ASPECTS OF RESISTANCE TO PHENOXY HERBICIDES

IN NODDING THISTLE (*CARDUUS NUTANS* L.)

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ABSTRACT

A nodding thistle (*Carduus nutans* L.) population had been reported from Argyll in Hawkes Bay, New Zealand, which had poor susceptibility to MCPA and 2,4-D. Plants from the Argyll population were grown beside another Hawkes Bay nodding thistle population in a glasshouse and their dose response curves for MCPA were compared in three separate experiments. The Argyll population was significantly less susceptible to MCPA in all experiments, though the magnitude of resistance varied between experiments from 5-fold to 14-fold. When grown beside each other in the field, the Argyll population was 7 times more resistant to MCPA than the other population.

A range of other herbicides was applied to the Argyll nodding thistle population. Cross-resistance was detected for 2,4-D and MCPB, but no significant decreases in susceptibility were detected with mecoprop, clopyralid, picloram, dicamba, paraquat/diquat or glyphosate. A significant reduction in susceptibility to tribenuron-methyl was measured in a field experiment, but this difference was not apparent when the experiment was repeated in a glasshouse. The cross-resistance to MCPA, MCPB and 2,4-D meant selective control of nodding thistle at Argyll in clover-based pastures was now very difficult to achieve.

Nodding thistle populations from 20 Hawkes Bay and 7 Waikato properties were tested for resistance to MCPA, and significant levels of resistance were detected in 14 of these populations. Interviews of property owners indicated that resistance had developed where 2,4-D or MCPA had been applied annually for many years, whereas properties without resistance had been sprayed less regularly.

Resistant and susceptible nodding thistle seedlings were grown together at a 1:1 ratio under conditions of nutrient stress to determine whether herbicide-resistant nodding thistle plants are less competitive than normal. No difference was detected between the resistant and susceptible biotypes used.

Under some conditions, susceptible plants were more likely to have high trichome densities on their leaves, but this trait was found to be too variable and not correlated closely enough with herbicide susceptibility to be useful in distinguishing between resistant and susceptible biotypes.

Significant differences in susceptibility to MCPA were maintained between resistant

and susceptible biotypes even when leaf surfaces were damaged to allow better foliar penetration of the herbicide, or when herbicide was applied to plants via the root system. Thus the mode of resistance did not appear to involve difficulties with foliar uptake.

Studies with radiolabelled 2,4-D confirmed that resistance did not relate to poor leaf penetration. These experiments indicated that 2,4-D was broken down more rapidly in resistant plants. Other findings were that 2,4-D or its metabolites were released in greater quantities from the root systems of susceptible plants, and that herbicide molecules were more difficult to extract from the interior of susceptible plants, possibly due to increased binding.

Reasons why resistance to phenoxy herbicides has developed in nodding thistle are discussed, and techniques for controlling resistant populations selectively in pastures and preventing further resistance from developing are also analysed.

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LIST OF PESTICIDES

The chemical names of pesticides mentioned in this text are:

amitrole	3-amino-1,2,4-triazole
bentazone	3-isopropyl-2,1,3-benzothiadiazin-4-one 2,2-dioxide
bromofenoxim	3,5-dibromo-4-hydroxybenzaldehyde 2,4-dinitrophenyloxime
bromoxynil	3,5-dibromo-4-hydroxybenzonitrile
chlorsulfuron	*N*'-(2-chlorobenzenesulfonyl)-*N*-(4-methoxy-6-methyl-1,3,5-triazin-1-yl)urea
clopyralid	3,6-dichloropyridine-2-carboxylic acid
cyanazine	2-chloro-4-(1-cyano-1-methylethylamino)-6-ethylamino-1,3,5-triazine
2,4-D	2,4-dichlorophenoxyacetic acid
dicamba	3,6-dichloro-2-methoxybenzoic acid
dichlorprop	(±)-2-(2,4-dichlorophenoxy)propionic acid
dinoseb	2-(1-methylpropyl)-4,6-dinitrophenol
diquat	9,10-dihydro-8a,10a-diazoniaphenanthrene
DNOC	2-methyl-4,6-dinitrophenol
etridiazole	5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole
glyphosate	*N*-(phosphonomethyl)glycine
ioxynil	4-hydroxy-3,5-di-iodobenzonitrile
MCPA	4-chloro-2-methylphenoxyacetic acid
MCPB	4-(4-chloro-2-methylphenoxy)propionic acid
mecoprop	(±)-2-(4-chloro-2-methylphenoxy)propionic acid
metsulfuron	2-[3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)ureidosulfonyl]benzoic acid
paclobutrazol	(2*R*,*S* 3*R*,*S*)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1*H*-1,2,4-triazol-1-yl) pentan-3-ol
paraquat	1,1'-dimethyl-4,4'-bipyridylium
picloram	4-amino-3,5,6-trichloropicolinic acid
simazine	2-chloro-4,6-bisethylamino-1,3,5-triazine
tribenuron	2-[4-methoxy-6-methyl-1,3,5-triazin-2-yl(methyl)carbamoylsulfamoyl]benzoic acid
trifluralin	2,6-dinitro-*N*,*N*-dipropyl-4-trifluoromethylaniline

CHAPTER 1: INTRODUCTION

1.1 OBJECTIVE

Nodding thistle is a spiny herbaceous biennial weed which invades many New Zealand pastures. MCPA and 2,4-D will control this species, but poor results are sometimes obtained (Popay *et al* 1989). Plant size and time of application are known to influence effectiveness (Hurrell *et al* 1983). However results from a 1981 Hawkes Bay trial (discussed in Section 1.2.4) suggested there are also other factors involved (Harrington and Popay 1987).

The objective of this project was to gain an understanding of why MCPA and 2,4-D consistently give poor control of nodding thistle in parts of Hawkes Bay. Understanding this phenomenon might lead to improved recommendations for the control of this weed. As the project progressed, research concentrated on the herbicide resistant biotypes that were discovered.

Before describing this work, discussion of the following topics is appropriate:

- nodding thistle
- MCPA and 2,4-D
- factors affecting the efficacy of foliar-applied herbicides.

1.2 NODDING THISTLE (*CARDUUS NUTANS* L.)

Information on the biology and control of nodding thistle has been fully reviewed recently (Desrochers *et al* 1988; Popay and Medd 1990). Therefore a further complete review of the literature pertaining to this species is not appropriate. However some aspects particularly relevant to this study are discussed below as an introduction to the project.

1.2.1 Importance in New Zealand

Livestock avoid eating nodding thistle because of its spines. This enables the species to compete with and dominate surrounding pasture plants. Kelly and Popay (1985) found that although thistle rosettes shade only one-third of the area within a circle circumscribed by the longest leaf, they can cover as much as 59% of the ground within

Hawkes Bay pastures. Nodding thistle is also probably competitive because of its tap root that can penetrate the soil to 40 cm or more (Popay and Medd 1990), giving access to water below the shallower rooting systems of other pasture species in summer. Reductions in pasture production due to competition have been measured in Waikato pastures where, for every 1000 nodding thistle plants per hectare, pasture production was reduced by 8% between October and May (Thompson *et al* 1987). Although there are about 15 thistle species in New Zealand pastures (Webb *et al* 1988), nodding thistle is generally considered to be one of the most aggressive (Matthews 1975).

As well as reducing pasture production, thistles reduce pasture utilization by grazing animals (Hartley and James 1979). Thompson *et al* (1979) found pasture production directly beneath vegetative rosettes of nodding thistle was reduced by 40%, but even this pasture would have remained unused as livestock seldom graze near thistle rosettes.

Dense mature stands of nodding thistle obstruct livestock, and dried fragments and spines may cause physical injury or adhere to wool, lowering its value (Popay and Medd 1989). Its presence in wool and hay also makes these unpleasant to handle (Delahunty 1961).

Although well established in Otago, Canterbury, Hawkes Bay and Waikato, there are many areas in New Zealand where nodding thistle does not occur, or is present only in small numbers. It is therefore gazetted as a Noxious Plant in all parts of New Zealand (Congdon 1978) to minimize spread of the weed into new areas. It is one of three weed species in New Zealand which prevent certification of crop seed if seed of the weed is found during laboratory examination of seed lots or if plants are found in the crop at field inspection (MAFQual 1987). This ruling has applied since 1967/68 (Smith 1971). These legislative measures have no doubt reduced the rate of spread of nodding thistle within New Zealand. However they have also increased the significance of this weed relative to many other weed species as its presence means that control must be attempted even if population numbers are relatively low.

Thus pastoral farmers may control nodding thistle to improve pasture production, increase pasture utilization by livestock, improve stock movement and health, increase wool and hay quality, or to meet legal requirements. The benefits of control must be compared with the cost of this control. Direct costs are those of the herbicide and application. However the main cost may well be the loss in pasture quality and production caused by damage to the legume component of swards by herbicide application, as demonstrated by Hartley (1983) for Scotch thistle (*Cirsium vulgare*

(Savi) Ten.) control with MCPA. This effect on clovers may be severe particularly in areas where clopyralid is used on the phenoxy-resistant biotypes of nodding thistle discovered during this project.

Attempts have been made to develop a mathematical model to study the economic implications of controlling nodding thistle infestations under New Zealand conditions (Moore *et al* 1989). However this exercise has probably served only to highlight the large amount of research into the biology of a weed needed to produce meaningful results from such a model. Much of this research has yet to be done for nodding thistle.

Nodding thistle is generally considered a weed of pastures in New Zealand, but it also causes problems in arable crops (Delahunty 1961), especially for crops grown for certified seed as nodding thistle can influence certification as discussed above. Nodding thistle is particularly troublesome in lucerne (Taylor 1981) as crop dormancy in winter allows seedlings to establish and most herbicides applied in late winter give poor control of this weed once it has established (Atkinson and Meeklah 1980).

Although nodding thistle is generally an unwanted species, bees produce high quality honey from the flowers which was worth $500,000 for the Hamilton district (New Zealand) alone during the 1981-82 season (Reid 1982).

1.2.2 Biology of Nodding Thistle

1.2.2.1 **Seed Physiology**

The physiological characteristics of seeds are very important in determining the success of annual and biennial pasture weeds. Although nodding thistle seeds (actually achenes) generally germinate readily at 15-30^{o}C when moist, enforced and innate dormancy mechanisms help ensure successful establishment of seedlings (Popay and Medd 1990).

Enforced dormancy results from exposure to high ratios of far-red to red light, as found in the shade of green vegetation (Medd and Lovett 1978a). This prevents germination in situations where strong competition by existing vegetation would normally jeopardize survival of the seedlings. This enforced dormancy is evident in experiments which show how seed germination occurs freely in bare soil but is greatly inhibited by the presence of vegetation (Popay 1978; Phung and Popay 1981; Popay *et al* 1987; Martin and Rahman 1988a). Seedlings germinate in pastures mainly in autumn (Popay

and Kelly 1986) when the pasture has not yet recovered from summer drought, over-grazing or insect attack, but when adequate moisture is present following the first autumn rains. Considerable germination can also occur in spring if pasture is absent (Popay and Kelly 1986; Popay *et al* 1987).

Darkness also causes enforced dormancy, which prevents deeply buried seed from germinating (Medd and Lovett 1978a). Once seeds are buried in the soil, they can remain viable for many years, allowing reinfestation of sites once the seeds are brought back up to the soil surface. Popay *et al* (1987) found that, although seed buried in the top 2 cm of soil survived only 2-3 years, seed at 4-6 cm survived 18-28 years, and at 19-21 cm, 34-77 years.

Nodding thistle seeds initially have a period of innate dormancy lasting for several months (Medd and Lovett 1978a; Popay *et al* 1987). This ensures some of the seed shed in summer becomes buried or covered by pasture species before the first effective rains of autumn, so that enforced dormancy prevents these seeds from germinating in autumn and allows a store of seed to build up in the soil (Popay *et al* 1987).

1.2.2.2 **Growth and Development**

Despite dormancy mechanisms assisting nodding thistle seed to germinate at times when establishment is most likely to be successful, population studies have shown that many of the seedlings do not survive to produce flowers. Popay and Thompson (1980) found that only 19% of the 821 individual seedlings they tagged in July survived to flowering in Hawkes Bay. Competition from pasture plants probably contributes to the demise of these seedlings. Although 36% of seedlings transplanted into paraquat-treated pasture survived to flower, only 9% of those transplanted into undamaged pasture flowered (Edmonds and Popay 1983). Popay and Kelly (1986) measured a mean survival rate of 8% for nodding thistle germinating in autumn, and 3% survival for those germinating at other times. They suggested that seedlings germinating in winter or spring would be subject to greater pasture competition than those germinating in autumn, and those germinating in late spring and summer would be subject to drought stress.

The seedlings of nodding thistle develop into rosettes of spiny, lobed leaves (Fig 1.1). The diameter of these rosettes depends on plant age and growing conditions, sometimes reaching over 80 cm but usually measuring less than 40 cm (Popay *et al* 1979). The rosette diameter of individual thistles can decrease during times of slow growth such as in winter (Edmonds and Popay 1983) or summer (Popay *et al* 1979).

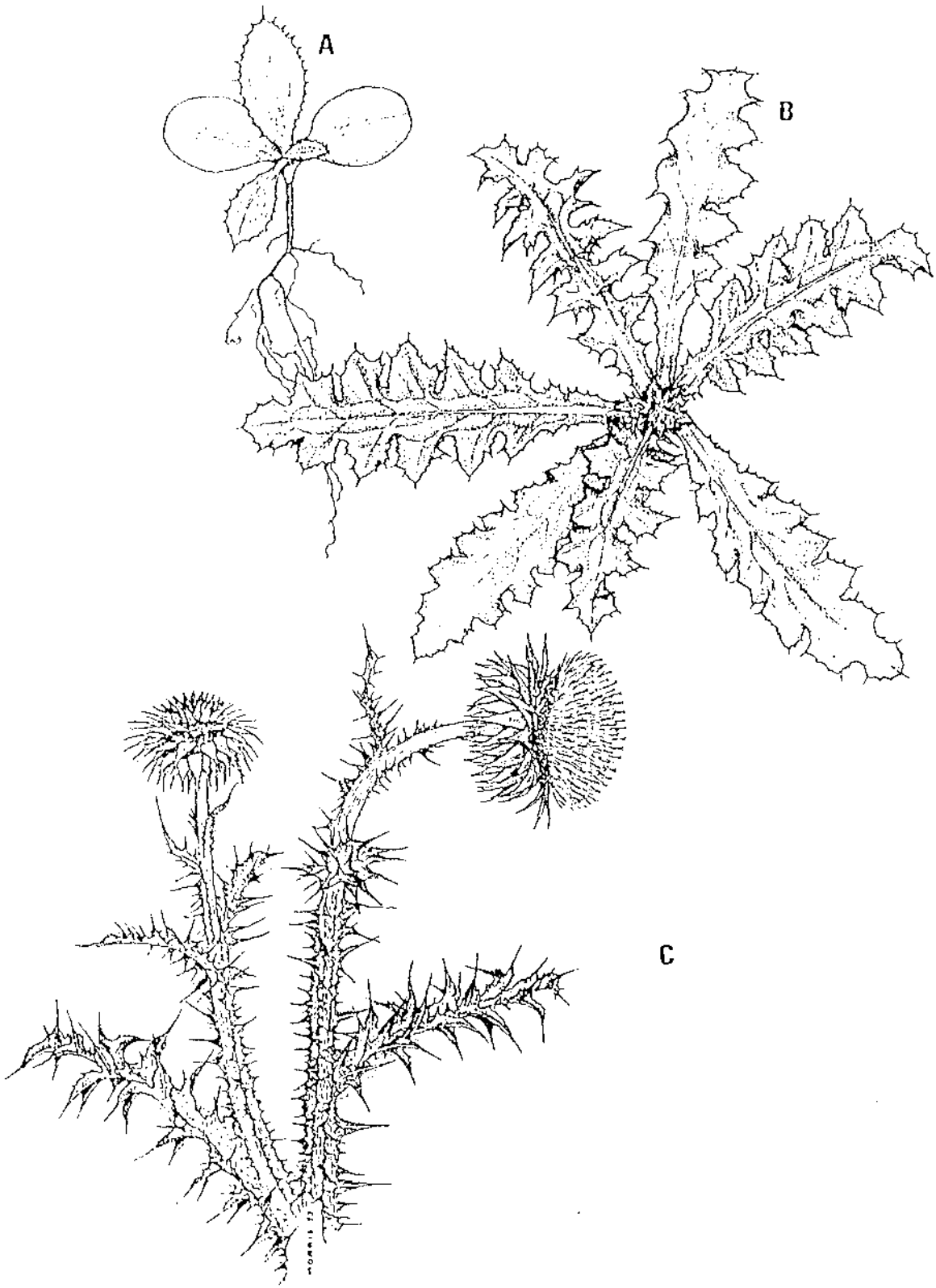


Fig 1.1: Line drawings of a nodding thistle (a) seedling, (b) rosette and (c) flowering branch. After Hyde-Wyatt and Morris (1980).

