinated (Table 4.4). At 7 days most of the seeds had germinated when kept at 15, 20 and 25°C. The seedlings kept at 15°C ranged from 1 – 5 cm in length, whereas for seeds kept at 20 and 25°C, the seedlings ranged from 5 – 13 cm in length and had well developed leaves. By 14 days almost all of the seeds had germinated at these three temperatures. At 10°C the normal seedlings did not emerge until 14 days had passed and ranged from 0.5 – 5cm in length. The few normal seedlings which germinated at 5°C germinated later, by 28 days and were very small, only 0.5 – 1 cm long.

Table 4.4: Average number of normal seedlings (out of 25 seeds) which germinated after 7, 14 and 28 days at each temperature.

	Days			
Treatment	**7**	**14**	**21**	**28**
5°C	0.0	0.0	0.0	4.0
10°C	0.0	21.3	0.3	0.0
15°C	20.0	0.7	0.0	0.0
20°C	19.0	1.0	0.0	0.0
25°C	19.7	0.3	0.0	0.0
LSD (P<0.05)	1.7	1.4	NS[1]	1.4

[1]NS = not significant (P<0.05)

4.3.3 Discussion

More normal scarified seeds germinated than non-scarified. This indicates that *C. silvatica* seeds are likely to need to have their seed coat broken before germination can occur and resembles what Brummitt (1963) found. It is the same with *C. sepium*, *C. tuguriorum* and *C. arvensis*, all three species need scarification (Steinbauer & Grigsby 1959; Weaver & Riley 1982; Burrows 1996; Parsons & Cuthbertson 2001). In the field this scarification could come from insects, microbial action or abrasion from movement down riverbeds (Burrows 1996).

Some of the non-scarified seeds also germinated normally but this is likely to have occurred because those seeds may have been scarified by other means in the field e.g. insects, prior to testing. The other non-scarified seeds were not scarified in any way and they were recorded as either hard seeds or fresh ungerminated. Seeds which were hard did not have their seed coat broken whereas those which were fresh but ungerminated would have been imbibed but were unable to germinate normally under the current conditions of the test. This could be because the seeds were not scarified enough.

The scarified seeds had higher amounts of dead seeds than the non-scarified. This would have been because many of the non-scarified seeds did not have their dormancy broken and therefore it was not able to be tested how much of those seeds were also dead.

There was also a timing difference between scarified and non-scarified seeds. Scarified seeds germinated within 15 days whereas the non-scarified seeds took twice as long. This indicates that the breaks in the seed coat that were present in these seeds, were much smaller than the scalpel cut on the scarified seed and the water may have not penetrated easily, which resulted in delayed germination.

Due to a limited amount of seeds, it was decided not to look at non-scarified, non prechilled seeds as the focus was on the main factors affecting germination, which appeared to be factors relating to scarified seeds, so non-scarified seeds were not studied in further detail. treatment.

For some species, prechilling can often give an improvement in germination, but in the case of this species, 7 days of prechilling gave no improvement.

Temperature however is important for germination. Normal seed germination occurred best at 20 and 25^{o}C. The total amount of normal germinated seedlings was not significantly different from those kept 10 and 15^{o}C but germination occurred faster at 20 and 25^{o}C. Normal seeds also germinated when kept at 5^{o}C, which is the same low temperature *C. arvensis* seedlings are said to germinate at (Brown & Porter 1942), but it was a slow process with only small seedlings present after 28 days.

There were also differences in the number of fresh ungerminated seeds between those kept at 5^{o}C and the other temperatures. This would be because the seeds had imbibed with water but were unable to germinate within 28 days due to conditions which were too cold for all but a few of the *C. silvatica* seeds able to germinate. There were also less dead seeds in those kept at 5^{o}C than those kept at 20 and 25^{o}C and this would be because many of the seeds weren't able to reach the stage where it was possible to tell whether they were dead or ungerminated.

Because *C. silvatica* germination is best at higher temperatures, this could partly explain why no seeds germinated in the plots of the field trial (Chapter 2). Competition in many of the plots could also have affected the successful establishment of germinating seedlings anyway, but in those areas where there were bare spots, temperature may have affected germination. When regrowth recordings were made in August and September, average soil temperatures for these months were only 8.9 and 9.9^{o}C respectively. The seed coat of those in the field may also have not yet been

broken or perhaps there were very few seeds in the soil anyway. This will be further investigated in Section 4.5.

Normal seedling germination ranged from 69.0 – 86.7% when the non-scarified seeds and those kept a 5^{o}C are excluded. This level of germination is similar to that found by Brummitt (1963), where 75% of scarified *C. silvatica* seeds germinated after being placed in potting compost.

4.3.4 Summary

This section and the previous section (Section 4.2) sought to answer the two objectives:

- To determine how frequently viable seeds are formed within *C. silvatica* flowers

- To determine how best to break the dormancy of *C. silvatica* seeds

Sampling in Section 4.2 showed that *C. silvatica* plants can produce seeds, with an average of 2.3 seeds per capsule of which 1.5 seeds per capsule appear to be healthy. Of those scarified seeds normal germination occurs at about 60 – 80% (Section 4.2) and therefore in this trial it was found only about one seed per capsule is viable and likely to germinate. Therefore *C. silvatica* plants do not produce a lot of seeds.

The results in Section 4.3 also confirm that *C. silvatica* seeds are likely to need to be scarified and shows that *C. silvatica* seeds can germinate at 5, 10, 15, 20 and 25^{o}C if scarified, with the best and quickest germination occurring when the seeds were kept at 20 and 25^{o}C. In the field the seeds are therefore likely to germinate in spring and summer periods when temperatures are warmer and in warmer areas germination could occur year round, if seeds are present in the soil layer.

4.4 The viability of seeds from established *Calystegia silvatica* treated with herbicides

Calystegia silvatica subsp. *disjuncta* can produce seed (Section 4.2) and it is possible those seeds might be affected by herbicide application. Herbicides may be absorbed by the seeds as they are developing and herbicides applied at initial seed set can affect seed germination. This was found to be the case with glyphosate, where applications to *Xanthium strumarium* and *Sesbania exaltata* during the initial seed set stage reduced the number of the seeds, the seed weights and seedling emergence (Clay & Griffin 2000). Metsulfuron has also been found to affect ragwort (*Senecio jacobaea*)

and nodding thistle (*Carduus nutans*) seed production and germination (James et al 1999). Thus the herbicides applied to the established plants in the field (Chapter 2) may have affected the *C. silvatica* seeds. The objective for this experiment was to test whether the seeds that were found on the *C. silvatica* plants eight weeks after treatment had been affected by the herbicide application.

4.4.1 Materials and methods

Seeds were collected from the field trial (Chapter 2), treated and untreated plots, on 16 June 2012, eight weeks after herbicide application. Seeds could not be collected from three plots as in two of the plots, one metsulfuron treatment and one aminopyralid treatment, there were no seeds and the other plant, an untreated control, had been earlier removed by cows. The treatment information can be found in Section 2.2.3.

Any seed capsules collected that were not full or were open were discarded. Each of the full unopened seed capsules were opened and the seeds counted and assessed as either being healthy, shrivelled, very shrivelled, underdeveloped, insect damaged or had evidence of fungi.

Due to a limited amount of healthy seeds available only four replicates of 10 seeds of the untreated control could be used. All other treatments had four replicates of 25 seeds made up of all the plots for that treatment in a completely randomised design. The seeds were placed onto anchor germination paper (45.5 x 15.0 cm) that was moistened with water, the seeds were dusted with thiram and scarified with a scalpel. The seeds were then kept at 25^{o}C and assessed 7, 14, 21 and 28 days after incubation and the number of normal germinated seeds, abnormal seedlings, dead seeds, hard seeds and fresh ungerminated seeds were counted.

SAS was used to perform an analysis of variance of the data collected and least significant differences were calculated where significant treatment differences were found.

4.4.2 Results

The proportion of normal, underdeveloped, shrivelled or very shrivelled seeds in the treatments, and those affected by other factors such as fungi or insects (Table 4.5), was similar to those sampled from the waste area (Section 4.2). There were also no significant differences between the proportion of the normal, shrivelled or underdeveloped seeds between the herbicide treatments and the untreated control. There was a lower proportion of normal seeds found in the metsulfuron treatment but

this was not significantly different from the untreated control. The proportion of herbicide treated seeds with fungi or insect presence was also not significantly different from the untreated controls. The proportion of very shrivelled seeds in the glyphosate and triclopyr/picloram/aminopyralid was significantly less than the proportion of very shrivelled seeds found in untreated control plots. There were also no significant differences in the average numbers of seeds per pod between the herbicide treatments and the untreated control and the average number of seeds per pod ranged from 1.9 – 2.9.