Growing conditions and the time of seedling emergence influence whether this species behaves as a winter annual, a biennial or (more rarely) a summer annual. Medd and Lovett (1978b) showed that, in Australia when interspecific competition was absent, thistles emerging in autumn always flowered the following summer. Those emerging in June or July flowered in either the first or second summer, and those emerging in late winter or spring did not flower until the second summer. Popay and Thompson (1979) found in New Zealand that thistles germinating in pasture in autumn can flower in either the first or second summer (if they survived), and Popay *et al* (1979) showed that growth rates during the late winter and spring are important in determining when a thistle will flower. Nodding thistle requires vernalization for flowering (Medd and Lovett 1978b) although this requirement appears to be quite variable (Popay and Medd 1990). Popay and Thompson (1979) recorded plants emerging in September or October near Taihape (600 m altitude) in New Zealand which behaved as summer annuals, and they assumed that temperatures were low enough early in the life of these thistles to provide the conditions necessary for vernalization. Although nodding thistle usually behaves as an annual or biennial, Doing *et al* (1969) claimed that interference with its normal development (*eg* spraying, grazing) can cause the species to behave as a short lived perennial.

Nodding thistle begins the reproductive phase when the apex of the rosette elongates ("bolts"), with some rosette leaves being carried up on the stem while the remainder die. An erect, branched stem forms rapidly, occasionally reaching a height of 150 cm but usually much shorter.

1.2.2.3 **Reproduction and Dispersal**

Flower heads up to 6 cm in diameter consist of many hundreds of tubular crimson florets (Fig 1.1). Each floret bears male and female organs, but fertilization is mostly through outcrossing (Popay and Medd 1990). Flowering begins in late November or December and continues until late autumn, with peak flowering occurring in January (Popay and Thompson 1979). Seeds are shed 1 to 2 months after flowering, depending on weather conditions (Doing *et al* 1969).

Popay *et al* (1984) estimated seed production by a reasonably dense (3.8 flowering stems per m^2) stand of nodding thistle in a Hawkes Bay pasture at 8600 seeds per m^2 from 29 flower heads (thus 300 seeds per head). Seed viability was 65%.

Each seed is attached to a pappus of numerous simple white hairs up to 2 cm long

(Popay and Medd 1990). Many seeds separate from their pappi before these are blown away from the plant, and those seeds that do leave the plant detach from pappi within a few metres of the mother plant (Doing *et al* 1969). Kelly *et al* (1988) examined the dispersal of seeds by using nets at several distances from an isolated patch of plants. They found that only 21% of the pappi caught 10 m from the plants carried seeds and, at a distance of 100 m, this figure had fallen to 7%. However seed still attached to pappi after travelling 100 m may well travel for some distance further. With seed production reaching over 8000 per m^2 in dense stands (Popay *et al* 1984), wind dispersal should probably not be dismissed as unimportant for the spread of this species on to neighbouring properties.

For long distance travel between New Zealand provinces however, movement with crop or pasture seed has probably been very important, especially prior to 1967/68 when nodding thistle seed was still permitted in certified seed. Nodding thistle was introduced into Australia in 1950 from New Zealand in contaminated seed (Popay and Medd 1990). As discussed by Smith (1971), some movement is still likely nowadays in uncertified seed, and transport of hay and lime can also spread seed.

There seems to be some confusion as to whether seed can also be spread by grazing animals. There are some reports that nodding thistle has established in new areas following the arrival of livestock from infested areas (Guthrie-Smith 1953; Smith 1971). Matthews (1971) claimed that seed ingested by livestock (species not stated) is normally destroyed in the gut, whereas Parsons (1973) stated that heads are often eaten by sheep and the seeds pass through the animals without losing viability. The latter author also felt that the pappi of seeds help them become attached to animals. Presumably flower heads still containing seed could also become entangled in the woolly fleeces of sheep after detaching from a plant. Popay (pers comm) claims livestock normally eat flower heads in full flower before seeds are mature and so are unlikely to survive passage through the gut. Germination tests conducted on saffron thistle (*Carthamus lanatus* L.) seed, after passing through goats in Australia which fed on mature flower heads, showed that no seeds were viable (Pierce 1990).

1.2.2.4 **Habitat**

The ecological requirements for the success of nodding thistle have been discussed in detail by Doing *et al* (1969), and their findings are summarised below.

The vernalization requirement of this species is thought to limit its distribution to temperate climates. It is found in Europe (where it is native), USA, southern Canada,

Argentina, south-eastern Australia and New Zealand.

It thrives in areas prone to seasonal drought as this weakens existing vegetation cover, allowing seedlings to establish. However moisture is needed in summer when the plants are flowering to ensure good seed production. Under Australian conditions, alternating dry and moist summers are postulated to give best conditions for nodding thistle. However New Zealand tends to have a more humid climate than Australia and summers that are considered dry by New Zealand standards are probably moist enough to allow adequate seed production.

Nodding thistle generally occurs where soil fertility is high, especially where top-dressing has increased phosphate levels, where nitrogen levels are high due to pasture legumes or fertilizer application, and where soils have high exchangeable calcium. Nodding thistle was found to act as an annual only when soil nutrition was very high. High fertility would enable plants to grow large enough for vernalization while conditions were still cool.

This species generally occurs on deep soils without very compact horizons, and where waterlogging does not occur. It is typically found on hilltops and slopes, presumably due to the lack of waterlogging, susceptibility of pasture cover to drought damage and high fertility from livestock transfer.

1.2.3 Non-Chemical Control of Nodding Thistle

Nodding thistle has not yet established in many areas where it probably could thrive. In such areas, techniques to prevent introduction are obviously very worthwhile. In New Zealand, the Government has used legislation to help minimize spread into areas by declaring nodding thistle a Noxious Plant and by not certifying seed lots found to contain nodding thistle seed (see Section 1.2.1). Individual farmers can help minimize spread by using only certified seed, by carefully monitoring the movement of equipment and materials such as hay which may contain weed seed on to their properties, and by ensuring any nodding thistle plants that do establish are killed before seed is produced.

Once nodding thistle has established, the following control options are available.

1.2.3.1 **Pasture Management Techniques**

Germination of nodding thistle seeds is reduced by the presence of green vegetation, even if pasture is short (Phung and Popay 1981). Seedlings that do germinate within existing pasture are likely to be killed by competition (Edmonds and Popay 1983), especially for light (Medd and Lovett 1978a).

Therefore one of the most effective ways of controlling nodding thistle is to ensure the pasture remains dense and competitive throughout the year. Popay (1986) suggested this could be done by:

- selecting suitable pasture cultivars, *eg* using "Grasslands Wana" cocksfoot, "Grasslands Nui" and Ellett ryegrasses rather than "Grasslands Ruanui" ryegrass for areas subject to summer drought

- avoiding over-grazing, especially in summer, possibly by providing alternative feed sources such as lucerne

- controlling insects, such as grass grub and porina.

Apart from keeping pasture dense and competitive, the other main pasture management technique for controlling weeds is to encourage defoliation by livestock. Unfortunately sheep and cattle generally avoid grazing nodding thistle foliage, although Popay (1986) claims some control may be obtained using rotational mob stocking.

The palatablilty of nodding thistle can be increased to these classes of livestock. The palatability of weeds can increase following the application of such herbicides as MCPA and 2,4-D, a phenomenon which has led chemical companies to recommend keeping livestock out of sprayed pasture following grazing to prevent poisoning by weeds such as ragwort (*Senecio jacobaea* L.) and hemlock (*Conium maculatum* L.) (O'Connor 1989). In Australia, thistles and other pasture weeds are sprayed with sub-toxic rates of MCPA to increase their palatability, and then grazed intensively by sheep at 7-10 days after application (Nufarm 1989). Known as "spray-grazing", this technique is claimed to control thistles without damaging pasture legumes. Although the "spray-grazing" technique has not been used in New Zealand, nodding thistle in Waikato sprayed with MCPA in May or June was better controlled if mob stocked 3 weeks later (Sanders 1990). At least one Hawkes Bay farmer has used a spray of molasses to increase rosette palatability and claims successful control was obtained (Popay, pers comm).

Bendall (1973) showed that slender winged thistle (*Carduus pycnocephalus* L.) and winged thistle (*Carduus tenuiflorus* Curt.) rosettes were readily eaten by sheep in Australia if they were growing in rank pasture. Competition for light in the tall pasture made the thistles etiolated and lush with softened prickles. The growing points of the thistle rosettes were approximately 2-3 cm above ground level because of the etiolation and most of the thistles did not regrow after removal of these meristems. This system could also be used with nodding thistles, though pasture density and growth may be adversely affected, which might lead to further germination of weed seeds through removal of shading. Mob-stocking of nodding thistle during winter in Waikato with sheep bared the ground and further nodding thistle seedlings then established (Sanders 1990).

Martin and Rahman (1988b) attempted to control nodding thistle using various combinations of hard and lax grazing. They allowed pasture covers to reach 2500-3500 kg DM/ha in summer before grazing down to 1100-1500 kg DM/ha, and found that the survival of both small and large rosettes differed little from that under continuous grazing pressure. On the basis of this result, they felt there was no benefit to be obtained from allowing pasture to become rank prior to grazing. However it could be argued that they did not give the technique a fair test. A pasture cover of 2500-3500 kg DM/ha can hardly be described as rank. The residual pasture cover of 1100-1500 kg DM/ha does not really represent hard grazing either.

These same authors also looked at various grazing strategies over winter. With some of these treatments, pasture cover prior to grazing did reach 4500 kg DM/ha, though it was as low as 1200 kg DM/ha in some plots. The pasture was then grazed by sheep down to residual covers of 500-800 kg DM/ha at various times during winter. For small rosettes (3-10 cm diameter), 29% mortality occurred under continuous grazing, whereas the rotational grazing treatments resulted in 42-57% mortality. Larger rosettes were generally less affected. If the pasture was allowed to become more rank prior to grazing, more effective control of nodding thistle may be possible.

Unlike sheep and cattle, goats graze thistles readily. Rolston *et al* (1981) showed that goats prevented Scotch thistle (*Cirsium vulgare*), marsh thistle (*C. palustre* (L.) Scop.) and Californian thistle (*C. arvense* (L.) Scop.) from producing mature seed heads. Although the thistles were not grazed as rosettes, defoliation became intensive once the reproductive phase began with bolting. Scotch thistles and marsh thistles that bolted were eaten to ground level by autumn. Six goats per hectare prevented almost any thistle seed production. Although nodding thistle was not present in this trial, Holst (1980) has reported that goats readily eat this species in Australia. Goats have also

been used in New Zealand to control nodding thistle (Stevenson 1989).

1.2.3.2 **Biological Control**

Nodding thistle is native to Europe, Siberia, Asia Minor and North Africa (Clapham *et al* 1962). It has only been introduced to New Zealand within the last 100 years (Allan 1940). There is good potential to use biological control for nodding thistle in this country, introducing insects and fungi that feed on it in its native countries but which were left behind when the weed was brought here.

Considerable research has been conducted in North America on the biological control of nodding thistle. The two main species evaluated so far are weevils. A receptacle weevil, *Rhinocyllus conicus* Frohlich, was released in Canada in 1968 and USA in 1969, while a crown-feeding weevil, *Trichosirocalus horridus* (Panzer) (syn *Ceuthorynchidius horridus* Panzer), was released in USA in 1974 and Canada in 1975 (Wapshere *et al* 1989). They have also been more recently introduced to other countries where nodding thistle causes problems. *R. conicus* was released in New Zealand in 1972 and *T. horridus* in 1985 (Jessep 1989a).

R. conicus adult weevils lay eggs on the exterior of seed heads, from where the larvae move into the seed head and feed within the receptacle, cutting off the food supply to the developing seeds (Jessep 1975). The insect was spread throughout New Zealand both by farmers and Noxious Plants Officers (Popay *et al* 1984) and also with natural dispersal by the flying adult (Jessep 1981). As a result it has now become established throughout New Zealand wherever nodding thistle grows (Jessep 1989a).

However a study by Popay *et al* (1984) suggests that this insect may not be destroying enough nodding thistle seed to make much impact on the weed in this country. The peak of egg laying appears to end before the peak of flower head production, so that flowers produced later in summer are not affected by the weevil. Over the two seasons that Popay and co-workers studied an infested thistle population in Hawkes Bay, seed production was only reduced by 38%. Even on the assumption that just one larva in a head would eliminate seed production from that head, total seed production would have been reduced by about 76%. This would still have resulted in 2000 mature seeds per m^2, theoretically enough to maintain a large seed bank in the soil. Only 3% of nodding thistle seed was lost to *R. conicus* at a Canterbury site in 1988-89 (Kelly *et al* 1990). Flowering occurred four weeks later than at Hawkes Bay and so presumably did not coincide with egg-laying.

T. horridus adult weevils lay eggs from late summer until the end of the following spring on the leaves of nodding thistle rosettes (Jessep 1989a). The newly hatched larvae crawl down to the crown where their feeding damages the central growing points and the base of older leaves (Jessep 1989b). Feeding can destroy the crown and damage lateral regrowth. Jessep (1989b) claims regrowth from damaged rosettes is soft rather than prickly, and so is more likely to be eaten by sheep.

The impact of *T. horridus* on nodding thistle in New Zealand is not yet known as the first release in 1985 was at Ashburton only, and more widespread releases were not made until 1989 (Jessep 1989b). However Cartwright and Kok (1985) found that not only was this insect of minimal use in controlling nodding thistle in USA, it could also increase seed production from plants that it attacked. When insect numbers were low, the destruction of apical dominance by insect feeding resulted in plants producing more stems and larger crowns than usual. Under certain conditions, this could lead to an increase in seed production. Results in a New Zealand trial conducted by Jessep (1989b) suggested that *T. horridus* can considerably reduce seed production, but insect numbers were artificially high.

Other organisms are being evaluated for their potential as biological control agents for nodding thistle overseas (Popay and Medd 1990), some of which may be released one day in New Zealand. These agents should complement organisms such as birds and mice which are already feeding on nodding thistle and its seeds (McCallum and Kelly 1990). Their combined predation should at least reduce the soil seed population and vigour of rosettes, which may make nodding thistle more susceptible to other control techniques.

1.2.3.3 **Mechanical Control**

Popay and Medd (1990) suggested several mechanical techniques for controlling nodding thistle. Hand grubbing is commonly practised for infestations of low density or as a follow-up operation after broadcast treatment of denser or larger infestations. Unless plants are grubbed 5 to 10 cm below the surface, buds contained on the crown and upper root tissues will resprout. Flowering nodding thistles are often mown or slashed. This improves the appearance of thistle-infested pastures, assists the movement of stock and improves access to fodder. Plants cut before the appearance of the terminal bud are likely to regrow, and viable seeds can be produced from heads severed as early as two days after anthesis (Popay and Medd 1990).

1.2.3.4 **Summary**

As has been shown above, there are techniques for controlling nodding thistle which do not make use of herbicides. However they all have drawbacks. Preventing nodding thistle arriving and establishing on a property is very worthwhile in areas without the weed at present, but it is obviously too late to use this technique in those parts of the country where it is well established. Keeping pasture dense and competitive throughout the year will prevent the seed from germinating, but this can be very difficult to achieve in dry summers, especially on ridges and stock camps. Goats will eat nodding thistle once it begins flowering, but this control strategy requires changes in farming policy on affected properties. There may be some potential to increase damage to thistles using sheep, but the techniques involved require further research under New Zealand conditions. Biological control is a long term control strategy which may not show any effects on nodding thistle infestations for many years yet. Grubbing is only practical for very low densities and mowing is probably too ineffective to be worthwhile.

As a result, control with herbicides has been the favoured option in New Zealand since several inexpensive products kill nodding thistles.

1.2.4 Chemical Control of Nodding Thistle

Popay *et al* (1989) showed that MCPA, 2,4-D, MCPB and MCPB/clopyralid mixtures control nodding thistle under appropriate conditions, and all are used in New Zealand. MCPB is the only one of these herbicides that does not damage clovers, but it is also less effective than the rest at some times of the year (Delahunty 1960). MCPA at 1.0 kg ai/ha and 2,4-D at 1.5 kg ai/ha are equally effective (Popay *et al* 1989). MCPA has become the preferred herbicide for nodding thistle in the South Island whereas 2,4-D is the most commonly used herbicide in the North Island (Popay and Medd 1990). MCPB ester/clopyralid mixtures at 0.75 + 0.02 to 1.0 + 0.03 kg/ha give significantly better control than MCPA or 2,4-D where control is difficult, though they damage clovers more (Rutherford *et al* 1981).

Clopyralid alone, picloram and dicamba are all extremely effective against nodding thistle, but are very damaging to clovers and can persist in the soil (Popay and Medd 1990). Picloram, dicamba and glyphosate applied to bolted nodding thistle plants with rope wick applicators give some control of the plants without serious pasture damage, but up until recently this has been less effective than spray applications (Thompson 1983; Jentes 1985). However improvements in wiper application technology are

resulting in more effective control of nodding thistle with this technique (Martin *et al* 1990).

Although MCPA and 2,4-D have been recommended for nodding thistle control in New Zealand pastures for 30 years (Delahunty 1960), research has continued on the effective use of these herbicides. Timing of application has been one of the main factors studied.