Table 4.5: The proportion of normal, shrivelled, very shrivelled and underdeveloped *C. silvatica* seeds across different herbicide treatments. The proportion of seeds with the presence of either insects or fungi is also included.

Treatment	Normal	Shrivelled	Very shrivelled	Insect presence	Fungi presence	Under-developed
2,4-D/dicamba	56.7	5.6	14.8	2.9	20.1	0.0
aminopyralid	57.0	7.3	9.7	9.0	17.0	0.0
triclopyr/picloram /aminopyralid	71.3	4.1	6.6	0.8	17.1	0.0
metsulfuron	39.9	1.2	24.2	17.0	13.6	4.2
glyphosate	79.0	3.0	5.5	0.5	12.0	0.0
clopyralid	62.4	5.1	9.0	8.7	4.4	10.3
untreated	63.0	5.3	19.7	6.8	5.1	0.0
LSD (P<0.05)	NS[1]	NS	12.0	NS	NS	NS

[1]NS = not significant (P<0.05)

When the percentage of germinated seeds is compared (Table 4.6), it can been seen that both the aminopyralid and metsulfuron treatments had only 62% and 64% normal seed germination whereas the glyphosate treatments had 79% normal seeds germinate. These treatments were not significantly different though and show the variability of normal seed germination.

Table 4.6: Percentage of normal and abnormal seedlings and dead, fresh ungerminated or hard *C. silvatica* seeds in a germination trial of seeds collected from different herbicide treatments.

Treatment	Normal seedlings	Abnormal seedlings	Dead	Fresh ungerminated	Hard
2,4-D/dicamba	72.0	0.0	28.0	0.0	0.0
aminopyralid	62.0	0.0	38.0	0.0	0.0
triclopyr/picloram/ aminopyralid	72.0	0.0	27.0	1.0	0.0
metsulfuron	64.0	0.0	35.0	1.0	0.0
glyphosate	79.0	0.0	20.0	1.0	0.0
clopyralid	75.0	0.0	23.0	2.0	0.0
untreated	70.0	0.0	30.0	0.0	0.0
LSD (P<0.05)	NS[1]	NS	NS	NS	NS

[1]NS = not significant (P<0.05)

4.4.3 Discussion

The herbicides didn't appear to have any effect on the number of seeds which appeared to be normal or the number of seeds which were shrivelled, underdeveloped or showed the presence of other organisms. The differences in the proportions of very shrivelled seeds are likely to be because of the variability of the seeds collected.

The herbicide treatments also didn't influence the germination of the seeds. Although the number of normal seeds which germinated in both the aminopyralid and metsulfuron treatments was lower than other treatments and the untreated control, the results were not significant and this shows the variability in the results. Also the percentage of normal germination was similar to those sampled from the waste area in Section 4.3.

It was therefore shown that the herbicides did not affect the germination of *C. silvatica* seeds. This could be because the herbicides were applied to plants which already had flowered and were beginning to senesce, as they were dying back before winter and therefore most of the seeds would have already have been formed and filled and it is unlikely that the plant would have been translocating sugars to the seeds. Therefore the herbicides were also unlikely to have been transported, by the plant, to the seeds.

The amount of seed from these plants would not likely provide a major increase to the soil seed bank.

4.5 Seed presence in the soil near established *C. silvatica* and the germination ability of those seeds

Little is known about whether *C. silvatica* seeds build up in the soil (Williams 2009). To investigate this, soil cores were taken from beneath established dense infestations of *C. silvatica* to discover how many seeds were present and how viable the seeds were.

4.5.1 Materials and methods

Soil cores were collected from two sites. The first site was the same untreated area used for seed collection (Section 4.2). The second site was the area next to the orchard at the Plant Growth Unit which was used for collecting plants in Chapter 3. The soil cores were taken from beneath dense infestations of established *Calystegia silvatica*.

In September 2012, 500 soil cores were taken from beside the Turitea Stream and 100 soil cores were taken from the area behind the Plant and Growth Unit. The tiller corer used had a diameter of 5.3 cm and a depth of 3.2 cm. A few soil cores with a depth of 30 cm were also taken in September 2012, but as no seed was found and because the *C. silvatica* seeds are unlikely to be deep in the soil layer as they have no burial mechanism and are quite large seeds (Brummitt 1963), no further soil cores were collected at this depth.

The soil cores were washed in a sieve with 2.5mm x 2.5mm mesh openings. Any seeds found in each sample were collected and recorded.

To test the viability of any recovered seeds, they were placed onto anchor germination paper (45.5 x 15.0 cm) which was moistened with water, dusted with thiram, scarified with a scalpel and kept at 25^{o}C. The number of seeds which germinated were counted after 7 days.

The number of seeds per m^2 was calculated using the size of the corer (5.3 cm diameter) and converting that to m^2.

4.5.2 Results

Only seeds which appeared healthy were recovered from the soil core samples. No shrivelled seeds were found. The number of seeds in each soil core varied from a maximum of seven seeds per core to a minimum of no seeds per core. The first site, near the Turitea Stream, had an average of 22.7 seeds per m2and the second site, behind the Plant and Growth Unit, had an average of 18.1 seeds per m^2. The amount

of seed recovered from both areas sampled worked out to be 21.9 seeds per m^2. All of the seeds recovered had germinated by 7 days.

4.5.3 Discussion

This work aimed to find out how much viable seed is present in the soil under dense infestations of *C. silvatica*. It was found that while there were some seeds within the top layer of soil, there was only a small amount. Some seed banks can contain hundreds or thousands of seeds per m^2 (Baskin & Baskin 2001) including *Convolvulus arvensis* which is estimated to have a seed bank of up to 2,000 seeds per m^2 (Parsons & Cuthbertson 2001). In this study there were estimated to be only 21.9 *Calystegia silvatica* seeds per m^2. This may be because the plants were not there the previous season, the plants rarely produce seed or because predators removed the seeds. The seeds of *C. silvatica* were found to be quite large (Section 4.2) and although this would enable them to grow new seedlings and from greater depths, it also means that the seeds are more likely to suffer from predation as they have no burial mechanism and therefore may just sit on the top of the soil (Brummitt 1963) where they would be easier to find by rodents and other animals (Tilman 1988).

The seeds in the soil could germinate in bare areas or areas with low levels of competition if it is warm enough and the seeds are sufficiently scarified. *C. silvatica* seeds could also germinate in areas of moderate competition as the seeds are quite large and the seed reserves could provide the seedling with enough resources to germinate and survive. In Chapter 2 it was observed that no seeds germinated in the bare soil after the landslip. This could be because there were no seeds in that area or because the seeds in the soil bank were so few and unlikely to be that deep. A *C. silvatica* plant could drop seeds in that area though and the seeds could germinate on the bare soil. As the seeds have no specialised structures most of the transport would be from either gravity or water (Williams 2009). Therefore the areas beneath or near the parent plant would need to be bare or only covered in moderately competitive plants for the seeds to germinate. Otherwise the seeds could wash away with flooding, into waterways, and colonise new sites along the waters path in a similar way to *C. silvatica* rhizomes (Williams 2009).

CHAPTER 5: Identification of *Calystegia* growing locally

5.1 Introduction

There has been much confusion over *Calystegia silvatica* taxonomy and identification in the past and confusion remains around some of the subspecies of *Calystegia* which could be present in New Zealand (Chapter 1.1.2). As it stands now it appears as if there are four native *Calystegia* species within New Zealand, *Calystegia tuguriorum*, *Calystegia soldenella*, *Calystegia marginata* and *C. sepium* subsp. *roseata*. This last species, although also found throughout Europe, does appear to be native to New Zealand, with the Maori name of "pohue", as it differs in many characteristics from other pink flowered *Calystegia* species found overseas (Ogden 1978). Although *Calystegia silvatica* subsp. *disjuncta* has obviously arrived in New Zealand, there is less certainty over whether *Calystegia silvatica* subsp. *silvatica* is widely present here. There has also been uncertainty expressed over whether *C. sepium* subsp. *sepium* has arrived in New Zealand (New Zealand Plant Conservation Network 2010), though other references state that it is here (Ogden 1978; Wilson-Davey et al. 2009) and thus was also included in the discussion below.

To easily identify these species in New Zealand a key (Figure 1.1 and 5.1) was developed based on the New Zealand taxonomy and species information found in Ogden (1978), Sykes et al (1988) and Popay et al (2010) and the international taxonomic information found in Brummitt (1963) and Brown et al (2009). The key splits the species based on the broadest and easiest characteristics to compare. *Convolvulus arvensis* was not included in the key but can be easily separated from most of the *Calystegia* species as it has arrow-shaped leaves and can be separated from *Calystegia silvatica* and *Calystegia sepium* by its small leaves (<40 mm long). Both *Calystegia silvatica* and *Calystegia sepium* have leaves up to 180 mm long (Sykes et al. 1988; Popay et al. 2010).

The plants found around Massey University farms and those trialled in Chapters 2, 3 and 4, were identified, using this key, as *Calystegia silvatica* subsp. *disjuncta*. To find whether this species of *Calystegia* is widespread the objective for the work described in this chapter was:

- To discover the identity of the *Calystegia* plants which commonly grow in the local areas

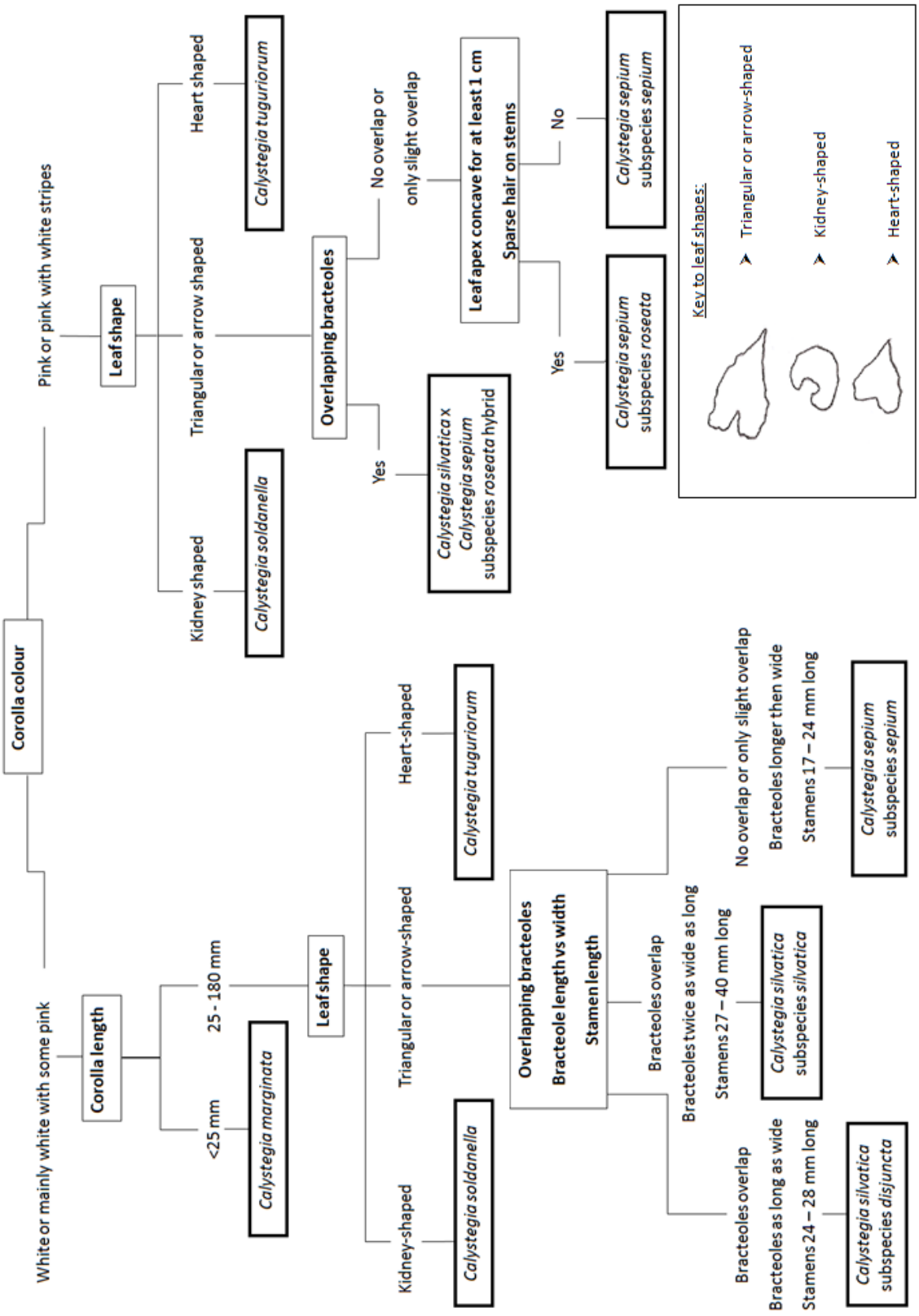

Figure 5.1: Taxonomic key for New Zealand *Calystegia* species. Information from Brummitt (1963), Ogden (1978), Sykes et al (1998), Brown et al (2009) and Popay et al (2010).

5.2 Materials and methods

Calystegia plants were sampled from 11 December 2012 to 22 February 2013, once the plants were flowering. They were sampled from the local area ranging from Napier, Hawke's Bay, New Zealand to Levin, Horowhenua, New Zealand. Plants were also sampled from Hastings, Clive, Havelock North, Waipawa, Dannevirke, Ashhurst, Feilding, Awahuri, Palmerston North, Shannon and Foxton, New Zealand. For a full list of locations, including street names and descriptions, please see Appendix 1. The plants were selected by driving through random parts of each locality and sampling plants observed in these areas.

From each *Calystegia* plant sampled, five whole leaves and five whole flowers including bracteoles were removed from the plant to examine. If there were less than five flowers on the plant than all of the flowers were taken. Fifty *Calystegia* plants were sampled in total.

The length and shape of each leaf collected was recorded. The length of each corolla and stamen length was measured. Corolla colour was also recorded. Bracteoles were checked to see whether they overlapped or not and the length and ½ width of the bracteoles were measured to check whether the length was smaller or the same size as the width. Only the ½ width of the bracteoles was measured on the plant, which is one common practice (Brummitt 1963) available if the bracteoles are folded in a centreline and can't be flattened out, which the bracteoles in this case were. The ½ widths were then multiplied by two to represent a whole width and then compared to the length of the bracteoles. These measurements were then compared to those found in Figure 5.1 and the species identified.

5.3 Results

Out of the 50 bindweed plants sampled, 238 flowers were measured and 250 leaf shapes recorded. Of the 238 flowers 201 were white. There were also 15 flowers with white corollas with pink tinges and 22 corollas were pink with white stripes.

Of those flowers that were white or white with pink tinges, the length of the corollas varied but all were over 43 mm (Figure 5.2). The leaf shapes of those flowers were all triangular or arrow-shaped.