Popay *et al* (1989) discussed how timing is important for three main reasons. Herbicide should be applied after all seedlings have germinated for the season. In most parts of New Zealand, almost all germination occurs after autumn rainfall in drought-damaged pastures. Pasture cover is adequate to prevent germination occurring at other times of the year. Therefore a late autumn application should kill almost all of the seedlings for the season. However in some parts of the country, especially Otago, hard winter grazing may result in further germination in spring. Applying herbicide annually in late autumn would not affect these seedlings until many months later when they would be quite large.

The second factor influencing timing of herbicide application discussed by Popay *et al* (1989) is that nodding thistle becomes less susceptible to herbicides as it gets larger. As rosettes increase in size, higher application rates are required for effective control. Thistles are particularly tolerant once they reach the reproductive stage. In Hawkes Bay, spring application of herbicides can be less effective than autumn application because the mild winter climate can result in rosettes being quite large by spring, and flowering can begin quite early in this area.

In addition to these two considerations, phenoxy herbicides such as MCPA and 2,4-D are less effective when plant growth is slow (Muzik 1976). This will be discussed in more detail in a later section. This may be the reason why nodding thistle is less susceptible to these herbicides applied in July and August (Popay *et al* 1989).

Poor control of nodding thistle cannot always be explained in terms of these factors. In 1981, a trial was conducted to compare the susceptibility to herbicides of nodding thistle claimed to be hard to control at Argyll (Hawkes Bay, New Zealand) with that at another farm 31 km away at Matapiro where nodding thistle was easily controlled (Harrington and Popay 1987). Individual nodding thistle rosettes at both sites were pegged and treated with either MCPA (potassium salt) or 2,4-D (butyl ester). Both herbicides were applied at 20 mg ai/plant in 10 ml of solution from a modified drench gun with a solid cone nozzle. Each treatment was applied at six different dates to eight plants, giving a total of 192 plants treated in the trial. The average diameter of plants

was 24 ± 10 cm at treatment. The proportion of plants that died after each treatment was recorded. The soil type at both sites was a Matapiro silt loam, and the annual rainfall was similar (899 mm at Argyll and 995 mm at Matapiro).

MCPA and 2,4-D were both significantly less effective at Argyll than at Matapiro (Table 1.1). This difference occurred in autumn, winter and spring with plants of similar sizes, making an explanation using the factors discussed earlier quite difficult. As discussed in Section 1.1, these results led to the present study being initiated in 1985 to discover more about factors influencing the efficacy of MCPA and 2,4-D on nodding thistle.

Table 1.1: Effect of MCPA and 2,4-D on the control of nodding thistle grown at Argyll and Matapiro (from Harrington and Popay 1987). All values are % of plants killed.

Time of application	MCPA		2,4-D	
	Argyll	Matapiro	Argyll	Matapiro
March	40	100	38	100
April	43	100	21	100
May	88	100	43	88
June	50	100	14	63
July	50	75	0	88
September	20	100	0	100
Mean*	48.5 b	95.8 c	19.3 a	89.8 c

*Treatment means followed by the same letter are not significantly different ($p = 0.05$) using an F-test.

1.3 MCPA AND 2,4-D

1.3.1 Properties

The chemical structures of MCPA and 2,4-D are shown in Fig 1.2. The only difference in their structure is the substitution of the methyl group attached to the phenyl ring of MCPA for a second chlorine atom in 2,4-D. The molecular weight of MCPA is 200.6 and of 2,4-D is 221.0.

They are commonly referred to as phenoxys or phenoxyacetic herbicides because they comprise a phenyl ring attached to an oxygen atom, in turn attached to acetic acid. They are also often known as hormone herbicides because of their similarity in structure and activity to the plant hormone indoleacetic acid.

MCPA has a solubility in water of 825 ppm and 2,4-D is 620 ppm (Worthing and Walker 1983). This low solubility necessitates the formulation of these herbicides as either salts or esters (which are then emulsified) so that they can be diluted in water prior to application (Klingman and Ashton 1982). Anderson (1977) outlines some of the salts and esters of these chemicals that can and have been used as herbicides, and also some of the properties of these compounds. Ester formulations tend to be more effective herbicides than salt formulations because they are better able to penetrate the waxy cuticles of plant foliage. However ester formulations are volatile, unlike the salts, and so are more likely to cause damage to non-target plants.

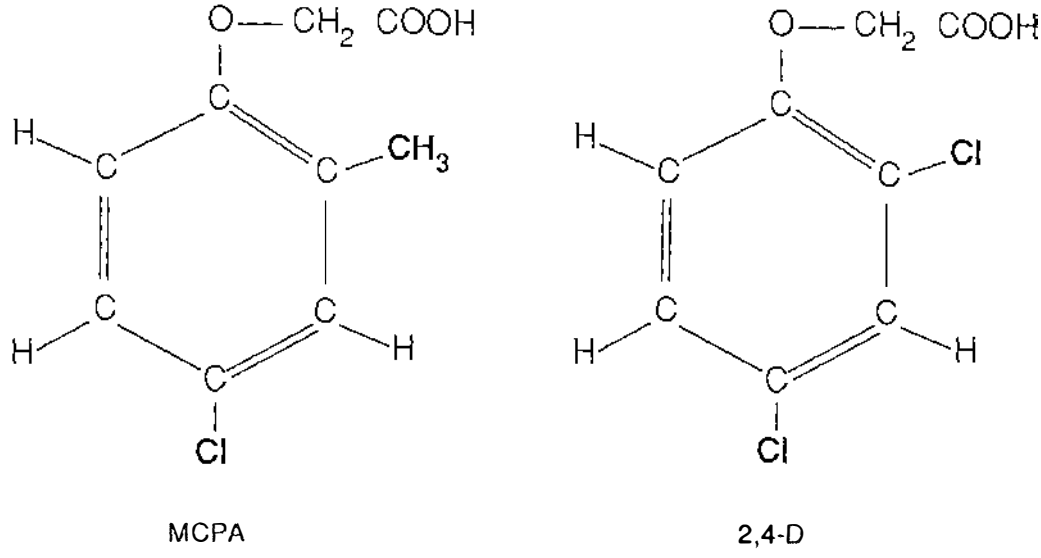


Fig 1.1: The structure of MCPA and 2,4-D molecules

These two herbicides are generally used only as foliar-applied herbicides because they are rapidly degraded in the soil by microorganisms, low rates being decomposed in one to four weeks in a warm, moist loam soil (Klingman and Ashton 1982). Because they are relatively small organic acids, they dissociate to form a negatively charged ion in soil solution that is not strongly attracted by soil colloids, and therefore can be leached (Ross and Lembi 1985).

MCPA and 2,4-D have moderately low toxicities, with the acute oral LD_{50} for rats being 700 mg/kg for MCPA and 375 mg/kg for 2,4-D (Worthing and Walker 1983). Thus in New Zealand they are categorized as Class 3 poisons (O'Connor 1989).

1.3.2 History and Uses

As outlined by Ivens (1980), the discovery of MCPA and 2,4-D in the early 1940s signalled the start of the herbicide industry as we know it today. Prior to the 1940s, chemicals available for weed control had many drawbacks, such as the toxicity of sodium arsenite, DNOC and dinoseb, the corrosiveness of sulphuric acid and the unreliability of copper sulphate and related salts. Research into plant growth substances in the early 1940s lead to one group of British scientists discovering the herbicidal properties of MCPA, and another British group plus an American team both discovering 2,4-D.

MCPA and 2,4-D were developed primarily for their ability to kill dicotyledonous weeds at low application rates without damaging grass species. This allowed them to be used selectively to control weeds in cereal crops, and they have also been used for selective weed control in turf and pastures (Fletcher and Kirkwood 1982).

These new herbicides had major advantages over any chemicals used previously for controlling weeds. They were relatively non-toxic, neither staining nor corrosive, active against a wide range of weeds (including some important perennials) yet selective in grass crops, and their action was less dependent on weather conditions after application. They were also cheap to use in relation to the yield increases obtainable as a result of effective weed control.

Ivens (1980) states that the average size of farms in many parts of the world was increasing in the 1940s and 1950s while farm labour was becoming scarcer and more expensive so that there was a growing need for the type of weed control offered by herbicides. As a result MCPA and 2,4-D came into extensive use for cereal production in a very short time. In 1945, approximately 800 tonnes of 2,4-D was produced in

USA, then 2000 tonnes in 1946, 10,000 tonnes in 1949, 23,000 tonnes in 1950 and 43,000 tonnes in 1961 (King 1966). Although 2,4-D was the main chemical used in USA where the chlorophenol needed for its production was available, MCPA was the main chemical used in Britain since chlorocresol, the starting point for MCPA production, was more available (Norman *et al* 1950).

The success of these two chemicals led to a number of chemical companies initiating programmes of herbicide development, leading to the development of the array of organic herbicides now available (Ivens 1980).

The two herbicides came into common use in New Zealand in 1948 (Neill 1952). A measure of their success and effectiveness is that they are both still commonly used in New Zealand today, in spite of the development of hundreds of other herbicides, many of which have not survived on the highly competitive herbicide market.

Reference to the recommendations for these two chemicals for 1990 in New Zealand (O'Connor 1989) indicates their niche within the market. MCPA is available by itself only as a potassium salt for selective weed control in pastures, cereals and linseed. Three forms of 2,4-D are available. The butyl ester of 2,4-D is marketed for weed control in pastures and waste areas. The amine salt of 2,4-D is sold for weed control in cereals as it is less damaging to cereal plants than the butyl ester formulation. This can also be used in pastures but is less effective. A dust formulation of the 2,4-D sodium salt is also available for use in pastures.

MCPA and 2,4-D are sold in combination with other chemicals to increase the range of weeds controlled. MCPA is sold in combinations with mecoprop, dichlorprop, dicamba, ioxynil, bromoxynil or cyanazine for weed control in cereals and turf. Dicamba and picloram are the two main additives to 2,4-D in products marketed for controlling herbaceous and woody perennial weeds in pastures (spot applications only), turf and waste places.

Matthews (1975) has discussed the susceptibility of New Zealand's weed species to MCPA and 2,4-D. Generally they control a similar range of weed species, most of which are dicotyledonous. However some species are better controlled by 2,4-D while others are more susceptible to MCPA. Where selectivity is not important, 2,4-D ester tends to be more effective and therefore more commonly used for perennial weed control than salt formulations of MCPA. Although grass species are relatively tolerant of MCPA and 2,4-D, some damage can occur. Application to cereal plants prior to the 5-6 leaf stage or after stem elongation can result in deformities. MCPA is less damaging than 2,4-D, and salt formulations less damaging than esters (Fryer and

Makepeace 1977). As a result, the MCPA potassium salt either by itself or with other herbicides is used much more commonly than 2,4-D for weed control in New Zealand cereal crops (Close and Noonan 1984), although the 2,4-D amine salt is occasionally used. Likewise fine turf grass species such as browntop (*Agrostis capillaris* L.) are more susceptible to damage by 2,4-D than MCPA, so the latter herbicide is more commonly used for weed control in lawns (Matthews 1975).

Both MCPA and 2,4-D damage the clover component of pasture. Maclean (1957), Meeklah (1958), and Thompson and Saunders (1984) all documented how both of these herbicides cause a temporary check in clover growth, with MCPA being slightly more damaging to white clover than 2,4-D at equal application rates. Hartley (1983) showed that MCPA could reduce live weight gains of sheep grazing treated pasture by as much as a Scotch thistle density of 1.67/m^2.

1.3.3 Absorption Into Plants

Factors affecting the absorption and translocation of the phenoxy herbicides have been extensively studied. The effect of environmental factors, stage of growth and herbicide formulation have been investigated for many different species, using intact plants, excised leaves, tissue segments and isolated cuticles (Ashton and Crafts 1981).

To enter the foliage of plants, herbicides must penetrate a lipoidal, non-cellular, non-living membrane called the cuticle. This minimizes water loss from the plant and also restricts the entry of exogenous materials, particularly those of a polar nature (Fletcher and Kirkwood 1982).

Ashton and Crafts (1981) have outlined the process by which phenoxy penetration of this cuticle occurs. Whether applied as a salt or ester, 2,4-D molecules diffuse into the cuticle, move through it into the aqueous apoplast, and finally enter the living cells by penetrating the plasmalemma. Negatively charged cuticle surfaces repel the negatively charged phenoxy anions of highly dissociated sodium or potassium salts, which therefore are not readily absorbed. The salts of weak bases such as ammonium or the amines are less completely dissociated. The esters readily dissolve in the nonpolar cuticle and pass through it.

The pH of the spray solution influences cuticle penetration as weak acids penetrate the nonpolar cuticle best at low pH values when the molecules are largely undissociated (Bovey 1980). However pH is not thought to affect penetration of ester formulations since they are not readily dissociated (Ashton and Crafts 1981).

Penetration of the cuticle can also be aided by the addition of surfactants, and very few foliar-applied herbicides are formulated without one or more surfactants (McWhorter 1985). Jansen (1965) discussed the principles for the selection of surfactants for various forms of 2,4-D.

Absorption of phenoxy herbicides increases with increasing temperature within the physiological range, *ie* below 30-35^{o}C, presumably due to increased disorganization of the lipid materials arranged in micelles in the cuticle and plasma membrane, with consequent increased cuticle and membrane permeability (Loos 1975).

High relative humidity enhances absorption of the phenoxy herbicides because of increased droplet drying time and probably increased cuticle permeability (Ashton and Crafts 1981). Stomata will also be open but herbicide penetration via stomata is minimal because of cuticle lining inner walls of stomatal pores and the low surface tension required for liquids to enter pores (Hess 1985).

Absorption is also enhanced by an increase in illuminance (Sargent and Blackman 1972). Hull (1970) has reviewed the factors influencing penetration of pesticides into foliage in some detail. Other factors include leaf age and development, differences between species, rainfall, moisture stress and the physical nature of the leaf surface.

Phenoxy herbicides are readily absorbed by roots, apparently primarily by an active process since they are accumulated in roots against a concentration gradient. Low temperatures, anaerobic conditions and metabolic inhibitors markedly reduce their uptake (Donaldson *et al* 1973).

1.3.4 Movement Within Plants

Many authors, including Robertson and Kirkwood (1970), Loos (1975), Hay (1976), Richardson (1977), Ashton and Crafts (1981), and Fletcher and Kirkwood (1982) have reviewed research into the movement of 2,4-D and MCPA within plants.

As discussed by Ashton and Crafts (1981), numerous studies have shown that the phenoxy herbicides are translocated in the phloem. Thus the pattern of distribution from a treated leaf is both acropetal and basipetal from source to sink, from photosynthesizing leaves to areas of high utilization of photosynthate (apical meristems; developing leaves, stems, flowers, fruits, roots; and root meristems) bypassing mature photosynthesizing leaves.

However the amount of herbicide moved from treated tissue into the stem is quite small, and the amount reaching the shoots is often negligible (Hay 1976). Robertson and Kirkwood (1970) discussed the reasons for this. The transport route can be damaged by these materials when applied at "normal agricultural" rates, explaining why translocation is often better in physiological experiments that use lower concentrations. Translocation is also reduced by herbicide molecules being adsorbed to surfaces within the plant, forming immobile complexes or being absorbed by phloem parenchyma cells. Retention of the phenoxy herbicides seems to be minimized by rapid translocation processes, so movement into the roots is best achieved during periods of active root growth when the herbicide will be drawn with the assimilates into this sink (Loos 1975).

However MCPA and 2,4-D can also be moved from the phloem to the xylem during translocation (Field and Peel 1971). Hay (1976) argued that very little herbicide reaches the roots because it moves into the apoplast once in the stem and is carried back up into treated leaves or into developing leaves from which transport does not occur.

The movement of phenoxy herbicides from plant roots following absorption from the soil is very restricted despite the ability of these herbicides to move within the xylem (Loos 1975). There is probably strong retention of the herbicide by living cells in its passage through the symplast to reach the xylem.

In experiments looking at movement of foliar-applied phenoxy herbicides into plant roots, the herbicide often leaks out of the roots into the surrounding medium (Ashton and Crafts 1981).

The translocation of phenoxy herbicides from foliar applications is much more restricted in monocotyledons than in dicotyledons, apparently due to intercalary meristems in the stems and leaves of grasses acting as a barrier (Robertson and Kirkwood 1970).

The movement of 2,4-D within plants has been more often investigated than that of MCPA. Their behaviour is probably very similar, though MCPA is more mobile in plants than 2,4-D (Ashton and Crafts 1981).

Loos (1975) and Richardson (1977) discussed the effect of environmental conditions on the translocation of the phenoxy herbicides. In general, environmental conditions such as illuminance, relative humidity, soil moisture and temperature that favour high

rates of photosynthesis and growth result in maximum rates of translocation.

1.3.5 Symptoms and Mode of Action

MCPA and 2,4-D have profound effects on the growth and structure of plants. Epinastic bending may follow within minutes of foliar application, growth may cease within hours, and over days of exposure, formation of tumours, secondary roots and fasciated structures may be pronounced (Ashton and Crafts 1981).