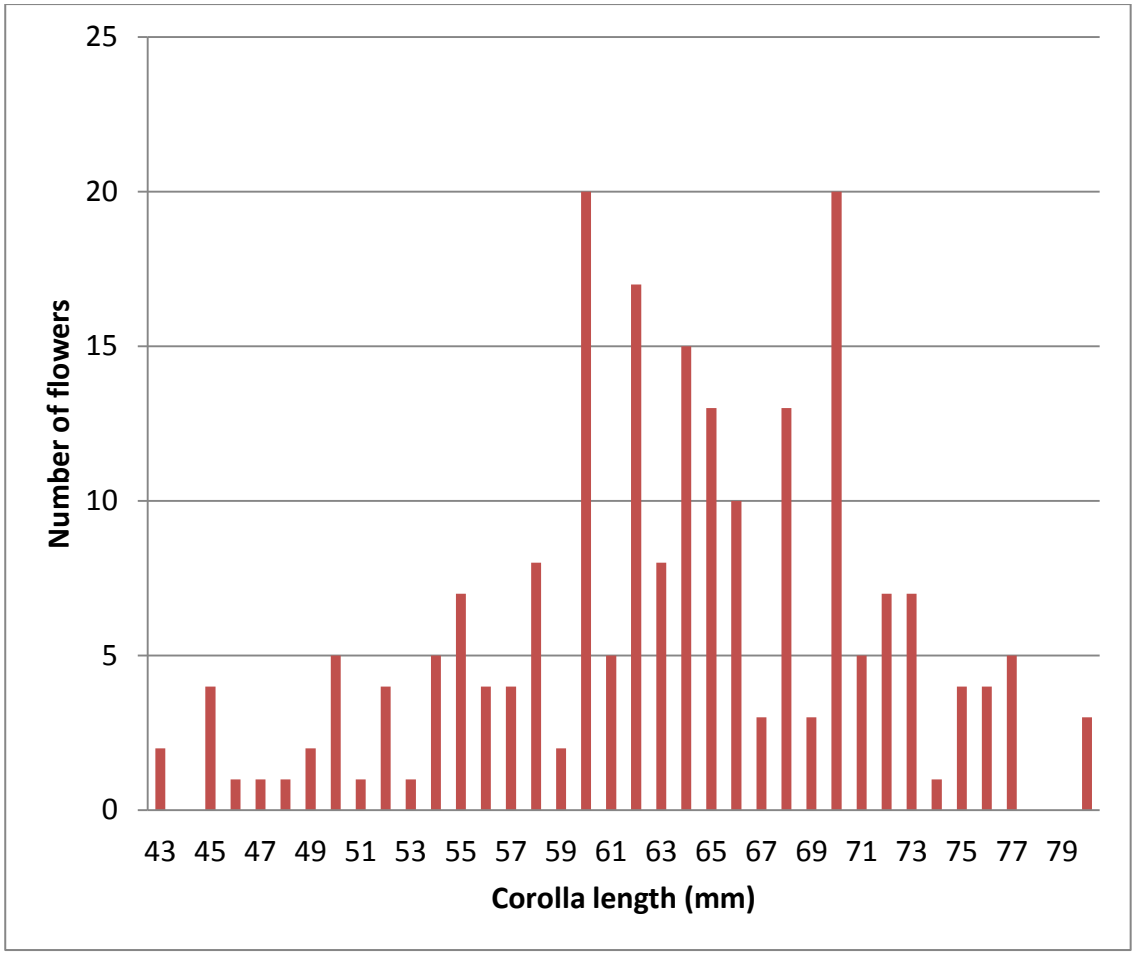

Figure 5.2: The total length of the corollas which were either white or white and pink tinged.

All of the white flowers and white flowers with pink tinges had bracteoles which overlapped (Plate 5.1) and the width of the bracteoles was equal to or near equal size to the length of the bracteoles. The stamen lengths of those white flowers ranged from 20 mm to 34 mm with most of them ranging from 27 – 31 mm in length (Figure 5.3).

All of this information resulted in those flowers with white or white with pink tinged corollas being identified as *Calystegia silvatica* subsp. *disjuncta*.

Plate 5.1: *C. silvatica* subsp. *disjuncta* flowers with white corollas and overlapping bracteoles.

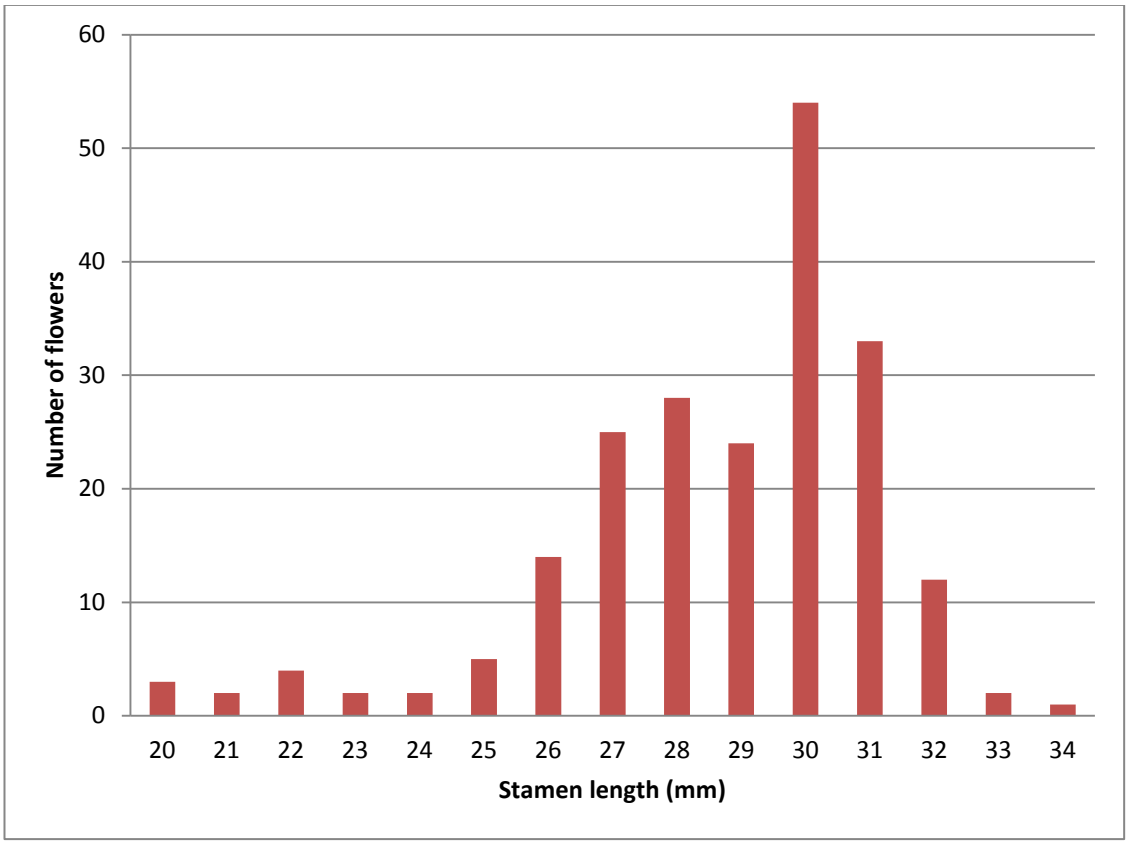

Figure 5.3: The total length of the stamens of white or white/pink tinged.

Of those plants sampled, three had flowers that were pink with white stripes (Plate 5.2). All of those plants had leaves that were triangular or arrow-shaped. Out of those plants, two from the Hawke's Bay region and one from the Manawatu/Horowhenua region, had bracteoles which overlapped. These results indicate that those three plants could be a hybrid between *Calystegia silvatica* and *Calystegia sepium*.

Plate 5.2: Hybrid flowers with pink and white striped corollas and overlapping bracteoles.

The other two plants sampled which had pink flowers with white stripes had triangular or arrow-shaped leaves and bracteoles which did not overlap (Plate 5.3). The leaves from one of the plants had concave leaf apexes and the plant was concluded to be *Calystegia sepium* subsp. *roseata* (Plate 5.3). The other specimen did not have concave leaf apexes, and as the stems were not compared, no identification could be made.

Plate 5.3: *C. sepium* subsp. *roseata* flowers with non overlapping bracteoles and a leaf with a concave leaf apex.

The *Calystegia silvatica* subsp. *disjuncta* plants sampled were found to be growing near railways (Plate 5.4), roadsides, in reserves, walkways (Plate 5.5), riverside or lake side (Plate 5.6), gardens, waste areas, parking lots, shelterbelts and in wetland reserve areas. The plants were growing over a variety of shrubs and trees including native plants and weeds including blackberry (*Rubus fruticosus* agg.).

Plate 5.4: *C. silvatica* subsp. *disjuncta* growing over a fence-line near the railway line in Dannevirke.

Plate 5.5: *C. silvatica* subsp. *disjuncta* on an embankment in Karituwhenua Stream walkway in Havelock North.

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Plate 5.6: *C. silvatica* subsp. *disjuncta* growing with pink *Lathyrus odoratus* (*sweet pea*) in a Lake Horowhenua Domain Park in Levin.

5.4 Discussion

Most of the *Calystegia* species sampled within the local area were *Calystegia silvatica* subsp. *disjuncta*. This is the same species that was identified and worked with in Chapters 2, 3 and 4.

The key (Figure 5.1) allowed easy identification of most of the species, though there were a few outliers on some of the measurements. For stamen length some of the flowers had stamen lengths over 30 mm. However they were still identified as being from *C. silvatica* subsp. *disjuncta* and not the subspecies *silvatica* as the bracteole widths were equal to or near equal to the length of the bracteoles. There are different limits given in some of the references (Brummitt 1963; Brown et al. 2009), ranging from 24 – 28 mm to 26 – 34 mm. Most of the stamen lengths that were looked at in the local area had stamens which fit within these upper and lower limits. The key could be amended to include a broader range of 24 – 34 mm. Those few stamens which were below 24 mm in length were likely to have just been anomalies, perhaps because the flowers had developmental problems. All of the flowers with shorter stamens were from the same plants as flowers with stamens within a 24 – 34 mm range. No grouping of flowers collected from a single plant had stamens all beneath the lower limit, or above the upper limit and therefore they were not identified as another species or

subspecies because on average the stamen lengths fitted those of *Calystegia silvatica* subsp. *disjuncta*, as did the other characteristics of those flowers.

It was a similar situation with the corolla lengths of the white and white pink tinged flowers. All were identified as *Calystegia silvatica* subsp. *disjuncta*, although the corolla lengths of some flowers were below 47 or 50 mm which is the lower limit given for *Calystegia silvatica* (Brummitt 1963). However the flowers which had corolla lengths below this lower limit were part of the same plant with flowers over this limit and therefore they are still concluded to be *Calystegia silvatica* subsp. *disjuncta*. This corolla length was not in the key as the key was based on the broadest categories available which would easily separate out the different *Calystegia* species. Although it could not be found if *C. tuguriorum*, *C. soldenella* or *C. marginata* have white flowers with some pink tinges, white flowers with some pink tinges was included in the classification key as *Calystegia silvatica* flowers can be pinkish, although this is considered to be rare (Sykes et al. 1988; Popay et al. 2010), and *Calystegia sepium* subspecies *sepium* may also be white with pink tinges (Plate 5.7) . Those flowers found with pink tinges in the current survey, had fully white corollas with only a slight dusting of pink on the folds on the outside of the corolla, similar to the *C. sepium* subsp. *sepium* in a photograph obtained from Osborn (2009) and shown in Plate 5.7. The hybrid can be separated from pink tinged plants of *C. silvatica* or *C. sepium* as most of the hybrids corolla is pink, with white stripes, and not white with a small amount of pink.

Plate 5.7: *Calystegia silvatica* (left) and *Calystegia sepium* subspecies *sepium* (right). *C. sepium* has a white corolla with some pink tinges (Source: Osborn 2009).

The bracteole measurements gave the best indication of whether the *C. silvatica* subspecies was either *disjuncta* or *silvatica* as there is a clear difference. The ½ widths were measured as they were deemed to be best in this case. This is because *C. silvatica* species have bracteoles with a centre line which cannot be easily folded out to

measure the full width and therefore measuring a ½ width is the next best acceptable practice (Brummitt 1963).

Three of the plants sampled, with pink and white striped flowers and overlapping bracteoles, most probably were the hybrids between *Calystegia silvatica* and the native *Calystegia sepium* subsp. *roseata*, as suggested in Ogden (1978). Only flower colour and overlapping bracteoles could be used to identify these hybrids as there is limited, clear information available about the other characteristics which could separate them, such as stamen length. This could be because of natural variation (Brummitt 1963; Ogden 1978).

The other species found was probably the native *Calystegia sepium* subspecies *roseata* because it had pink and white striped flowers, triangular or arrow-shaped leaves, and non-overlapping bracteoles and because the apex of the leaves appeared to be attenuate or concave for at least 1 cm (Plate 5.3). This distinguishes *C. sepium* subsp. *roseata* from *C. sepium* subsp. *sepium* as the latter has non attenuate leaf apexes (Brummitt 1963). It was not known before sampling that *C. sepium* subsp. *sepium* can have pink and white striped flowers. Because this was unknown, the stems of the plants were not collected. The stems may have confirmed that the *Calystegia sepium* subspecies *roseata* thought to be found was indeed this species and the unidentified species may have been identified.

The key (Figure 5.1), provided easy identification of the *Calystegia* species sampled and would be easy for those not well versed in taxonomic descriptive terms to identify species present. The key however, does not have all the measurements and limits available for each of the species so if there were plants present in which the flowers and leaves did not fit exactly within the categories provided, further sources would need to be consulted to provide exact or approximate identification. More extensive classification information and taxonomic identification can be found in Brummitt (1963) or for New Zealand species, Sykes et al (1988), although this book doesn't separate out the different subspecies. The key also does not take into account the full range of hybrids which could be present, as many of the *Calystegia* species can hybridise (Brummitt 1963; Ogden 1978; Brown et al. 2009).

As most of the information about the subspecies is taken from international sources, those species in New Zealand could be different, particularly *C. sepium* subsp. *roseata* which is claimed to be a native, and not all of the characteristics are identical to the information from overseas sources (Ogden 1978). To amend this and truly identify what species of *Calystegia* are in New Zealand a taxonomic review would need to be completed, probably with the aid of DNA sequencing.

5.5 Summary

Most of the *Calystegia* found in the local area was *Calystegia silvatica* subsp. *disjuncta*. *Calystegia sepium* subsp. *roseata* also appears to be present. To tell the different species of *Calystegia* apart, a key was developed based on the broadest categories. However it may not be the definitive answer as the whole issue of the taxonomy does not appear to be clear-cut. *Calystegia* species and subspecies in New Zealand may have different characteristics from those found in overseas information and a large majority of this key was developed from international information.

CHAPTER 6: Discussion

6.1 Introduction

Farmers and regional councils have invested significant amounts of resources, money and time into riparian zones and native plantings which can help to improve water quality (Williams 2009; Wilson-Davey et al. 2009). *Calystegia silvatica* has been found growing in these riparian zones, where it can adversely affect the native plantings by growing over the plants, competing with them and harming the establishment and survival of new plantings (Wilson-Davey et al. 2009).

However, as shown in Chapter 1, there is little information available on the control of *C. silvatica* with herbicides. Those studies which have been completed have either not been formal trials (Hornby 2011) or the herbicides were applied to *C. silvatica* at a time when the herbicides may not have been able to fully translocate throughout plants and into the plants' underground organs (Rahman & Sanders 1992), where herbicide control is the most important. Other information available is in the form of recommendations from different sources including councils, books or the New Zealand Agrichemical Manual. Because of the limited trial work done on *C. silvatica* and because it is an important weed to control, the work in Chapters 2 and 3 was undertaken. To obtain more information about *C. silvatica*, work on the biology and taxonomy was conducted, as shown in Chapters 4 and 5.

6.2 Most effective herbicides

Trials in Chapters 2 and 3 showed that no herbicide trialled, at the rate they were applied at, controlled 100% of *C. silvatica* plants. However three herbicides, triclopyr/picloram/aminopyralid, 2,4-D/dicamba and aminopyralid showed almost complete and long lasting control. Increasing the rate of these three herbicides or applying a second application could increase the level of control and could kill the *C. silvatica* plants. This however wasn't trialled in these experiments due to limited plants available and limited time.

6.2.1 Triclopyr/picloram/aminopyralid

A triclopyr/picloram/aminopyralid mixture was one of the most successful herbicides and showed long lasting control. There was minimal regrowth (Chapters 2 and 3), particularly in the glasshouse trial (Chapter 3) where there was no regrowth in several of the pots 37 weeks after herbicide application. Hornby (2011) found that triclopyr/picloram showed good efficacy and long lasting control of *C. silvatica*. In the

current trials the herbicide was also just as effective when applied to 50% or 90% of the plant, or to the basal leaves or the upper parts. It did not show 100% control in either the field or the glasshouse when 90 + 30 + 2.4 g ai/100 litre was applied once, in autumn. The rate used was similar to that used on other weeds (Young 2012), but increasing the rate or doubling the rate could have increased the efficacy and given complete control. However increasing the herbicide rate could also increase the amount of off target damage that could occur. Triclopyr/picloram/aminopyralid would be safe to use in riparian zones if it is applied in a careful manner to only the *C. silvatica* leaves and vines, such as that done in Chapter 2, and spray drift is minimised.

A further consideration, not yet discussed in this thesis, is the relative costs of each herbicide, and these are now presented in Table 6.1.Triclopyr/picloram/aminopyralid is the cheapest out of the three herbicides which showed the most potential. If the rate was increased by 1.5 times, then the price of product per 100 litre would still only be $67.50 which is still less than the price of Banvine (2,4-D/dicamba).

Table 6.1: A summary of the approximate costs of the product needed per 100 litre for each herbicide. All prices are inclusive of GST.

Trade name*	**Cost**	**Rate per 100 litre**	**Price per 100 litre**
Answer	$246.0/1.125 kg[1]	0.075 kg	$16.40
Banvine	$73.00/1 litre[1]	1.2 L	$87.59
Glyphosate 360	$65.00/5 litres[2]	1.5 L	$22.41
Pasture Kleen Xtra	$150.00/5 litres[3]	1.15 L	$34.50
Starane	$205.30/5 litres[2]	0.5 L	$23.61
Tordon Brushkiller XT	$150.00/1 litre[1]	0.3 L	$45.00
Tordon Max or T-MAX	$450.00/5 litres[1]	0.6 L	$54.00
Versatill	$130.00/ 1 litre[1]	0.3 L	$39.00

*Active ingredient information for each product can be found in Table 2.1 and Table 3.1

[1]Prices obtained from RD1

[2]Prices obtained from AgriPlus

[3]Price obtained from RD1 for Pasture Kleen Xtra, which has a higher g ai/litre than that used in the trials. The rate of product per 100 litres was adjusted to an equivalent amount

6.2.2 2,4-D/dicamba

Another herbicide which showed good long lasting control of *C. silvatica* in both the field and the glasshouse (Chapters 2 and 3) was 2,4-D/dicamba. In the glasshouse where 240 +120 g ai/100 litres of 2,4-D/dicamba was applied to *C. silvatica* (Chapter 3), only a few *C. silvatica* plants grew back and several pots had no regrowth at 37 weeks.