As described by Hanson and Slife (1961), spraying susceptible plants with these herbicides causes rapid changes in the normal growth pattern. Meristematic cells cease dividing and elongating cells stop growing in length but continue radial expansion. In mature plant parts, parenchyma cells swell and begin to divide, producing callus tissue and expanding root primordia. Root elongation stops and root tips swell. Young leaves stop expanding and develop excessive vascular tissue, very compact mesophyll low in chlorophyll, and often fasciation. Roots lose their ability to absorb water and salts, photosynthesis is inhibited, and the phloem becomes plugged. All such disruptions contribute to the death of plants. At the cellular level, 2,4-D and MCPA prevent immature cytoplasm from maturing, cause reversion of mature cytoplasm to the immature stage, and increases the number of ribosomes.

Cherry (1976) summarised the hypothesis of how MCPA and 2,4-D kill plants as follows. They act as synthetic auxins, and appear to control nucleic acid biosynthesis as well as controlling some aspects of cell wall loosening, deposition and relaxation. The net result of treating target cells of sensitive plants with these herbicides is a large increase in ribonucleic acid production, including messenger RNA. MCPA and 2,4-D act in the cell like indoleacetic acid by enhancing RNA polymerase activity. The resulting synthesis of RNA and protein are accompanied by such a massive proliferation of growth, swelling and appearance of gall-like areas on the roots and leaves that the vascular tissues are crushed causing death.

Although this disruption of the vascular system is usually quoted as the primary mechanism of action for the phenoxys, this has not been proven conclusively despite the many years of research on these herbicides (Bartels 1985). The significance of other mechanisms of action within the plant remain unclear. For example, Robertson and Kirkwood (1970) argue that the significance of inhibitions of the Hill reaction and oxidative phosphorylation by these chemicals should not be underestimated. Any inhibition of these processes must influence the efficiency of energy-dependent functions including mineral ion uptake, photosynthate translocation and indeed

absorption and translocation of the herbicides.

Likewise Ashton and Crafts (1981) outlined the research on the importance of ethylene production in the death of sprayed plants. Plants treated with 2,4-D have stimulated ethylene production. Many of the symptoms of the phenoxy herbicide action such as stem and leaf epinasty are also caused by ethylene. This stimulation in ethylene production probably causes many of the herbicide-induced symptoms but most evidence suggests that ethylene does not actually kill the treated plant.

The relative roles of the various mechanisms of action may change when the phenoxy herbicides are applied at high rates. Ashton and Crafts (1981) state that high rates cause excessive contact injury to plant foliage, resulting in little translocation. Although low levels of 2,4-D stimulate cell proliferation, high levels inhibit both RNA production and cell growth.

Loos (1975) surmises that the reason for the greater sensitivity of dicotyledonous plants to phenoxy herbicides is determined primarily by differences in plant structure and rate of herbicide translocation. Phloem in monocotyledons is scattered in bundles, each surrounded by protective schlerenchyma tissue. The vascular bundles of these plants also have no auxin-sensitive cambium or pericycle. These differences probably help monocotyledons tolerate phenoxy herbicides. Intercalary meristems in the stem and leaves of these plants are thought to act as a barrier to herbicide translocation. There is usually less retention of herbicide on the leaves of monocotyledons which tend to be more upright and narrower than dicotyledonous leaves. At least some monocotyledons may be capable of rapidly metabolizing these compounds.

1.3.6 Degradation Within Plants

The phenoxy herbicides behave similarly to indoleacetic acid within plants. The main difference between them in sensitive plants relates to their degradation (Loos 1975). Indoleacetic acid is easily degraded by plants, allowing concentrations of the hormone to be strictly regulated. Conversely, many plant species are unable to degrade MCPA or 2,4-D and so become damaged when these chemicals are applied at toxic concentrations.

However some plant species can degrade phenoxy herbicides and are thus resistant to their effects. The following summary of how plants degrade phenoxys is taken from a review by Ashton and Crafts (1981).

Numerous reports have shown that plants are able to alter or cleave the side chain of 2,4-D. These reactions account for significant degradation in some plants, but are not considered to be the major pathway of degradation in most plants. However they are the predominant pathway for bacteria.

Hydroxylation of the phenyl ring is common in higher plants. The hydroxyl group often replaces the chlorine atom located opposite the carboxyl group on the phenyl ring. The displaced chlorine atom then rejoins the ring adjacent to the hydroxyl group. Following hydroxylation, the resulting molecule often conjugates with glucose.

Another method of deactivation is the conjugation of the herbicide molecule with an amino acid via the $-NH_2$ group of the amino acid and the -COOH of 2,4-D. Amino acids involved with such conjugation include aspartic acid, glutamic acid, alanine, valine, leucine, phenylalanine and tryptophan. Although it is known that cleavage of the side chain and hydroxylation render the herbicide inactive, there has been some dispute over whether amino acid conjugates are also inactive. However this conjugation does appear to render them immobile.

The possibility of binding or conjugation of 2,4-D with proteins has been proposed from time to time but has not been generally accepted.

1.4 FACTORS INFLUENCING EFFECTIVENESS OF FOLIAR-APPLIED HERBICIDES

1.4.1 Environmental Factors

1.4.1.1 **Climate**

Muzik (1975) reviewed research on the influence of climatic factors on the susceptibility of weeds to herbicides. His findings are summarised below.

Growth of plants for several weeks under high irradiance, low humidity and high temperature will lead to a greater degree of pubescence and a thicker cuticle. A dense pubescence and a thick cuticle will make the leaf less wettable and consequently lead to a reduced penetration of foliar-applied herbicides and therefore reduced phytotoxicity.

Wind action can lead to leaf, stem and root injury and, in turn, to increased susceptibility to herbicides. Wind can damage the cuticle, especially by the abrasive

action of dust particles, leading to improved entry of herbicides. Rain prior to treatment can enhance phytotoxicity through cuticle damage.

Conditions favouring rapid growth at time of application also appear favourable for herbicidal activity. The movement of foliar-applied herbicides along with the photosynthates away from the leaf maintains the gradient between levels of herbicide inside the leaf and the level of herbicide on the leaf surface necessary for rapid penetration. Ambient temperatures close to 30^{o}C seem best for rapid entry and translocation of these herbicides. Irradiance levels near the saturation point for the particular species leads to increased phytotoxicity.

Plants which have recently undergone severe water stress to the point of wilting will be more resistant to herbicides and should be allowed a week or two to recover before spraying. This is due to increased thickness and density of the cuticle. There will also be an accumulation of abscisic acid which causes stomatal closure, but this is probably of less importance (see Section 1.3.3).

Adequate soil moisture and a high relative humidity are critical to support hydration of the protoplasm and to avoid water stress. High relative humidity is not critical for growth and development so long as soil moisture is adequate, but in almost all instances rapid entry and transport of foliar-applied chemicals are favoured as relative humidity approaches 100%.

Rain falling soon after application reduces the effectiveness of most foliar-applied herbicides. Water-soluble herbicides penetrate leaf surfaces only slowly and can be washed off by rain occurring within 12 hours of application. Other herbicides such as 2,4-D esters penetrate very rapidly so their activity is not usually affected by rain falling soon after application. Light rain may enhance penetration of most herbicides by preventing drying of the spray droplets and hydrating the cuticle.

Low temperatures (5-10^{o}C) following treatment delay the expression of symptoms and, in most instances, decrease phytotoxicity. However freezing temperatures (below 0^{o}C) or drought conditions can increase the phytotoxicity of herbicides such as MCPA and 2,4-D. The herbicides may stress treated plants, making them more susceptible to frost or drought conditions.

1.4.1.2 **Other Environmental Factors**

Factors affecting plant sensitivity to herbicides other than those directly relating to climate are discussed by Aberg and Stecko (1975). They showed how weeds are most susceptible to herbicides immediately after germination, with tolerance increasing with age. However with established perennial weeds, herbicides may be most effective when applied at a time when recent rapid growth has depleted root reserves and when photosynthates are moving down into the roots to replenish these reserves.

Rapid growth rates at the time of application have been shown in the preceding section to be beneficial to phytotoxicity. Aberg and Stecko showed that herbicides such as 2,4-D work best on plants growing under conditions of high soil fertility and optimal pH levels. Rapid growth of unaffected crop plants can also increase competition on sprayed weed plants, making them more susceptible to the effects of the herbicide.

Physical damage to weeds before, during or after herbicide application may also influence the effectiveness of the herbicide. Damage to the cuticle of plants by tractor wheels during spraying can cause increased phytotoxicity due to increased cuticle penetration by the herbicide (Aberg and Stecko 1975). If weeds are defoliated prior to application of herbicide, there may not be enough leaf material present to intercept sufficient herbicide to kill the plant (Ross and Lembi 1985). Damage to the shoot system by application of excessive amounts of herbicide, or by defoliation, immediately after application will prevent translocated herbicides being moved into the root system and so reduce long-term control (Klingman and Ashton 1982).

1.4.2 Genetic Factors

Weedy species contain a large amount of variability within their gene pools (Holzner 1982). This variability means some individuals within a species are more tolerant of herbicides than others. A continuous selection pressure of many herbicide applications over a number of years can result in the development of herbicide resistant biotypes of a species (LeBaron 1982).

In 1985 when this project began, reported occurrences of herbicide resistance had been recently reviewed by Bandeen *et al* (1982) who discussed the cases of resistance that had been recorded as developing in North America up till the early 1980s, and Gressel *et al* (1982) likewise summarised the cases reported in the rest of the world. The majority of herbicide resistance cases involved triazine herbicides. Other herbicides had been involved but most cases reported for foliar-applied herbicides were of

variability between biotypes of a species with little being known of past spraying histories.

Gressel and Segel (1982) discussed the factors that influence how rapidly resistance to a herbicide builds up within a population. One or more alleles for resistance must be present at some level in the field population of a weed. The initial frequency of individuals with resistance when selection pressure is first applied by a herbicide is important. This is influenced by the number of genes involved, the dominance, and the ploidy. If a monogene-dominant phenotype is involved, initial frequencies are typically 1 in 100,000 to 1 in 1,000,000. However the frequencies will be much lower if resistance involves recessive genes or a herbicide with many modes of action each requiring a separate gene.

If there are genes for resistance, the higher the rate of kill, the more rapid the enrichment for resistance. Most herbicides are applied at rates giving 90 to 95% kill, so the 5 to 10% that survive will be a mixture of resistant and susceptible plants. For most non-persistent herbicides, another flush of susceptible seedlings may establish immediately after herbicide application and eventually produce seeds. This is why persistent herbicides, such as triazines, have caused resistance in many species whilst low-residual herbicides such as 2,4-D have not often led to resistance.

In most cases of pesticide resistance, the individuals selected when pressure is brought to bear on a wild population are less "fit". This has been found for bacteria resistant to antibiotics, fungi and insects resistant to their specific pesticides, rats resistant to warfarin and weeds resistant to triazines. Gressel and Segel state that the "wild-type" weed can be more fit that the selected individual at any one of a number of stages in the life cycle because of the following factors: (a) the proportion of seeds germinating at a given time; (b) the rate of germination; (c) success in establishment following self-thinning; (d) any of the physiological characters resulting in differences in growth rate; (e) plasticity; and (f) the seed size and yield per flower and per plant. The fitness differential can be an important factor in delaying the appearance of resistance.

The time taken for weeds to complete each generation by comparison with bacteria, fungi and insects is the main reason why resistance is a less common phenomenon with herbicides than the chemicals used to control these other organisms. Another factor discussed by Gressel and Segel is the dormancy of weed seeds. Every time a herbicide is applied, only a small proportion of the susceptible individuals of a species may in fact be growing and thus affected by the herbicide. Many of the "susceptible" individuals may exist as dormant seeds in the soil. Although they are not producing further offsprings while they are dormant, the fact that they are not being destroyed

helps to delay the build up of resistance for that species.

After discussing these factors affecting the rate of build up in resistant individuals within a population, Gressel and Segel (1982) commented on how resistance had not yet appeared at their time of writing despite monoculture with continuous use of 2,4-D or its phenoxyacid relatives for more than 30 years. They felt this was because phenoxy herbicides have a much lower effective kill than the triazines because of being rapidly degraded. The mechanism of action with these herbicides was also thought to be an important factor due to the complexity, and probably multisite nature, of their activity.

Since the mid-1980s, the number of herbicide resistance reports has increased markedly. It was estimated in 1991 that the number of herbicide-resistant weed biotypes had tripled since 1982 and that the area of land infested by such weeds had increased more than 10 times (LeBaron 1991). Resistance to triazine herbicides has continued to increase, the number of species with resistant biotypes having risen from 30 as reported by Bandeen *et al* (1982) and Gressel *et al* (1982) mainly in North America and western Europe to at least 57 species throughout the world in 1991 (LeBaron 1991). However a more worrying trend is the increase in types of herbicides to which resistance has now developed. LeBaron (1991) listed 14 herbicide groups to which resistant weed biotypes have developed, and many of the biotypes resistant to members of these groups have only been reported since the mid-1980s. Although some of these herbicides are persistent residual chemicals to which resistance might be expected to develop, many are also non-persistent foliar-applied herbicides. These include diclofop-methyl and other selective grass-killers such as fluazifop-butyl and haloxyfop-methyl (Heap 1991), paraquat (Matsunaka and Itoh 1991), phenoxyacetic herbicides (Harrington and Popay 1987), and sulfonylureas (Christopher *et al* 1992).

1.5 CONCLUDING COMMENTS

A number of issues relevant to the present project have been introduced in the preceding sections concerning nodding thistle, phenoxy herbicides and factors influencing herbicide activity. More detailed discussion of some points appear when appropriate in later chapters.

The literature review indicated that many factors could account for the difficulty in controlling nodding thistle at Argyll. However in 1985 when this project was initiated, environmental factors appeared more likely to be responsible for the poor control than genetic factors.

CHAPTER 2: TECHNIQUES FOR COMPARING THE SUSCEPTIBILITY OF POPULATIONS TO HERBICIDES

2.1 INTRODUCTION

The initial stages of the project involved development of techniques for comparing the susceptibility of nodding thistle populations to a herbicide.

If one population of individuals is less susceptible to a herbicide than another population, it should be possible to determine this by applying a single dose rate of the herbicide to a number of individuals from each population. However this application rate would need to be chosen carefully. If it was too low, neither population might be affected. Conversely of course, an application rate that was too high could kill all individuals from both populations.

Application of a number of different rates increases the likelihood that at least one rate will show whether one population is more susceptible than the other. As described by Fryer and Makepeace (1977), the use of several application rates allows the production of dose response curves for each of the populations whereby application dose is plotted against the subsequent response obtained from treated individuals (Fig 2.1). A comparison of two such curves will show which dose best differentiates between two populations in susceptibility to a herbicide. Such differing curves also allow an estimate of how much more tolerant one population is compared with another. This can be achieved by comparing the dose required to give the same level of response in each population.

The responses measured to produce such curves can either be quantal or quantitative (Finney 1978). With **quantal responses**, each individual is assessed simply on whether it has produced a certain response. This response is usually death, but it could also be some other characteristic response such as the development of a certain symptom. Conversely, if the response of each individual to a dose of herbicide involves measuring the magnitude of a particular trait such as reduction in growth or extent of necrosis, this is referred to as a **quantitative response**.

The design and analysis of dose response curve comparisons have been discussed fully by Finney (1978). If quantitative responses are to be used, an appropriate parameter needs to be selected which gives a good measure of the effect of the herbicide on the plant. Other considerations in the design of experiments include the number of doses

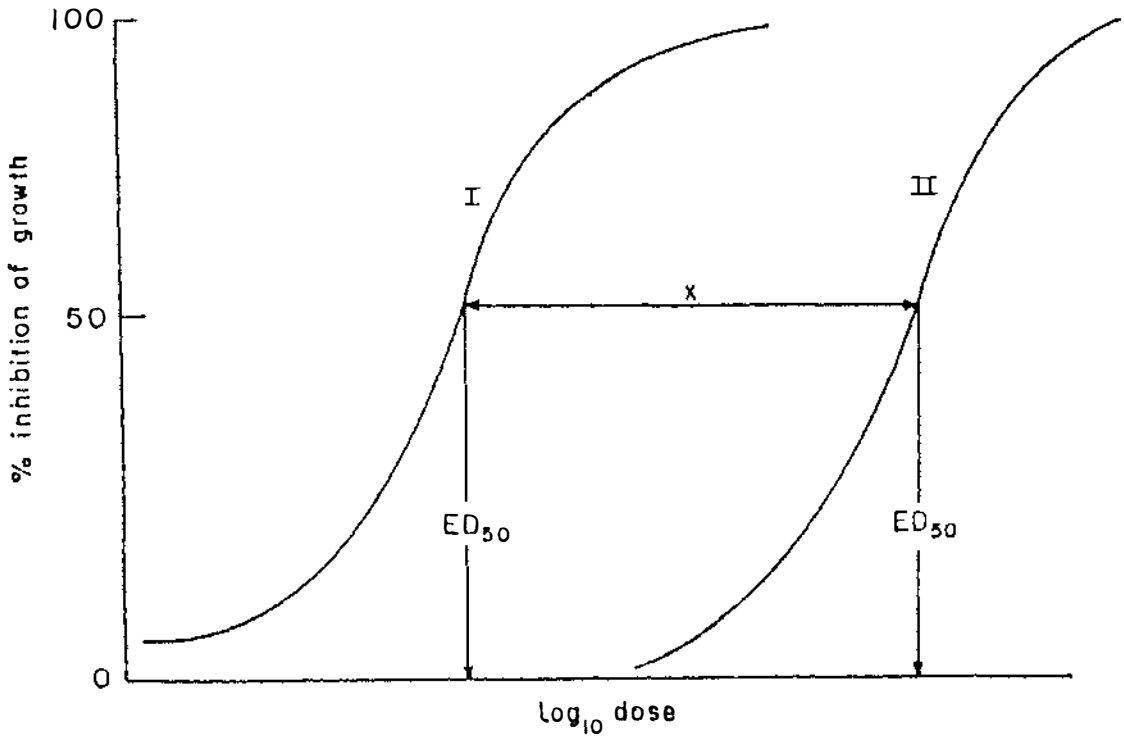


Fig 2.1: The dose response curve for two populations, I and II. An ED_{50} level is an equivalent dose for 50% response. So **x** gives an estimate of how much more tolerant population II is compared with population I (adapted from Fryer and Makepeace 1977).

to be used, the number of plants to be treated with each dose, randomisation of plants with respect to allocation of treatments, and selection of appropriate doses so that several dose levels for each population give values intermediate between total survival and total kill. Statistical analyses generally involve converting the dose response curve to a linear relationship so that a line of best fit can be derived and confidence limits attached to this line. Curves can then be compared with each other as will be discussed in more detail later.