It did not show 100% control of *C. silvatica* but increasing the dose may have given complete control. The rate selected for the trials (Chapter 2 and 3), 240 +120 g ai/100 litres, was the same rate recommended in the New Zealand Novachem Manual for use on *C. silvatica* (Young 2012). Out of all the herbicides tested 2,4-D/dicamba is the most expensive when the amount of product needed to replicate the rate we used is considered (Table 6.1). Increasing the rate would make it even more expensive than double the rate of triclopyr/picloram/aminopyralid.

6.2.3 Aminopyralid

Aminopyralid also showed good control of *C. silvatica*, particularly in the glasshouse trial (Chapter 3), where barely any shoots grew back after herbicide application. In the field there was also some long lasting control of *C. silvatica* (Chapter 2). The effects of aminopyralid differed though in that *C. silvatica* treated with either triclopyr/picloram/aminopyralid or 2,4-D/dicamba showed herbicide effects soon after herbicide application, aminopyralid treated plants did not. Initially there were few differences between those *C. silvatica* plants treated with aminopyralid and those plants which were untreated. This means that if aminopyralid is used on established *C. silvatica* plants in riparian zones then those plants covered with *C. silvatica* will not be released from competition as quickly as if 2,4-D/dicamba or triclopyr/picloram/aminopyralid were used. However aminopyralid did show long lasting control. Hornby (2011) also found long lasting control of *C. silvatica* when aminopyralid was used but the initial effects of aminopyralid weren't assessed. In both the glasshouse and in the field (Chapter 2 and 3) a rate of 18 g ai/100 litres of aminopyralid was used. This was a similar rate to that used by Hornby (2011). An increased rate may have given complete control of *C. silvatica* and 27 g ai/100 litres aminopyralid is still cheaper then 2,4-D/dicamba (Table 6.1).

6.3 Application technique

There is little information available about the use of triclopyr/picloram/aminopyralid, 2,4-D/dicamba and aminopyralid on or around native New Zealand plantings. One study applied aminopyralid to run off on the foliage of some native plants (Harrington & Schmitz 2007). The study found that aminopyralid showed harm to some species and less to others when a lower rate of 9 g ai/100 litres was used. These three herbicides could significantly affect native plantings if they were sprayed all over the plants or if the herbicides were applied to the soil near plantings, where they could enter the root zone. Application technique is therefore important and applying herbicides to *C. silvatica* that is growing in riparian zones could put native plants at risk of off target herbicide damage. This is an important consideration but as found in Chapter 2, careful spraying techniques can reduce off target damage. In Chapter 3 a

different application technique was trialled to test whether applying herbicides to just part of plant could still control *C. silvatica*. Although it was found for most herbicides that applications to the basal leaves did not control the plants any more than herbicide applications to the upper portions of the plants, what was found was that many of the herbicides showed the same level of control as in the field (Chapter 2) even though the herbicides were only applied to 50% of the plant. This means that herbicides could be carefully applied to *C. silvatica* which is growing in or around native plants by using equipment such as a backpack sprayer and carefully spraying *C. silvatica* that is between native plants. This would prevent spraying all over the native plant foliage and off target damage would be minimised. Another application technique which has been suggested is to paint the herbicide onto the leaves (Upritchard 2007), and this technique was used in the glasshouse (Chapter 3) to apply the herbicides in a way which would not cause the spray to drift to nearby *C. silvatica* plants receiving other herbicide treatments and to ensure each plant got the same dose. Using this technique in a riparian zone would be too time-consuming, particularly if the plants are dense or extensive. This technique could be used to control small plants in gardens though, or in other areas where only small plants are present.

6.4 Timing of application

As well as application technique, timing is also an important consideration as mentioned in Section 1.1.3. *C. silvatica* has extensive root and rhizome systems from which it can regrow. Herbicides need to translocate into these zones in order for complete control to occur. The best time to achieve this is when the plant is transporting sugars from the shoots to the roots which in *C. sepium* and *C. arvensis*, closely related species, occurs after early summer (Willeke et al. 2012). *C. silvatica* is also likely to be transporting sugars from the shoots to roots after summer as the plant begins to senesce and the aboveground parts die off before winter and thus it would be the best time to apply translocated herbicides, in late summer or autumn, to ensure that the herbicides are translocated to the roots. The best time to apply herbicides can vary amongst different herbicides in *C. arvensis*, as mentioned in Section 1.3.1, but the best time appears to be full bloom or late flowering and after early summer. In both the field and glasshouse trials (Chapter 2 and 3) the herbicides were applied in autumn. Autumn applications mean that if *C. silvatica* is already established then the native plants may have to be affected by competition for a least one season before herbicide application. However autumn herbicide applications will show more control than spring applications as the herbicides are more likely to translocate into the root systems of *C. silvatica*.

6.5 Other herbicides

Increasing the rate of the herbicides from the rates used in Chapters 2 and 3 could increase the level of control for the herbicides already mentioned and complete control could be achieved. For the other herbicides trialled, which didn't work as well, increasing the rate could increase the efficacy of these herbicides.

6.5.1 Glyphosate

Glyphosate was one of those herbicides which showed some control of the *C. silvatica* plants in the field but less control was achieved in the glasshouse. While those plants with herbicide applied to the upper portion of the plant showed some knockback, those plants with herbicide applied to the basal leaves and stem grew back. Glyphosate was applied at a rate of 540 g ai/100 litres and this rate could be doubled or tripled to achieve more control. If the rate used was three times that of what we used, then the cost would still be significantly cheaper than 2,4-D/dicamba. Previous trials have found that increasing the rate of glyphosate can increase the level of control of *C. arvensis* (Davison & Bailey 1974; Wiese & Lavake 1986; Matic & Black 1994). One consideration is that glyphosate is a broad spectrum herbicide and therefore it could also take out the ground cover species too. If the species beneath *C. silvatica* is another unwanted weed, such as blackberry (*Rubus fruticosus* agg.), then using glyphosate could help to remove that weed. However glyphosate could also remove the wanted ground cover beneath the plants, which should be maintained to help prevent further invasion of *C. silvatica* or other unwanted weed species by establishment of seedlings. Glyphosate could also affect the native species in the riparian plantings and so like the other herbicides already mentioned, the herbicide needs to be applied carefully so that only *C. silvatica* comes in contact with the herbicide and there is no off target damage. No off target damage was found in Chapter 2 when careful spray technique was used to apply the glyphosate to *C. silvatica*.

6.5.2 Metsulfuron

Metsulfuron was also trialled in both the field and the glasshouse (Chapters 2 and 3). In both trials it was found that there was significant regrowth in spring but in the glasshouse (Chapter 3) metsulfuron killed the aboveground parts of *C. silvatica* rapidly. *C. arvensis* treated with metsulfuron has also shown regrowth in previous trials (Heering & Peeper 1991; Matic & Black 1994). In an informal assessment of metsulfuron use on *C. silvatica* in New Zealand (Hornby 2011) the *C. silvatica* treated with metsulfuron did not appear to regrow within a year after herbicide application. Hornby (2011) used surfactant and twice the rate of metsulfuron as that used in the

field and glasshouse trials (Chapter 2 and 3). Therefore increasing the rate of metsulfuron and including surfactant could improve the level of control of *C. silvatica*. The metsulfuron would still be cheaper than several of the other herbicides used if the rate was doubled or even tripled (Table 6.1)

Metsulfuron is recommended for use on *C. silvatica* (Waikato Regional Council 2012) and can also be useful on other weeds that could be present in riparian strips, such as blackberry (Young 2012). However the highly recommended herbicides, 2,4-D/dicamba and triclopyr/picloram/aminopyralid, can also affect a number of other weeds.

6.5.3 Clopyralid

Clopyralid was the only other herbicide trialled in both the field and the glasshouse (Chapter 2 and 3). In the field clopyralid showed slow initial control of the aboveground parts but there was quite variable regrowth results. Some plants were smaller than their original size whereas one plant was twice the size of the original plant (Chapter 2). In the glasshouse clopyralid showed poor initial control of *C. silvatica* and there was significant regrowth. These results fit with what was found in other trials on *C. silvatica* (Rahman & Sanders 1992), *C. sepium* (Rahman et al. 2002) and *C. arvensis* (Matic & Black 1994). Hornby (2011) found that clopyralid showed good signs of knockback but formal regrowth assessments were not made. Hornby (2011) also used a higher rate (120 g ai/100 litres) than that used in the field and glasshouse where the rate used was 90 g ai/100 litres. Therefore increasing the rate of clopyralid used could increase the efficacy. However it is unlikely to provide good control of established *C. silvatica* and other herbicides already mentioned are better options. Clopyralid would be a safer option for use around native plantings then some of the other herbicides but the other herbicides can be applied in a way to reduce off target effects.