Initial experiments in this project concentrated on determining:

- how to apply different doses to individual plants accurately

- the most appropriate doses to apply

- whether an appropriate parameter could be found to allow quantitative responses to be measured.

2.2 DESIGN OF A SPRAYER

2.2.1 Introduction

A device was required to reliably apply a known amount of herbicide to nodding thistle plants. The necessity for both field and glasshouse experiments was recognized early in this investigation. Therefore application would be needed to plants growing both in paddocks and in pots. It was not necessary that both types of plant be treated by the same device, but if equipment needed to be designed specifically for this project, a sprayer catering for both situations would be useful.

2.2.2 Requirements for Field Trials

Plants sprayed in the field with various rates of a herbicide are often treated with specially designed small plot sprayers, and the design of some of these is described by Wiese (1977). In the early stages of this project, environmental differences between sites were thought to be most important in influencing the susceptibility of nodding thistle to phenoxy herbicides, as was discussed in Section 1.4.2. If this was the case, differences in microclimate across a site could be important in influencing the susceptibility of individual plants. Therefore in field experiments investigating the effect of environmental variables, all treatments within each replicate of a trial should be as close to each other as possible to minimise microclimate differences within a replicate. Otherwise careful monitoring of environmental differences between each plot would become necessary.

Plots sprayed with a boom-sprayer would need to be large enough to allow for drift of spray from adjoining plots. For dose response curves, the highest application rate may be at least 100 times that of the lowest rate, so such drift could be important.

Plot size would also be determined by the density of nodding thistle plants, preferably of uniform size. Nodding thistle plants vary with age in their tolerance to phenoxy herbicides (Popay *et al* 1989). To get plots large enough to overcome edge effects and containing sufficient plants of a particular size, one replicate of a trial with sufficient treatments to produce a worthwhile dose response curve could spread over several microclimate regions within a paddock.

One solution is to label and spray many individual plants rather than several plots each containing many individual plants. Plants to be labelled and treated could thus be selected for their uniformity in size. Spraying one plant at a time could reduce spray

drift by totally enclosing the plant during spraying. Although such a technique would require many more replicates, each replicate could be located within a small area to overcome problems with microclimate differences within a replicate.

Intensive labelling and recording of plants would be needed, but plants present in an area would be more efficiently used. In comparing the susceptibility of plants in areas with different microclimates, the number of plants of a particular size within a particular microclimate area might be limited. Every plant of suitable size could be utilised in an experiment where plants were treated individually. If the sprayed plot system was used, many plants close to the edge of plots might be wasted.

To test this individual plant spraying technique, a device was needed to apply herbicide to individual plants. This technique was used to generate the data in Table 1.1 comparing the susceptibility of nodding thistle populations to MCPA and 2,4-D at two field sites in Hawkes Bay in 1981. In this trial, a modified drench gun fitted with a solid cone nozzle was used, a system initially developed in New Zealand by Porter (1979). However the present author has observed that the spray droplets generated by this gun tend to be larger than those generated by traditional more highly pressurised spray equipment. One effect of microclimate on the susceptibility of plants to herbicides is the influence of illuminance, humidity and temperature on leaf pubescence and cuticle formation (Muzik 1975). In case penetration of herbicides into the leaves of plants is one of the main effects of microclimate on plant susceptibility to herbicides, droplet size of spray applied to plants in our trials should be as similar as possible to that generated by traditional spray equipment. This would reduce the possibility of interaction between droplet size and the penetration of herbicide through the different types of cuticle and pubescence.

Requirements for our herbicide applicator were that it should:

- be portable

- totally enclose each plant being sprayed to prevent drift

- apply droplets similar to that generated by traditional spray equipment

- accurately apply a constant amount of herbicide to each plant

- be easy to clean out to prevent contamination when changing to different dose rates.

2.2.3 Requirements for Glasshouse Trials

A number of devices have been designed and built for applying herbicide to plants grown in pots (Wiese 1977). Such devices vary in size and simplicity, from small enclosed chambers in which herbicide is blown from a 2 ml glass vial over a plant by an atomiser (Shaw and Swanson 1952), through to large spray booths in which a spray cart suspended from the roof of the booth moves nozzles across plants located on the floor of the booth (Bouse and Bovey 1967).

For this work, a spray device suitable for both field and glasshouse trials was designed and built.

2.2.4 Details of Sprayer

The sprayer designed and built for this project comprised a metal box 40 cm by 40 cm with a height of 60 cm. One side had a sliding clear perspex panel so the interior of the box could be seen. The box had no base so it could be placed over a thistle plant in the field. Herbicide could then be sprayed on to the plants from a solid cone nozzle on the ceiling of the box. The totally enclosed spraying environment ensured all the herbicide reached the ground. For glasshouse-grown plants, the box was placed over a tray to catch excess herbicide, and a metal grate was placed just above the tray to keep pots out of the spray accumulating in this tray.

In early field experiments with this sprayer, the nozzle was pressurised using air from a hand-pumped sprayer. Later field experiments made use of a cylinder of carbon dioxide. All experiments with glasshouse-grown plants used compressed air from the taps in a laboratory. With all of these sources of pressure, a regulator ensured an even pressure of 200 kPa, consistent with the pressure used with solid cone nozzles in more conventional spraying equipment (O'Connor 1989).

For each plant, 5 ml of herbicide solution was injected into the chamber immediately above the nozzle (Fig 2.2). A quick-snap air-lock coupler connected the chamber to a hose from the pressure source, and a valve between the chamber and the nozzle was opened to allow the herbicide to be propelled through the nozzle to the plant. Dose rates were varied by changing the concentration of the herbicide solution.

A problem encountered with this device involved the first and last portions of each herbicide sample being propelled directly into the middle of the plant below. As the

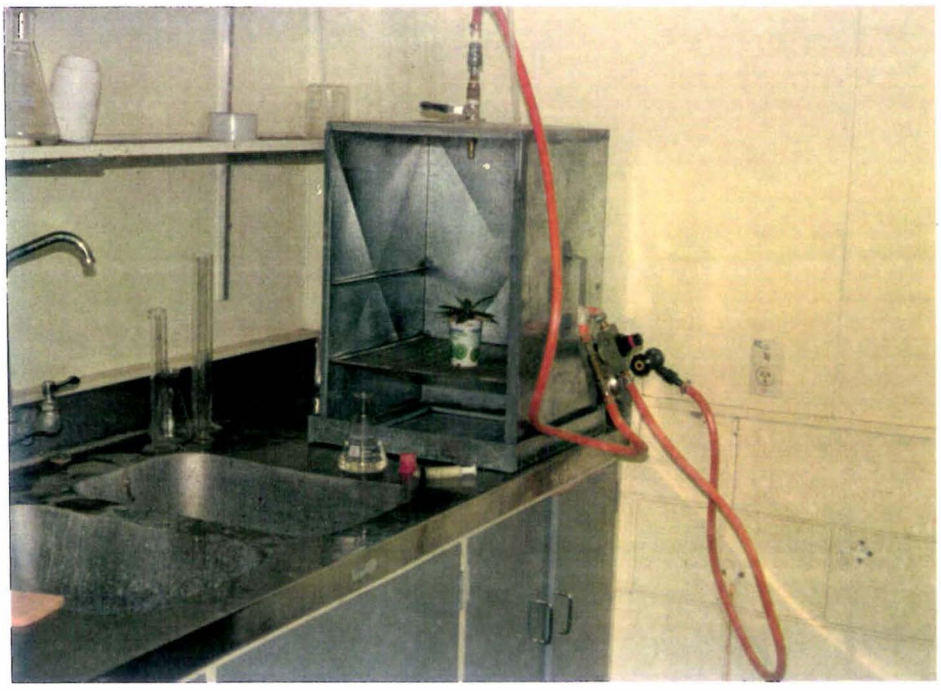


Plate 2.1: The sprayer, pressurised in a laboratory by compressed air, being used to treat a nodding thistle plant from a glasshouse.

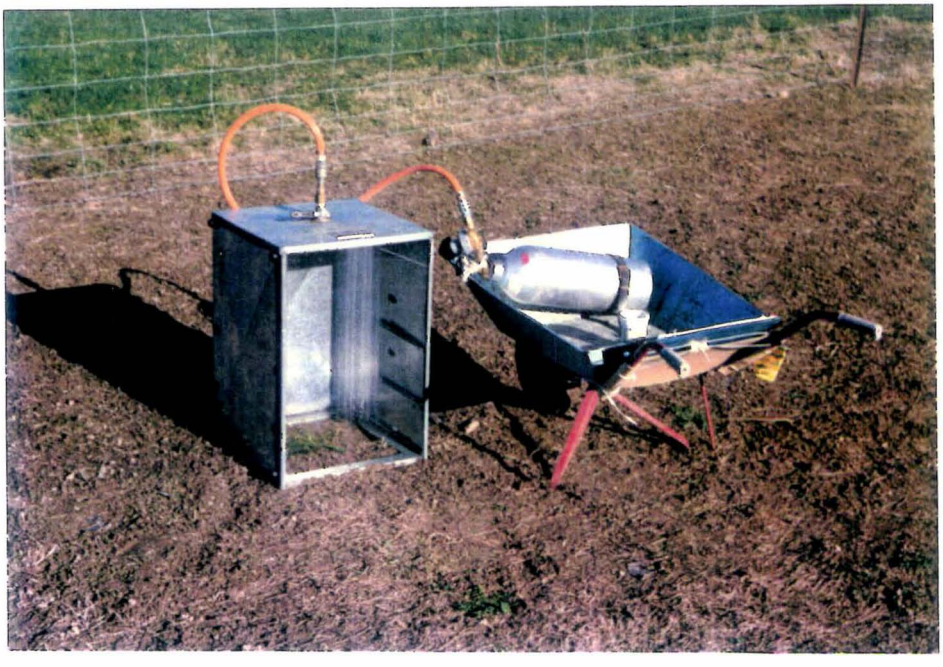


Plate 2.2: Use of carbon dioxide to pressurise the sprayer for treating a nodding thistle plant in the field.

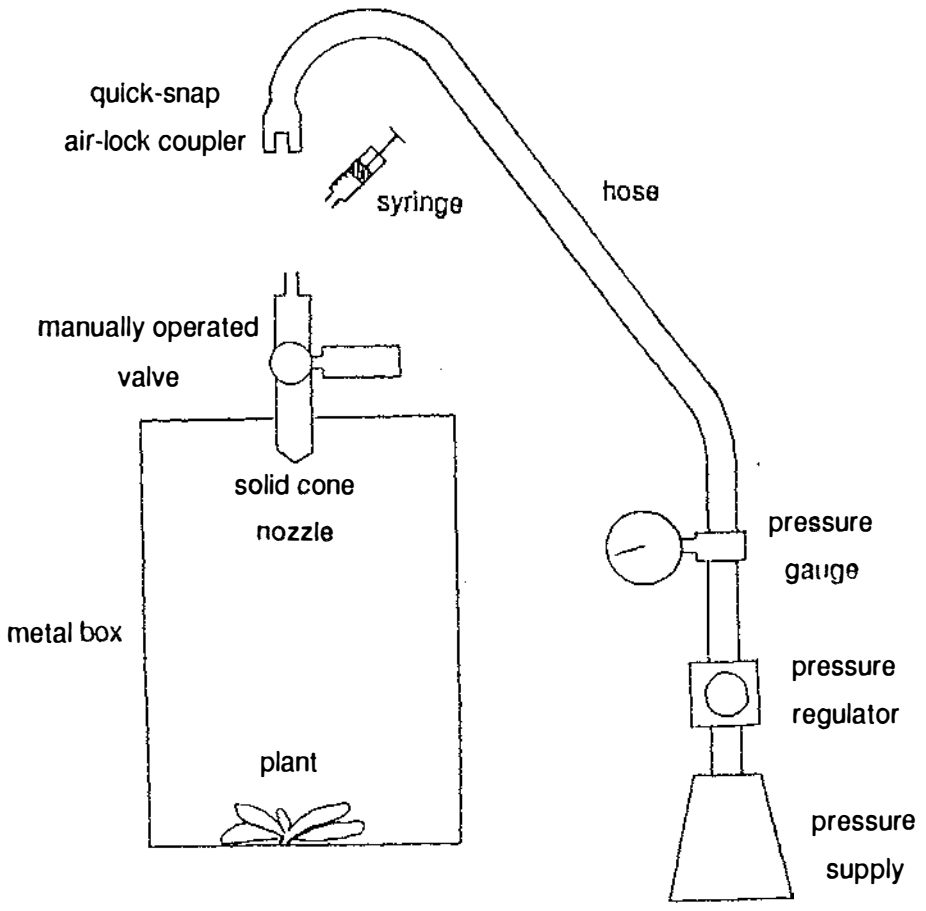


Fig 2.2: A diagramatic representation of the sprayer designed for applying herbicide solutions to nodding thistle plants in this project.

volume of the herbicide increased, the spread over a plant became more uniform because the proportion propelled into the middle of the plant became smaller (Table 2.1).

The 5 ml sample size was used in experiments as any increase in volume above 5 ml would have equated to abnormally high water rates for herbicide application. Herbicide is normally applied to thistles in no more than 200 l/ha of water. Higher volumes than this could influence the speed of droplets drying and penetration of droplets through layers of hairs and to lower leaves within a rosette. The 5 ml sample was equivalent to an average application rate of 312 l/ha. However the uneven application rate resulted in leaves directly below the nozzle intercepting the equivalent

Table 2.1: The percentage of herbicide solution injected into the chamber of the sprayer which was subsequently collected directly below the nozzle in a 250 ml flask with a horizontal surface area of 57 cm^2. The amount intercepted was determined by measuring the increase in weight of the flask following each application.

Volume of herbicide placed in chamber	% of spray collected directly below nozzle
1 ml	11.9%
2 ml	12.7%
3 ml	9.7%
4 ml	6.5%
5 ml	6.2%
6 ml	5.0%

of 540 l/ha, whereas leaves 10 cm and 15 cm from the centre received 184 l/ha and 114 l/ha respectively.

With a conventional spray boom, a thistle rosette covering twice the area of a second rosette would intercept twice as much herbicide. However the concentration of droplets to the centre of a rosette within the box sprayer resulted in rosettes receiving similar quantities of herbicide regardless of their size. Thus plants of similar size were used wherever possible in experiments to overcome possible problems from overdosing small plants.

The lack of uniform ground cover of spray droplets also presented problems with extrapolating doses applied with the box sprayer to application rates per unit area. This extrapolation was desirable when trying to equate results obtained using the sprayer with "normal" spraying practices.

The requirement for each plant to be directly below the nozzle meant that plants grown in pots had to be sprayed one at a time even if more than one could be fitted into the

box. The centre of the metal grate on which they stood was marked so that plants could be placed at the same position below the nozzle each time.

The sprayer required for this project had to apply accurately the same amount of herbicide solution to each plant so that dose response curves could be produced. Despite the drawbacks with the box sprayer, it appeared suitable for this task provided that plants to be sprayed were similar in size.

2.3 SCOTCH THISTLE FIELD EXPERIMENT

2.3.1 Introduction

Once the sprayer had been designed and built, a preliminary field test was required to resolve a number of issues before the research programme could begin. The ability of the sprayer to accurately apply set amounts of herbicide to plants in the field needed to be tested. Determination of herbicide concentrations which would give a range of responses in the thistles from no effect to total control was also required.

A system was needed for labelling each plant to be treated in the field. In the trial conducted in 1981 (Section 1.2.3.4), plants were individually marked using wooden pegs driven into the ground beside each plant. However livestock knocking over pegs resulted in a number of plants being "lost". An alternative system of marking plants was devised for this project and needed testing.

The other major objective of the initial field trial was to determine how to assess the effect of the herbicide on the plants. It was hoped to use quantitative responses (see Section 2.1), giving more information about the susceptibility of each plant than quantal responses, so that less plants would need to be treated to obtain a meaningful dose response curve. The use of quantitative responses depended on finding parameters that gave a reliable indication of how severely a plant had been affected by the dose of phenoxy herbicide to which it had been exposed.

Scotch thistle (*Cirsium vulgare* (Savi) Ten.) plants were used for this initial work as high densities were available close to Massey University, unlike nodding thistle. Their close proximity allowed the detailed monitoring of sprayed plants necessary to fully assess quantitative responses to MCPA. As the sensitivity of Scotch thistle and nodding thistle to phenoxys is similar (Matthews 1975), quantitative responses were also assumed to be similar.

2.3.2 Methods and Materials

Scotch thistle plants were individually marked using numbered plastic livestock ear-tags 7 cm wide by 10 cm long. These were fixed flat against the ground beside individual plants using two 15 cm long galvanised nails per tag so that the tip of the tag pointed toward the centre of the thistle and was 20 cm from its centre. A total of 70 Scotch thistle plants were labelled in this way. These plants were located in two adjacent paddocks on the Moginie Block of the Massey University Pasture and Crop Research Unit. The paddocks contained perennial ryegrass / white clover pastures rotationally grazed by sheep, and the soil type was a Tokomaru silt loam.

The length of the longest leaf of each rosette, measured and recorded as an indication of the relative size of each plant, varied from 8 cm to 28 cm. To evenly distribute any variability in herbicide susceptibility that might result from this size difference, a randomised block design was used so that plants of similar size were grouped in the same blocks and treatments were randomly allocated to plants within each block. There were ten blocks and seven treatments.

Since the 1981 trial (Section 1.2.3.4) showed variability in nodding thistle response to both MCPA and 2,4-D, MCPA was used for most of the work in this project as it seemed to be more effective at controlling nodding thistle than 2,4-D. Also, the 2,4-D used in New Zealand for controlling nodding thistle is normally a butyl ester formulation, whereas the MCPA used is a potassium salt formulation. As ester formulations are more volatile than salt formulations (Klingman and Ashton 1982), MCPA would be less likely to cause problems with drift of vapours from plants sprayed with heavy doses on to nearby plants with low doses, especially in glasshouse experiments.

The seven treatments consisted of an untreated control and six concentrations of MCPA. These were 0.75, 1.5, 3, 6, 12 and 24 mg ai / 5 ml of spray solution.

The treatments were applied to the plants using the box sprayer between 2.30 pm and 5.30 pm of 14 October 1985. The weather was fine with a light breeze.

2.3.3 Results and Discussion

2.3.3.1 **Plant Identification**

The system of marking plants using plastic ear-tags worked quite well. No tags were removed by grazing livestock. Many of the thistles killed by the herbicide had lost all of their foliage by the time final assessments were made two months later. The leaves had dried out and then disappeared either due to the wind or livestock. The only sign of their previous existence was a rotting crown 1-2 cm in diameter. Crowns were quite easy to find once a tag was located because they were 20 cm from the tag and the direction from the tag was known. This also avoided confusion with other nearby thistles which were still alive.

Tags were often difficult to find despite being quite large and brightly coloured because pasture species, especially white clover and pennyroyal (*Mentha pulegium* L.), grew over them. The tags also became covered in soil due to the action of earthworms and livestock. Only two of the 70 tags were lost permanently, though a lot of time was spent trying to locate some of the others.

2.3.3.2 **Quantitative Responses**

Detailed observations of quantitative responses failed to identify a trait which would give more information than quantal responses. Although growth rates of individual leaves were affected more by higher application rates, no technique to quantify growth retardation of leaves could be devised which would not require large amounts of work. The severity of damage symptoms was difficult to quantify by scoring because of the variable nature of symptoms. There was often a poor correlation between severity of symptoms and eventual fate of plants.

Weighing shoot mass following removal at ground level several months after herbicide application allowed reasonable differentiation between live, stunted and dead plants. However the large variability within these results meant that simply recording which plants had survived usually gave a better dose response curve.

2.3.3.3 **Quantal Responses**

The statistical analysis of dose response curves derived from quantal responses has been discussed fully by Finney (1971). By plotting percentage mortality on the y-axis

of a graph against the log transformed dose rate on the x-axis, a symmetrical sigmoid curve relationship is obtained, as shown in Fig 2.1. The requirement for a log transformation of the dose to obtain such a curve was the reason for selecting a geometric progression of dose concentrations for this and all later experiments. All dose response curves in this report will be shown using a logarithmic scale on the x-axis. However units on such logarithmic scales have been transformed back to arithmetic units to make it easier for readers to equate log-transformed data to actual doses used in the experiments.

Sigmoid curve relationships can be transformed into a straight line by converting the percentage mortality data on the y-axis into units known as **probits**. This is very useful for statistical purposes because it then becomes much easier to fit a regression line to the data. A weighting system used when fitting such lines makes response levels between 1% and 99% mortality more important in influencing the position of the regression line than those at 0% or 100% mortality. This has important implications with regard to design of experiments because dose rates need to be chosen to give as many points as possible in this range. Fig 2.3 shows that the data obtained in the Scotch thistle trial had only two points within this range. The weighting system gives greatest emphasis to points close to the 50% mortality level and correspondingly less weight to those approaching 1% or 99%.

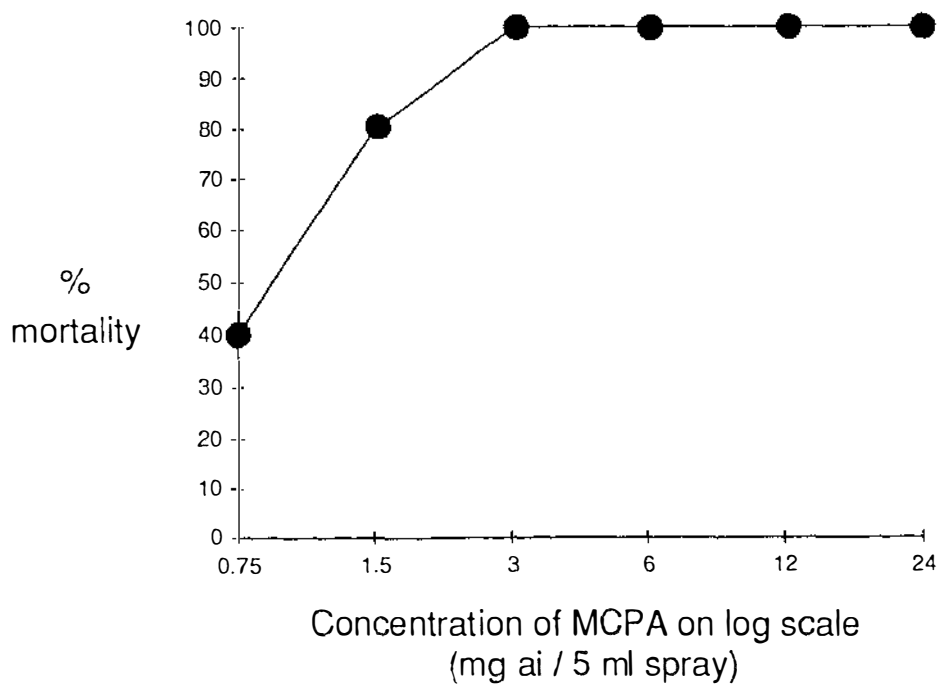


Fig.2.3: Mortality data for Scotch thistle plants sprayed with various concentrations of MCPA in the field near Massey University in October 1985.

One of the main objectives of fitting a line to such data is to obtain an estimate of the 50% level of response. Dose response curves are usually compared with each other at the 50% level as there tends to be less variability within a population in its response at this level than at levels approaching 0% or 100%. For example, consider the dose of herbicide required to kill a certain proportion of a population of plants. Within a population, a small number of plants may be exceptionally susceptible to the herbicide. Determining the dose of herbicide at which none of these plants die (*ie* 0% mortality level) is dependent on whether any such particularly susceptible plants are present. The figure obtained will be a measure of the susceptibility of the least tolerant plant in the population, and therefore does not accurately reflect how the bulk of the population would respond to the herbicide. The converse argument could be used with respect to the 100% level of mortality. Thus the best indication of the susceptibility of the entire population is given at 50% mortality. With mortality data, the dose giving this level of response is known as the LD50 (lethal dose for 50% of the population), and is the level of response that will be discussed in future experiments.

Once a dose response curve has been fitted using probit transformations, confidence limits can be calculated for this curve. These are usually calculated so that there is a 95% probability that the true position of the curve estimated from the data collected from a population lies between the defined upper and lower limits. Although these limits could be shown on a graph with the fitted curve, generally there is interest only in the limits at the 50% level. Therefore a bar is commonly included on graphs of the fitted curve showing the 95% limits for the 50% level only. The SPSS-X probit analysis computer package was used for analysing data in this project.

To illustrate some of the concepts discussed above, various stages of the probit analysis of the Scotch thistle data are shown in Figs 2.3 - 2.6. The raw data presented in Fig 2.3 was transformed into probits and are shown plotted in Fig 2.4. A regression line was then fitted to these data by the computer (Fig 2.4) and transformed back to units of percentage mortality and actual dose levels (Fig 2.5), though the log scale was retained on the x-axis. The confidence limits for the curve are also shown in Fig 2.5, but usually this information is presented as in Fig 2.6 with the 95% confidence limits shown only at the 50% level of mortality. The data points used to generate the curve are also presented because the curves are merely estimates of the true position of the response curve based on the data collected.

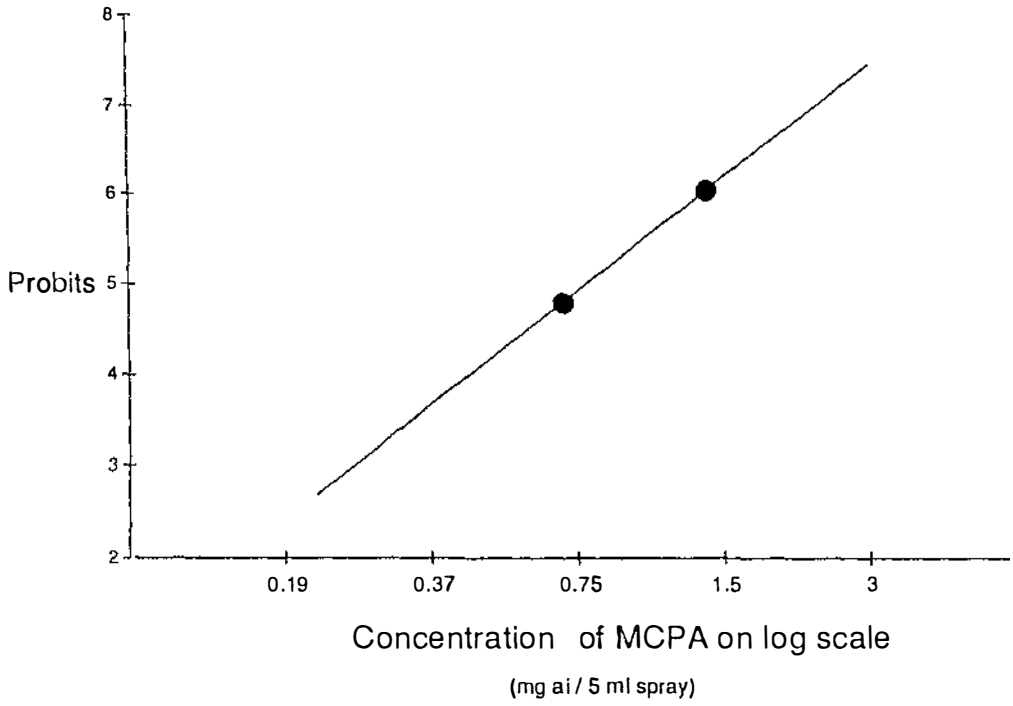


Fig 2.4: Probit transformation of Scotch thistle mortality data, and the computer-fitted regression line for these data.

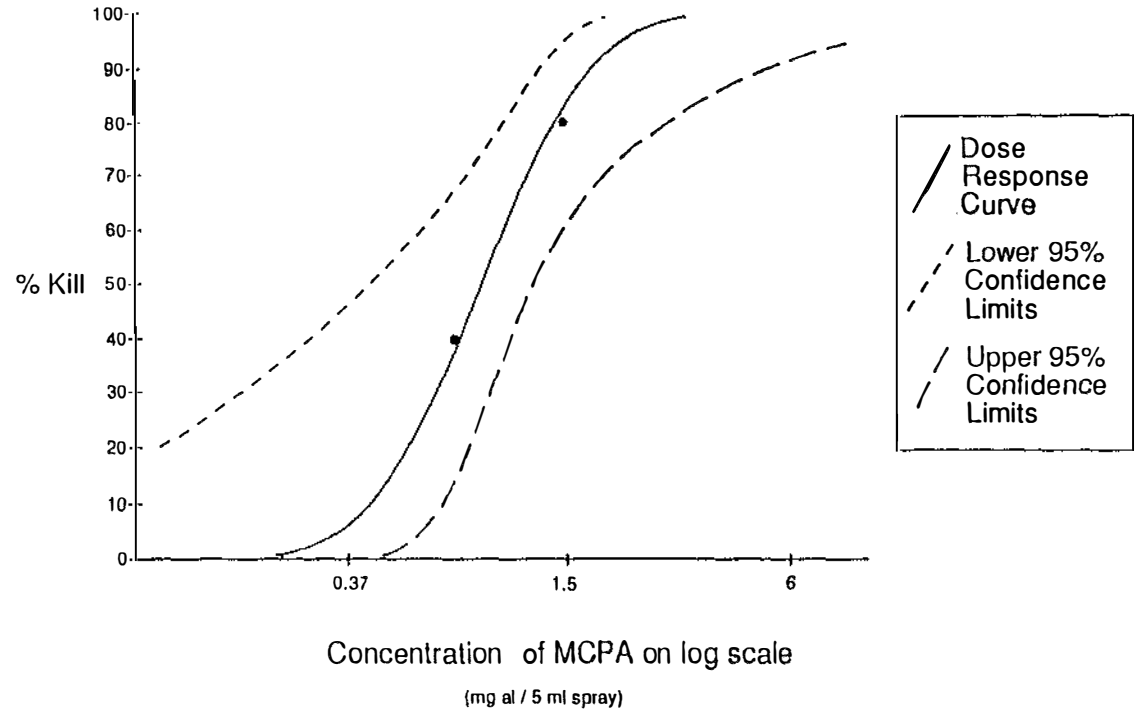


Fig 2.5: Computer fitted dose response curve of Scotch thistle for MCPA with the upper and lower 95% confidence limits for this curve.

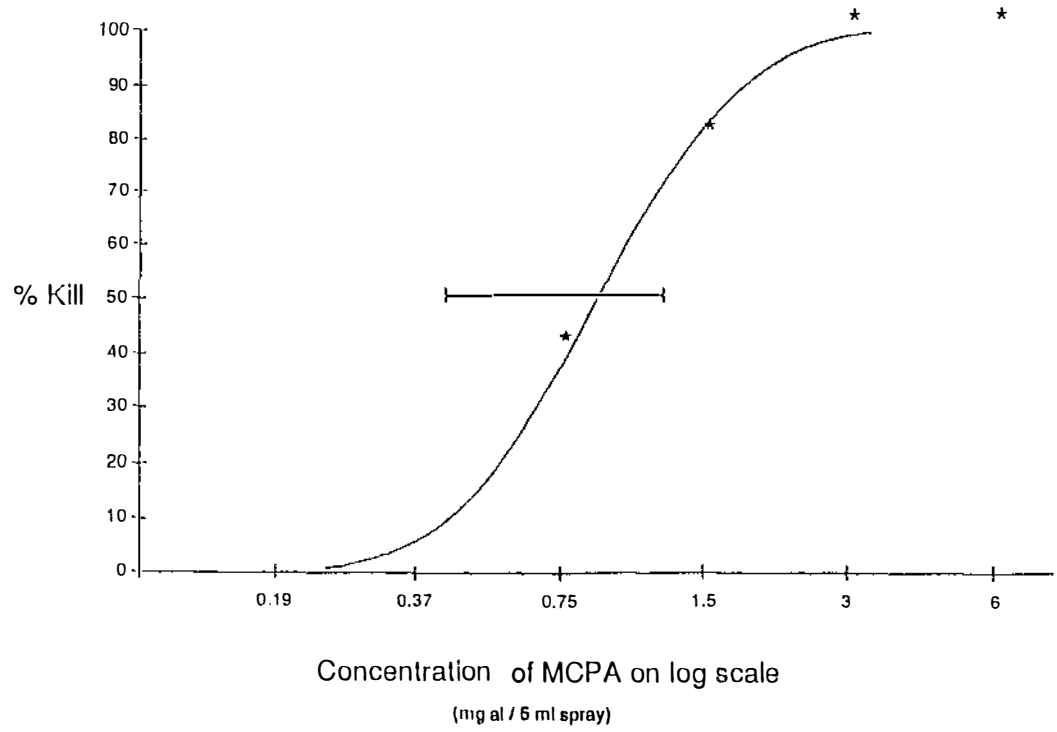


Fig 2.6: The traditional presentation of a dose response curve for Scotch thistle susceptibility to MCPA with the 95% confidence limits shown for the LD50 dose.