6.5.4 2,4-D

Because of limited plots available 2,4-D was not trialled in the field but it was trialled in the glasshouse (Chapter 3). Like metsulfuron, 2,4-D killed off the aboveground parts of *C. silvatica* quickly but there was significant regrowth in the spring. These results are similar to what was found in previous trials using 2,4-D on *C. arvensis* where in most cases there was significant regrowth long term (Wiese & Lavake 1986; Schoenhals et al. 1990; Matic & Black 1994). It is however recommended for use on either *C. silvatica* regrowth (MAFF 1957) or young seedlings (Young 2012). It appears as if this would be the best use of 2,4-D, on regrowth after the established plants have been treated with another herbicide or if seedlings emerge, particularly as, like metsulfuron, 2,4-D kills

off the aboveground parts quickly which would prevent seedlings from establishing extensive rhizomes.

6.5.5 Fluroxypyr

Fluroxypyr was recommended as a potential herbicide for young *C. silvatica* as it is used for control of other invasive vines (Wilson-Davey et al. 2009). It was only trialled in the glasshouse (Chapter 3), on younger vines, and not on the established plants in the field. While it initially affected the aboveground parts there was significant regrowth in spring and it showed poor control of *C. silvatica* when used at a rate of 100 g ai/100 litres. Matic and Black (1994) also found that fluroxypyr treated *C. arvensis* grew back after time. The rate of fluroxypyr could be increased to improve the efficacy however other herbicides already mentioned are better options for the control of established or young *C. silvatica* in riparian zones.

6.6 Surfactants

Surfactants could also increase the level of control of those herbicides used. In the glasshouse (Chapter 3) it was found that water beaded on the leaves, and then dripped off, and therefore surfactant was added to the herbicide mixtures before application. No surfactant was used in the field (Chapter 2). Hornby (2011) used surfactant and twice the rate of metsulfuron, than that used in the field and glasshouse (Chapters 2 and 3), and found better long term control than the current trials did. The surfactant could have contributed to the increased level of control as the herbicide would have been able to penetrate the leaves better. The Boost penetrant used in the glasshouse is recommended for use with Tordon Brushkiller and other brushweed herbicides (Young 2012) and this surfactant, or another compatible with the herbicides, could be used. The addition of surfactant to triclopyr/picloram/aminopyralid, aminopyralid or 2,4-D/dicamba would contribute to these herbicides providing complete control of *C. silvatica*, particularly if the rates of these herbicides were increased. The addition of surfactant could increase any off target damage if the herbicides came in contact with native plantings, but careful spray technique would minimise this risk.

6.7 Herbicide management

As no herbicide killed 100% of the *C. silvatica* in the trials (Chapter 2 or 3) it is likely that at least two herbicide applications are needed in riparian strips to remove established plants. An autumn application of triclopyr/picloram/aminopyralid, aminopyralid or 2,4-D/dicamba would likely take out most of the plants, particularly if an increased rate was used. Following this autumn application some plants may still emerge in spring, after winter dormancy. One of these three herbicides could then be

used again later in the season once the plants had started to transport sugars from the shoots to the roots, after the compensation point suggested by Willeke et al (2012) has been reached, which is around early summer in *C. sepium* and *C. arvensis*. By this time most of the root buds would have also sprouted (Willeke et al. 2012). If the second herbicide application is delayed until this time then the *C. silvatica* is likely to have grown back through much of the site, and may compete with the native plantings again. But if the herbicide is applied in early summer then the reserves in the rhizomes and roots are unlikely to have replenished and a second herbicide application should kill off the remaining plants because there would be movement back into the remaining rhizomes if the compensation point has been reached.

6.8 Blackberry control

Often *C. silvatica* is not the only problem weed growing in riparian strips. While looking for bindweed plants in Chapter 5 it was often found that blackberry grows with *C. silvatica* in different environments and *C. silvatica* is often twining around the blackberry stems. Triclopyr/picloram/aminopyralid could be used to initially control both the *C. silvatica* and the blackberry. The current trials used 90 + 30 + 2.4 g ai/100 litres or 300 ml of Tordon Brushkiller XT product. This rate is just above that recommended for use on blackberry (Young 2012). Autumn herbicide applications are also recommended for blackberry and thus an autumn application of triclopyr/picloram/aminopyralid would control both blackberry and *C. silvatica*. For both species a follow up herbicide application may be necessary and while triclopyr/picloram/aminopyralid could be used, so could metsulfuron. Metsulfuron, at the same rate used in Chapters 2 and 3, can also control blackberry. Therefore an autumn application of triclopyr/picloram/aminopyralid followed by an early or mid summer application of metsulfuron should control most of the blackberry and may control the *C. silvatica*. Glyphosate can also control blackberry, and showed some knockback of *C. silvatica*, but glyphosate would also harm the ground cover species and therefore careful spray technique would be needed.

6.9 *Calystegia silvatica* seeds

It appears as though it is regrowth from rhizomes that allows *C. silvatica* to re-establish in an area, particularly if it has had established plants. In the field (Chapter 2), no seedlings were found in the plots, although most had established ground cover, and only a few were found outside the plot areas. To investigate the seeds of *C. silvatica*, seeds were sampled and germination studies conducted in Chapter 4. It was found that *C. silvatica* can produce seeds but only about one seed per pod will germinate normally. They were also large seeds and thus likely to succumb to predation from things like mice, and few seeds were found in the upper soil layer beneath established

plants. Seeds are therefore unlikely to play a big role in the establishment of *C. silvatica* in areas which have good groundcover as few seeds are likely to be present, they succumb to predation and there is competitive pressure from ground cover. This suggests that maintaining a good groundcover beneath native plantings will help to prevent re-establishment of *C. silvatica* from seeds. If the groundcover is removed because of herbicides, such as glyphosate, then seeds could germinate. If seeds were to germinate then spot spraying them with either metsulfuron or 2,4-D could prove effective. The seeds are expelled from the pods onto the ground (Brummitt 1963) and it is possible that the transport of those seeds is in waterways as opposed to wind or animal transport (Williams 2009). Through water transport these seeds can gain access to new areas, but are unlikely to germinate unless bare and less competitive environments are found. Rhizome fragments can also transport through waterways and floods and pose a bigger threat (Williams 2009).

6.10 *Calystegia* taxonomy

To determine whether the *Calystegia silvatica* subsp. *disjuncta* trialled in Chapters 2, 3 and 4 was an anomaly or a common bindweed species in the local area, bindweed plants were sampled and identified (Chapter 5). It was found that *C. silvatica* subsp. *disjuncta* is a common weed in the local area. It was also found that *C. silvatica* subsp. *disjuncta* occupies a range of different areas including near railways, roadsides, in gardens, reserves and shelterbelts.

Those herbicides, which were found to provide control of *C. silvatica* subsp. *disjuncta* in Chapters 2 and 3, may also control other *Calystegia* species, particularly *C. sepium* as both species are similar in form. Few differences were found between the control of *Convolvulus arvensis* in previous trials and the control of *C. silvatica* in Chapters 2 and 3 and therefore aminopyralid and triclopyr/picloram/aminopyralid may also be effective on *Convolvulus arvensis*. The other herbicide which proved effective on *C. silvatica*, 2,4-D/dicamba, has been shown previously to be less effective on *C. arvensis* (Westra et al. 1992).

6.11 Important findings

The work conducted in Chapters 2, 3, 4 and 5 filled some gaps in the current knowledge about the control, seed biology and taxonomy of *C. silvatica*. No trials could be found on the use of 2,4-D/dicamba , triclopyr/picloram/aminopyralid or fluroxypyr on *C. silvatica*. Those trials in Chapters 2 and 3 therefore show some of the effects those three herbicides have on *C. silvatica*. Formal trials using aminopyralid or metsulfuron on *C. silvatica* could also not be found and thus the current trials and findings fill this gap in the knowledge. The results also show the efficacy of these

herbicides when applied in autumn. Also discovered was that herbicide applications to the upper portions of the plants did not control the *C. silvatica* plants any less than herbicide applications to the basal leaves when the herbicides were applied in autumn. This is likely to be because the whole plant is translocating sugars back to the underground organs before winter dormancy. Further information was also gathered about *C. silvatica* seeds including information about the temperatures in which *C. silvatica* seed can germinate in and the fact that the seeds don't need prechilling for germination to occur. This work also confirmed the need for *C. silvatica* seeds to be scarified before germination as found by Brummitt (1963). There was also some uncertainty over the seed production of this species and it was found that, on average, *C. silvatica* flowers can each produce approximately one viable seed. It was also discovered that most of the bindweed in the local area is *Calystegia silvatica* subsp. *disjuncta*.

6.12 Further work

Because the trials only had a limited amount of pots or plots available, only a few herbicides which showed potential, were recommended or were used before, were able to be trialled. Some herbicides which could show potential for use on *C. silvatica* including dicamba, triclopyr, MCPA or combinations of herbicides, were not used and future work could look at the efficacy of these herbicides or others on *C. silvatica*. Or those herbicides used could be trialled at a variety of rates as it looks likely that increasing the rates of triclopyr/picloram/aminopyralid, aminopyralid or 2,4-D/dicamba could provide complete control of *C. silvatica*. As this trial took place over only one year, a second application of herbicide was not able to be made and studied and could be a future area to pursue. Further work could also look into more properties of the seeds.

6.13 Summary

- Although no herbicide tested showed 100% of control of *Calystegia silvatica*, several herbicides showed very promising results which could probably be improved further by increasing application rates and adding surfactants

- Autumn applications of triclopyr/picloram/aminopyralid, aminopyralid or 2,4-D/dicamba showed the best control out of the herbicides trialled

- Metsulfuron and 2,4-D didn't show long term control of *C. silvatica* but rapidly killed off the aboveground parts of *C. silvatica* in the initial stages

- Glyphosate also provided some useful knockback and could be used as the sole herbicide treatment in some situations such as when home gardeners need control in waste areas as they have limited access to herbicides

- Clopyralid and fluroxypyr both showed poor control of *C. silvatica*

- Applications of triclopyr/picloram/aminopyralid, aminopyralid and 2,4-D/dicamba in autumn would help ensure translocation of the herbicide to the roots and rhizomes of *C. silvatica*. A follow up application of triclopyr/picloram/aminopyralid, aminopyralid or 2,4-D/dicamba could be applied to any regrowth in summer, once the plants begin to translocate sugars to the roots

- Herbicides should be applied carefully by spraying only the *C. silvatica* leaves and vines in between native plantings and other wanted plants

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APPENDIX 1: The location of *Calystegia* plants sampled within Chapter 5

Hawke's Bay District		
Location	**Habitat**	**Identified species**
Napier, corner of Milton Road and Tennyson Street	Waste area	*Calystegia silvatica* subsp. *disjuncta*
Napier, Meeanee Quay, State Highway 2, Ahuriri Estuary	Estuary	*Calystegia silvatica* subsp. *disjuncta*
Clive, Farndon Road	Riverbank	*Calystegia silvatica* subsp. *disjuncta*
Clive, Farndon Road	Shelterbelt	*Calystegia silvatica* subsp. *disjuncta*
Hastings, corner of Elwood Road and Karamu Road North	Fence-line	Hybrid *C. sepium* X *C. silvatica*
Hastings, corner of Gordon Road and Southland Road	Waste area	*Calystegia silvatica* subsp. *disjuncta*
Hastings, corner of South Hampton Street West and Townshend Street	Home garden	*Calystegia silvatica* subsp. *disjuncta*
Hastings, corner of Tollemache Road and Main Road	Railway	*Calystegia silvatica* subsp. *disjuncta*
Hastings, Gordon Road, Ebett Park	Residential park	*Calystegia silvatica* subsp. *disjuncta*
Hastings, Karamu Road North, Karamu Stream	Fence-line by stream	*Calystegia silvatica* subsp. *disjuncta*
Hastings, Pakowhai Road near the Hawke's Bay expressway, State Highway 50A	Fence-line	*Calystegia silvatica* subsp. *disjuncta*
Hastings, Ruahapia Road	Fence-line by stream	Hybrid *C. sepium* X *C. silvatica*
Hastings, Ruahapia Road	Shelterbelt	*Calystegia silvatica* subsp. *disjuncta*
Hastings, State Highway 2, Pekapeka Park	Hillside	*Calystegia silvatica* subsp. *disjuncta*
Hastings, State Highway 2, Pekapeka Park	Wetland	*Calystegia silvatica* subsp. *disjuncta*
Hastings, Tollemache Road	Shelterbelt	*Calystegia silvatica* subsp. *disjuncta*
Hastings, Tollemache Road outside plum orchard	Shelterbelt	*Calystegia silvatica* subsp. *disjuncta*

Hastings, Willowpark Road South	Parking lot	*Calystegia silvatica* subsp. *disjuncta*
Havelock North, Te Mata Road, Karituwhenua Stream walkway	Riverside	Calystegia silvatica subsp. disjuncta
Havelock North, Te Mata Road, Karituwhenua Stream walkway	Riverside	*Calystegia silvatica* subsp. *disjuncta*
Havelock North, Te Mata Road, Karituwhenua Stream walkway	Riverside	*Calystegia silvatica* subsp. *disjuncta*
Waipawa, Ongaonga Road, Waipawa River	Riverside	*Calystegia silvatica* subsp. *disjuncta*
Dannevirke, by Rawhiti Street and Nelson Street	Railway	*Calystegia silvatica* subsp. *disjuncta*
Dannevirke, Queen street	Railway	*Calystegia silvatica* subsp. *disjuncta*
Dannevirke, Queen street	Roadside	*Calystegia silvatica* subsp. *disjuncta*

Manawatu District		
Location	**Habitat**	**Identified species**
Feilding, Waughs Road	Railway	*Calystegia silvatica* subsp. *disjuncta*
Awahuri, Awahuri Feilding Road, Kitchener Park	Walkway	*Calystegia silvatica* subsp. *disjuncta*
Awahuri, State Highway 3	Fence-line	Hybrid *C. sepium* X *C. silvatica*
Ashhurst, Guildford St, Village Valley Centre	Parking lot	*Calystegia silvatica* subsp. *disjuncta*
Ashhurst, Napier Road, State Highway 3, Manawatu River	Parking lot	*Calystegia silvatica* subsp. *disjuncta*
Palmerston North, Aokautere Drive, Adderstone Park	Walkway	*Calystegia silvatica* subsp. *disjuncta*
Palmerston North, behind Dittmer Drive, Manawatu River	Riverside	*Calystegia silvatica* subsp. *disjuncta*
Palmerston North, behind Esplanade, Manawatu River	Riverside	*Calystegia silvatica* subsp. *disjuncta*
Palmerston North, James Line	Roadside	*Calystegia silvatica* subsp. *disjuncta*
Palmerston North, Old West	Riverside	*Calystegia silvatica* subsp.

Road, Turitea Stream		*disjuncta*
Palmerston North, Pioneer Highway, State Highway 56	Waste area	*Calystegia silvatica* subsp. *disjuncta*
Palmerston North, Sheriff Road	Roadside	*Calystegia silvatica* subsp. *disjuncta*
Palmerston North, Tennent Drive	Riverside	*Calystegia silvatica* subsp. *disjuncta*
Palmerston North, Tennent Drive	Roadside	*Calystegia silvatica* subsp. *disjuncta*
Aokautere, Staces Road	Maize crop	*Calystegia silvatica* subsp. *disjuncta*
Aokautere, Staces Road	Paddock/fence-line	*Calystegia silvatica* subsp. *disjuncta*

Horowhenua district		
Location	**Habitat**	**Identified species**
Foxton, Harbour Street, Manawatu River	Riverside	*Calystegia sepium subsp. roseata*
Shannon, Julyan Street	Railway	*Calystegia silvatica* subsp. *disjuncta*
Shannon, Nathan Terrace	Waste area	*Calystegia silvatica* subsp. *disjuncta*
Shannon, Plimmer Terrace	Railway	*Calystegia silvatica* subsp. *disjuncta*
Levin, Arapaepae Road, State Highway 57	Roadside	*Calystegia silvatica* subsp. *disjuncta*
Levin, Queen Street, Kowhai Park	Fence-line	*Calystegia silvatica* subsp. *disjuncta*
Levin, Queen Street, Kowhai Park	Garden, old riverbed	*Calystegia silvatica* subsp. *disjuncta*
Levin, Queen Street, Lake Horowhenua	Lakeside	*Calystegia sepium* subsp. *roseata*
Levin, Queen Street, Lake Horowhenua Domain Park	Garden	*Calystegia silvatica* subsp. *disjuncta*