2.3.4 Conclusions

As a result of the Scotch thistle field experiment, the following conclusions were reached:

- the box sprayer appeared to be suitable for applying doses of herbicide to individual plants

- the ear-tag labelling system was worthy of further use, though there were some problems with finding some of these labels after two months

- the range of herbicides used did not include rates low enough to encompass the entire dose response curve for Scotch thistle

- quantitative responses could be measured for phenoxy damage with thistles, but quantal responses had advantages which made further investigation of the use of percentage mortality data worthwhile

- techniques were available for statistically analysing percentage mortality data.

2.4 NODDING THISTLE FIELD EXPERIMENT

2.4.1 Introduction

A second preliminary field experiment was laid down in November 1985 once initial results from the Scotch thistle experiment had been obtained. This experiment was designed to further develop techniques for producing dose response curves. This experiment made use of nodding thistle plants to make sure there were no major differences between this species and Scotch thistle.

The main objective of this experiment was to determine whether our technique allowed differentiation between populations which varied in susceptibility to MCPA.

2.4.2 Methods and Materials

Five sites were selected with populations of nodding thistles large enough to generate a dose response curve. At the time this experiment was conducted it was thought that environmental factors were the main variables likely to influence susceptibility to herbicides in nodding thistle and that the entire project would involve determining the relative importance of these factors. Thus it was hoped that the sites differed enough from each other to affect the susceptibility of their nodding thistle populations to MCPA. Four of the five sites were located within 1 km of each other on a sheep farm in central Hawkes Bay. This was the property of Mr John Potter on Salisbury Road, 10 km west of Maraekakaho. The fifth site was in Manawatu on the property of Mr Roger Waugh, which is also a sheep farm and is located 5 km north-east of Colyton on Midland Road. The Potter property was selected because there were reasonable densities of nodding thistle plants present in many different habitats around the farm. The Waugh property was used because it was one of the few sites where a naturally occurring population of nodding thistle existed near Massey University.

A gully runs through the Potter property, and Sites 2 and 3 were positioned along the top edge of this gully where the soil was quite thin and stony. Both were prone to drying out in summer because of this, with Site 3 being quite uniform and Site 2 being more variable due to differences in soil depth. Sites 1 and 4 on the Potter property were located in reasonably flat paddocks where the pasture appeared more uniform and dense. Site 1 was near a shelter belt, and the pasture was laxly grazed throughout the trial by a small number of rams. Site 4 was more tightly grazed both before and after application of the herbicides, and was also more exposed. Site 5 was part-way up a hillside on the Colyton farm in a paddock set-stocked by sheep.

As this trial was more a test of techniques than an attempt to begin isolating the most important environmental variables influencing herbicide susceptibility, there was no attempt to control livestock or to monitor differences in environmental variables between the five sites.

Plant size was measured to determine whether this varied between sites and to allow statistical blocking, based on the distribution of variability in plant size (Finney 1971). Plant size, determined by length of the longest leaf (Kelly and Popay 1985), did not differ significantly between sites. The average was 13.5 cm, and the range 7 cm to 24 cm.

Seven concentrations of MCPA ranging from 1 to 64 mg ai per 5 ml of solution were applied to plants at each site, with ten plants per site receiving each treatment. An eighth treatment was an untreated control. All plants were labelled as in Section 2.3.2 (Plate 2.3). Treatments were applied on 14-15 November 1985 at Maraekakaho and 28 November 1985 at Colyton. The proportion of plants which died was determined two months later.

2.4.3 Results and Discussion

The raw data obtained from the five plots are presented in Fig 2.7. Probit analyses of these data estimated the 50% mortality levels for each site with confidence limits (Table 2.2). This indicated that the four Maraekakaho sites did not differ significantly ($p = 0.05$) in susceptibility to MCPA. However the Colyton plants had a significantly higher tolerance of MCPA than those at three Maraekakaho sites. Data for all four Maraekakaho sites were combined for a further probit analysis comparing the average Maraekakaho response with that for Colyton. The fitted curves and LD50 confidence intervals from this analysis appear in Fig 2.8. The Colyton population was estimated to be 2.3 times more tolerant of MCPA than the Maraekakaho thistles.

The results from this trial suggested that our technique for comparing the susceptibility of nodding thistle populations to MCPA was effective. Some plastic tags adjacent to treated plants were difficult to locate because of pasture plants growing over them. Using the quantal response of death rather than measuring quantitative responses hastened data collection once labels were located. The pooling of data from the four Maraekakaho sites in Fig 2.8 showed the effect on confidence limits of having 40 plants per treatment compared with ten for Colyton. Although the confidence interval was reduced with forty plants, ten plants per treatment still allowed satisfactory differentiation between populations varying in tolerance (Table 2.2).

Plate 2.3: A plastic label marking the position of a treated nodding thistle plant 20 cm from its tip (at end of pen) which has since died.

Plate 2.4: Potted nodding thistle plants in irrigation trays immediately prior to treatment in the experiment described in Section 2.5.

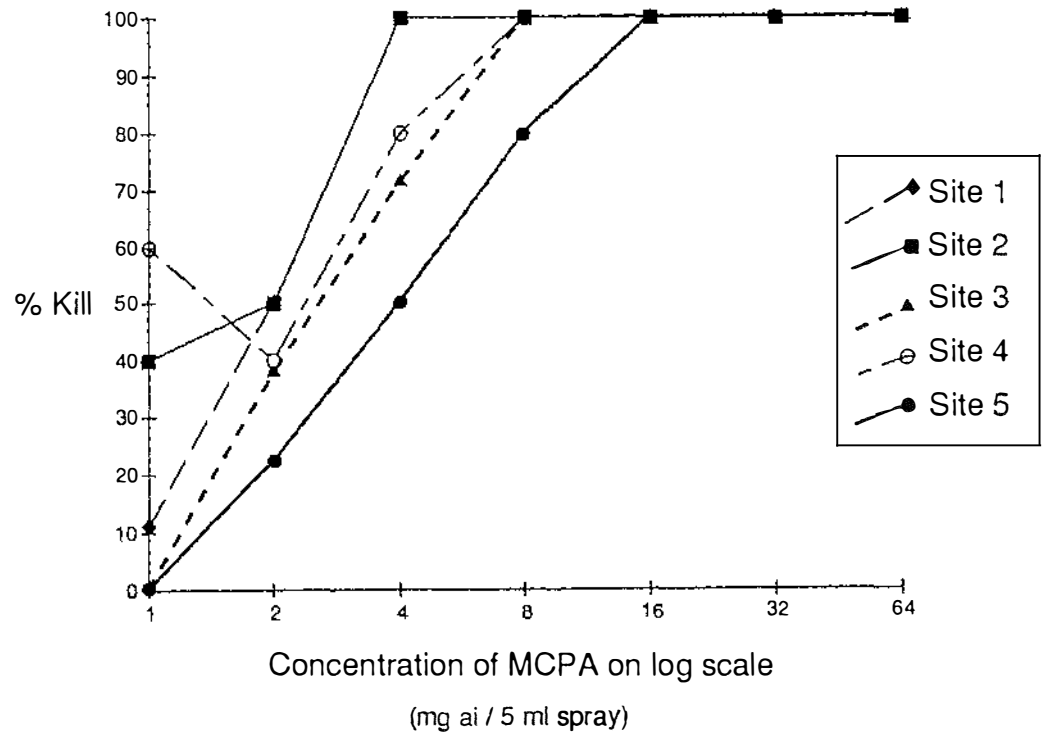


Fig 2.7: The dose response data obtained for nodding thistle at Maraekakaho (Sites 1-4) and Colyton (Site 5) in 1985.

Table 2.2: Estimates of the LD50 values for nodding thistle tolerance to MCPA at the five Maraekakaho and Colyton sites, with 95% confidence limits. All units are mg ai MCPA per 5 ml of solution applied to plants.

Site	LD50	Confidence Limits
1	1.77	1.23 - 2.50
2	1.42	0.97 - 2.02
3	2.61	1.77 - 3.82
4	1.57	1.09 - 2.22
5	3.96	2.83 - 5.52

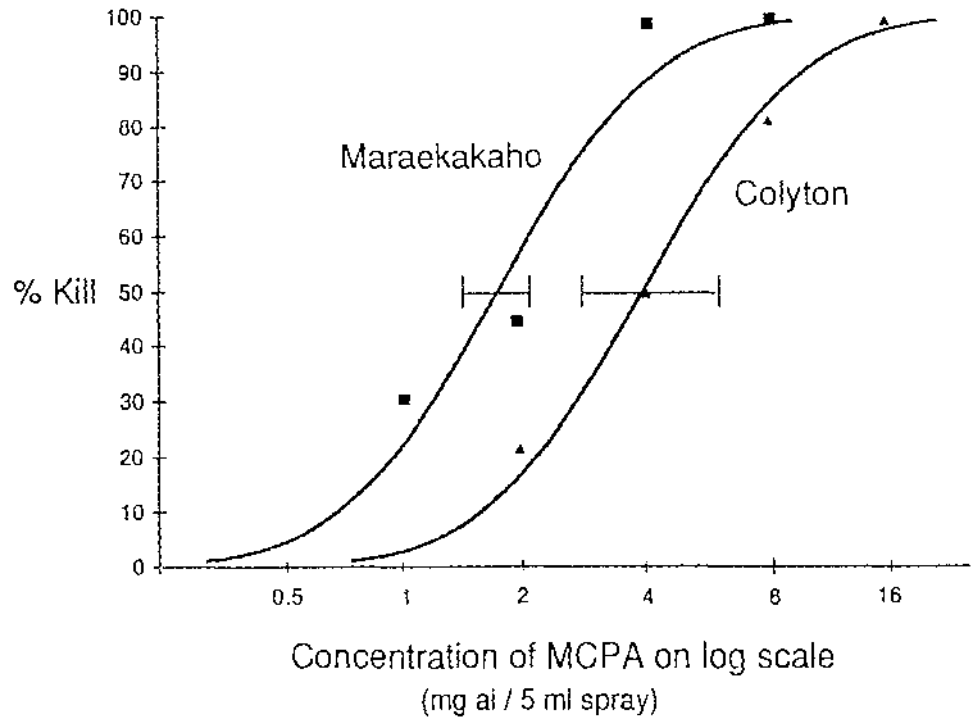


Fig 2.8: Computer fitted dose response curves for nodding thistle susceptibility to MCPA at Maraekakaho (combination of all four sites) and Colyton. The bars show 95% confidence intervals for the LD50 values.

2.5 NODDING THISTLE GLASSHOUSE EXPERIMENT

2.5.1 Introduction

Experiments were being conducted concurrently with the two field trials discussed above to determine how to grow nodding thistle populations in glasshouses and measure their susceptibility to MCPA.

Although production of healthy plants was desirable, a second objective was to grow large numbers of plants in the glasshouse to allow adequate replication and treatment numbers within experiments. Previous experience by the author with Californian thistle (*Cirsium arvense* L.) plants had shown that thistles can become very large under glasshouse conditions (Harrington 1983). Plant size could be limited by regular trimming of foliage, or by use of small pots. However both of these alternatives could affect plant susceptibility to herbicides. The latter alternative was tried in this initial experiment.

Sub-irrigation was considered preferable to overhead watering to ensure the small pots received adequate water once plants were well established. Capillary matting encourages plant roots to grow throughout the mats (Harrington 1983), so root damage occurs when plants are removed for spraying. The initial glasshouse experiment investigated a sub-irrigation system which would ensure roots remained within the pots.

Dose response curves had to be produced for glasshouse plants as well as field populations in case susceptibility to herbicide was affected by glasshouse conditions.

2.5.2 Methods and Materials

The nodding thistle seed used for many of the initial experiments in this project was obtained by Dr Ian Popay in 1981 from a roadside population at Ohiti Road, Hawkes Bay. Hereafter, this will be referred to as the Ohiti population.

Seed from this population was germinated on moist filter paper in petri dishes on 9 October 1985. Ten days later the seedlings were transplanted into 230 ml plastic pots containing a mixture of sand and slow-release fertilizer. Sand was used to enable easy extraction of plant roots from the medium.

The plants were placed in a series of metal trays 3 m long, 25 cm wide and 7 cm deep (Plate 2.4). The trays were filled with water twice daily by an automated irrigation system, and the water drained away after 30 minutes through holes in the tray floors. This allowed total soaking of the potting media, yet kept roots from growing out as the trays dried out between soakings.

Plants were blocked for size on 4 December 1985 based on the length of the longest leaf. The average was 8.6 cm, and the range 3.2 cm to 14.0 cm. Ten blocks of 12 plants were measured. Two plants from each block were harvested, dried and weighed to characterize plant size. The average dry weight (with range) for these 20 plants was 457 (98 - 723) mg for the shoots and 268 (28 - 546) mg for the roots. One plant from each block was left untreated and the rest were treated with one of nine concentrations of MCPA ranging from 0.015 to 3.84 mg ai / 5 ml solution (Fig 2.9). Treatments were allocated at random within blocks. Plants were sprayed individually with 5 ml of solution using the device described in Section 2.2. Temperature and relative humidity at time of spraying were 25^{o}C and 30% respectively. The final assessment of plant survival was on 25 March 1986.

2.5.3 Results and Discussion

All of the untreated plants survived until the final assessment nearly 4 months after application. This suggested that growing conditions were not too severe for the plants. However their growth was definitely limited by the pot size as plants grown concurrently in 2.7 litre pots grew much larger. Although the leaves were significantly shorter with the small pots, leaf number, shape and colour appeared unaffected.

Despite the range of herbicide concentrations selected for the experiment not extending high enough to encompass the entire dose response curve, four of the concentrations caused mortality between the 0 and 100% levels (Fig 2.9). Probit analysis of this data gave an estimate of the LD50 as 2.24 mg ai MCPA / 5 ml of solution. Table 2.3 compares this with LD50 estimates for the other trials discussed in this chapter. The glasshouse plants and the field plants appeared to be similar in susceptibility to MCPA. Scotch thistle was less tolerant to MCPA than the three nodding thistle populations, though these comparisons were probably not entirely valid as the populations were all growing under different environmental conditions. However Matthews (1975) did remark that Scotch thistle is more susceptible to MCPA than most other thistle species in New Zealand.

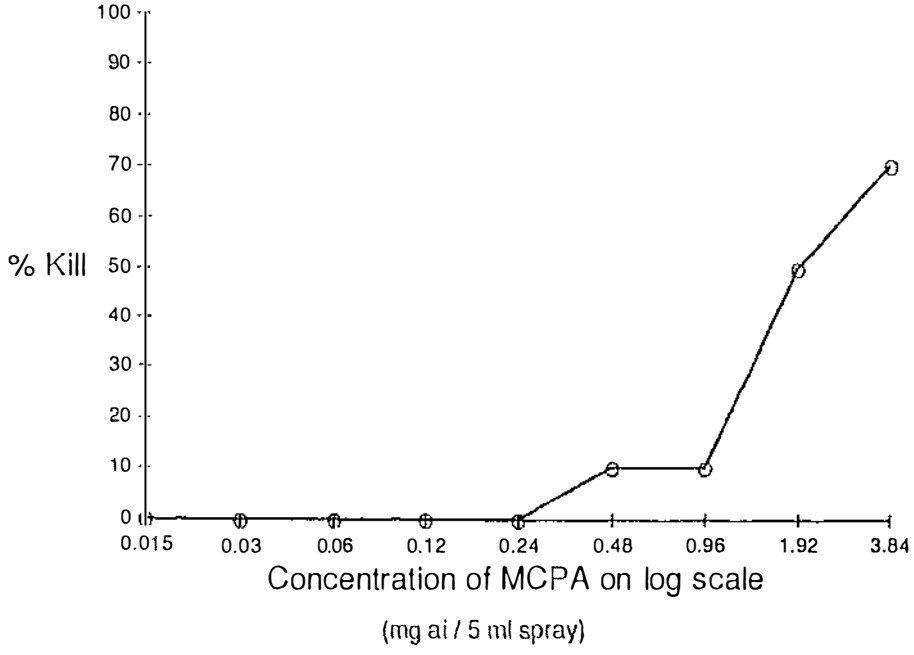


Fig 2.9: The percentage of glasshouse grown plants killed by MCPA applied in December 1985.

Table 2.3: Estimates of the LD50 (with confidence intervals) for the glasshouse-grown nodding thistle population, the field-grown Maraekakaho and Colyton nodding thistle populations, and the Massey University Scotch thistle population. All units are mg ai MCPA / 5 ml of solution applied to plants.

Population	LD50	Confidence Limits
Glasshouse nodding thistle	2.24	1.47 - 4.48
Maraekakaho nodding thistle	1.74	1.42 - 2.08
Colyton nodding thistle	3.95	2.80 - 5.56
Massey University Scotch thistle	0.88	0.43 - 1.22

Although the validity of comparing these initial results may be questionable, the values obtained did assist with selection of the most appropriate herbicide concentrations in future experiments. They also suggested that our techniques for comparing population susceptibility to herbicides were effective.

CHAPTER 3: GLASSHOUSE COMPARISONS OF POPULATIONS

3.1 INTRODUCTION

The literature reviewed in Chapter 1 suggested that environmental factors were more likely to be responsible for difficulties in controlling nodding thistle populations than genetic factors. However it was decided to test the involvement of genetic factors before proceeding further.

Populations from different sites were compared for genetic differences in herbicide susceptibility after being grown from seed in the same environment, thus eliminating any influence of environmental variability between sites. Initial comparisons of populations were conducted in a glasshouse environment for convenience.

3.2 FIRST GLASSHOUSE COMPARISON

3.2.1 Methods and Materials

Three nodding thistle populations were compared for genetic differences in herbicide susceptibility in the first glasshouse experiment. All three populations had been studied in past experiments:

(1) Argyll population (see Section 1.2.4)
(2) Ohiti population (see Section 2.5.2)
(3) Colyton population (see Section 2.4).

The Argyll and Colyton populations were selected for the tolerance of MCPA shown in past experiments. The Ohiti population had probably received minimal herbicide applications in past years due to its roadside location so was selected as a population likely to show low tolerance of MCPA. One of the Maraekakaho populations previously found sensitive to MCPA (Section 2.4) was also to be used for this experiment, but poor germination of seeds and high seedling mortality resulted in insufficient plant numbers for its inclusion.

Seed from these three populations were placed on moist filter paper in petri dishes on 8 May 1986. Seedlings were transplanted 10 days later into 230 ml plastic pots containing a mixture of sand, fertilizer and etridiazole (Terrazole) to control damping-off diseases. They were placed in the irrigation trays discussed in Section 2.5.2, but

received more frequent irrigation than plants in the 1985 experiment due to automation of water-filling.

Prior to treatment, plants of similar leaf number and therefore size were blocked together for each population and treatments were randomly allocated within each block. There was no difference between the three populations in plant size. Leaf length and dry weight were determined for 24 plants selected across all blocks. Longest leaf length averaged 11.3 cm (Plate 3.1), with a range of 9.5 cm to 17.0 cm for the 24 plants. The average (with ranges) dry weight of shoots was 2.49 g (0.96 - 3.93 g) and roots was 2.11 g (0.61 - 4.26 g). The plants were treated on 17 September 1986. Herbicide treatments consisted of 7 concentrations of MCPA ranging from 0.37 to 24 mg ai / 5 ml of applied solution (Fig 3.1). Each treatment was applied to 25 plants from each population, except that the lowest concentration was applied only to Colyton plants. There were also seven to nine untreated plants from each population. The final assessment of plant survival was on 29 January 1987.

3.2.2 Results and Discussion

Ohiti and Colyton plants were significantly more susceptible to MCPA than Argyll plants (Fig 3.1). This unexpected result suggested that the Argyll population differed genetically from the other two populations in tolerance of MCPA.

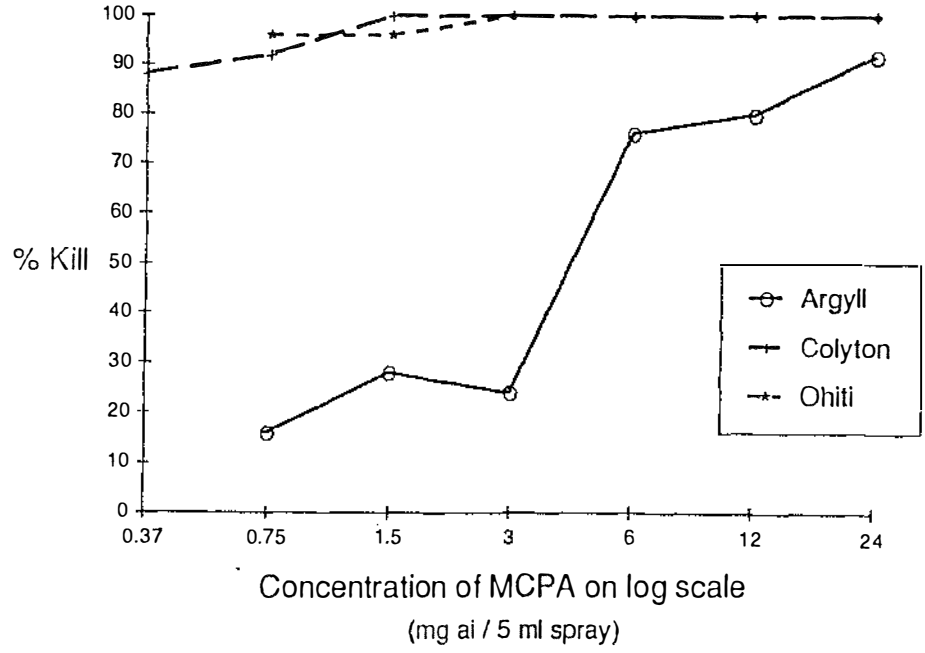


Fig 3.1 Percentage of nodding thistle plants killed from Argyll, Colyton and Ohiti populations grown and treated with MCPA in a glasshouse in September 1986.

Plate 3.1: Nodding thistle plants immediately prior to treatment in the first glasshouse comparison (Section 3.2).

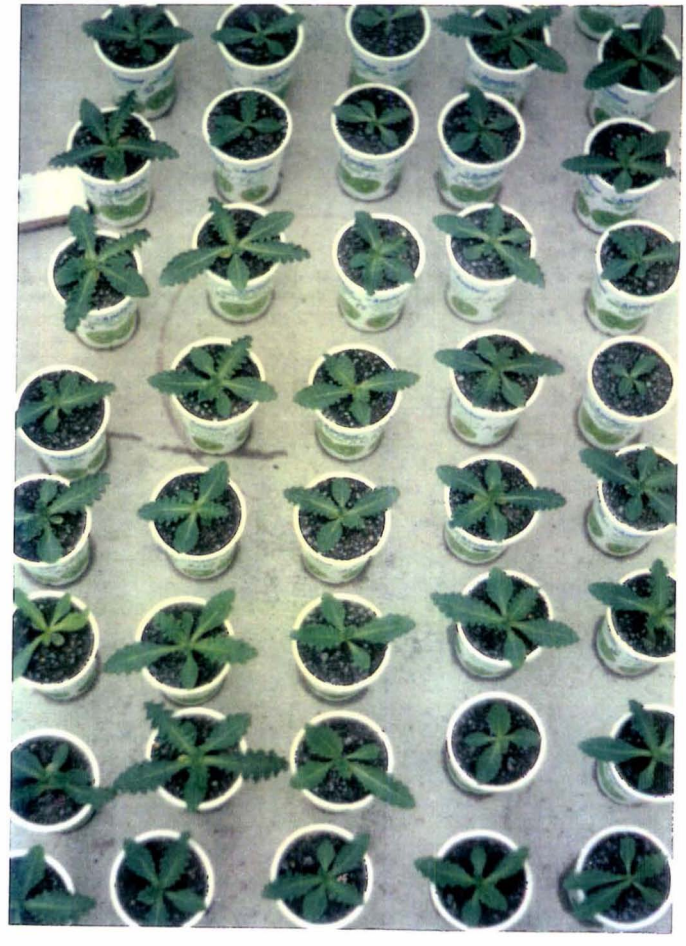


Plate 3.2: Plants from the second glasshouse comparison (Section 3.3) immediately prior to treatment.

As all seven untreated Ohiti plants survived, the high mortality of Ohiti plants was due to MCPA and not some other factor. However two of the seven (29%) untreated Colyton plants and two of the nine (22%) untreated Argyll plants did die. It was unfortunate that more plants were not left untreated to better measure this natural mortality.

Probit analysis of the Argyll population assumed that 22% of the treated Argyll plants succumbed to natural mortality. The LD50 for MCPA was estimated as 6.3 mg ai / 5 ml, with lower and upper 95% confidence limits of 4.1 and 8.6 mg ai / 5 ml respectively. The results depicted in Fig 3.1 suggest the LD50 for the Colyton population was below 0.37 mg ai / 5 ml, so Argyll plants were at least 17 times more tolerant of MCPA.

Likewise the Ohiti population apparently had an LD50 below 0.75 mg ai / 5 ml, yet this population had an LD50 at least three times higher at 2.2 mg ai / 5 ml in an earlier glasshouse experiment (Section 2.5). The size and age of plants when treated differed markedly between the two experiments. The 2 month old plants in the earlier experiment had mean shoot and root masses of 460 and 270 mg DW respectively, compared with 2500 mg and 2100 mg DW for the 4 month old plants in this experiment. Although older plants are generally more tolerant of MCPA (Popay *et al* 1989), the small pot sizes used in this experiment may have weakened the larger plants, making them more susceptible to the herbicide. Small pot size may also account for the natural mortality that occurred in this experiment but not in 1985.

3.3 SECOND GLASSHOUSE COMPARISON

3.3.1 Introduction

The discovery that tolerance of nodding thistle to MCPA was probably due to genetic factors resulted in further experiments being conducted to investigate this phenomenon instead of beginning research on the influence of environmental factors as had been planned.

The tolerance of the Argyll population to MCPA was compared with the Ohiti population in further glasshouse experiments to confirm the original findings and to obtain a better estimate of the magnitude of the Argyll tolerance. The Colyton population was eliminated from further comparisons as it appeared to be similar to the Ohiti population in susceptibility.

If small pot size did influence results in the first comparison, options for overcoming this problem were to use larger pots or to treat the plants before they became too large. An increase in pot size would result in fewer plants fitting into a glasshouse. Therefore the second experiment compared Argyll with Ohiti plants grown in the same sized pots as previous experiments but with earlier treatments. Using younger plants had an added advantage of reducing the time taken to conduct the experiment, which was small with only four application rates and 10 plants per treatment to check the suitability of young seedlings for susceptibility comparisons before conducting a larger experiment. It was possible that Argyll plants only tolerated MCPA once they were older.

3.3.2 Methods and Materials

Ohiti and Argyll seeds began germination in petri dishes on 12 November 1986 and were planted out into pots as described in Section 3.2.1. Overhead watering was used until the seedlings had successfully established as sub-irrigation was unable to keep the surface layers of sand moist.

Treatments were applied on 22 December 1986 when the average length of the longest leaf was 53 mm (range of 18 - 80 mm) (Plate 3.2). Twelve plants harvested that day had average (with range) shoot and root masses of 121 (27 - 324) mg DW and 54 (15 - 150) mg DW respectively. Treatments were 0, 0.19, 0.75, 3.0 and 12.0 mg ai of MCPA applied in 5 ml of water to each of 10 plants from each population. Temperature and relative humidity during application were 23^{O}C and 63% respectively. Average (with standard error) temperatures in the glasshouse during the experiment ranged daily from a maximum of 25 $\pm$ 2^{O}C to a minimum of 19 $\pm$ 2^{O}C. The final assessment of plant survival was on 10 March 1987.

3.3.3 Results and Discussion

Despite only four application rates being used, the four-fold difference between each rate resulted in a good spread of responses being obtained for both populations (Fig 3.2). Following probit analysis, the Argyll population was again found to be significantly more tolerant of MCPA than the Ohiti plants.

The Argyll and Ohiti populations differed in tolerance by an estimated 4.8 times, less than suggested by data from the first comparison. The Argyll dose response curves for the two experiments were similar, with LD50 values (and 95% confidence intervals)

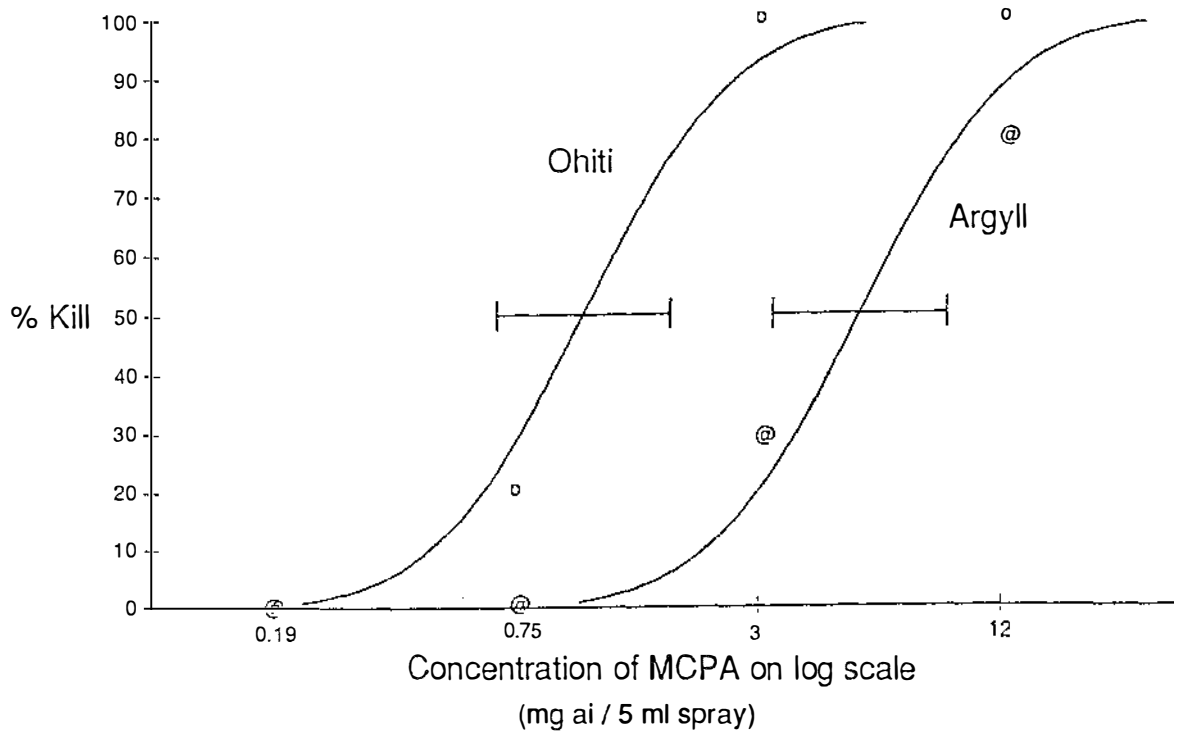


Fig 3.2: Percentage of nodding thistle plants killed from Argyll (@) and Ohiti (o) populations grown and treated with MCPA in a glasshouse in December 1986. Computer-fitted dose response curves are included with 95% confidence limits for each LD50.

for the first and second experiments of 6.3 (4.1 - 8.6) mg ai / 5 ml and 5.3 (3.2 - 8.7) mg ai / 5 ml respectively. However the Ohiti population appeared more susceptible to MCPA in the first glasshouse comparison when it suffered 96% mortality at 0.75 mg ai / 5 ml, compared with 20% mortality at the same concentration in the second experiment.

As none of the 20 untreated plants died in this second experiment, the results could be considered more reliable than in the initial comparison despite the reduced replication and number of application rates. However as this second experiment had been designed simply to confirm that younger plants could be used for tolerance comparisons, a third glasshouse experiment was then conducted to elucidate further the magnitude of tolerance differences.

3.4 THIRD GLASSHOUSE EXPERIMENT

3.4.1 Introduction

Although young plants were to be used in the third glasshouse comparison of population susceptibility, logistical difficulties resulted in treatments being delayed until plants were larger.

3.4.2 Methods and Materials

Germination of Ohiti and Argyll seeds began in late November 1986. Seedlings were planted into 230 ml plastic pots containing sand, fertilizer and etridiazole and received overhead watering four times daily. Once established, the plants were placed into metal trays and sub-irrigated daily from 12 January 1987.

Treatments were applied on 23 February 1987. Ohiti plants received 12 application rates of MCPA ranging from 0.023 to 48 mg ai / 5 ml solution (Fig 3.3). Nine of these treatments were replicated 10 times, but concentrations of 0.37, 0.75 and 1.5 mg ai / 5 ml were each applied to 20 Ohiti plants. Nine concentrations ranging from 0.19 to 48 mg ai / 5 ml were each applied to 10 Argyll plants. There were 40 untreated plants of each population, and a further 40 plants were harvested, dried and weighed at the time of treatment to characterize plant size. The average (with range) shoot and root masses were 1.85 (0.67 - 3.32) g DW and 1.94 (0.80 - 2.63) g DW respectively. Temperature and relative humidity varied between 22 - 25^{o}C and 35 - 60% respectively during application. Plant survival was assessed on 25 May 1987.

3.4.3 Results and Discussion

The Argyll population was again found to tolerate substantially higher concentrations of MCPA than the Ohiti population (Fig 3.3).

Only one of the 80 untreated plants had died by 25 May 1987, yet a number of plants treated with very low concentrations of herbicide also died. Symptoms of death in these plants were different from those affected by herbicide, with sudden wilting occurring due to rotting of the root system. Wilting was associated with wet rather than dry potting media. Fungal organisms isolated from damaged root systems included *Phytophthora* spp and *Alternaria tenuis* (A.G. Robertson, pers comm). Large numbers of fungus gnat larvae were also found feeding in the rotting root system.

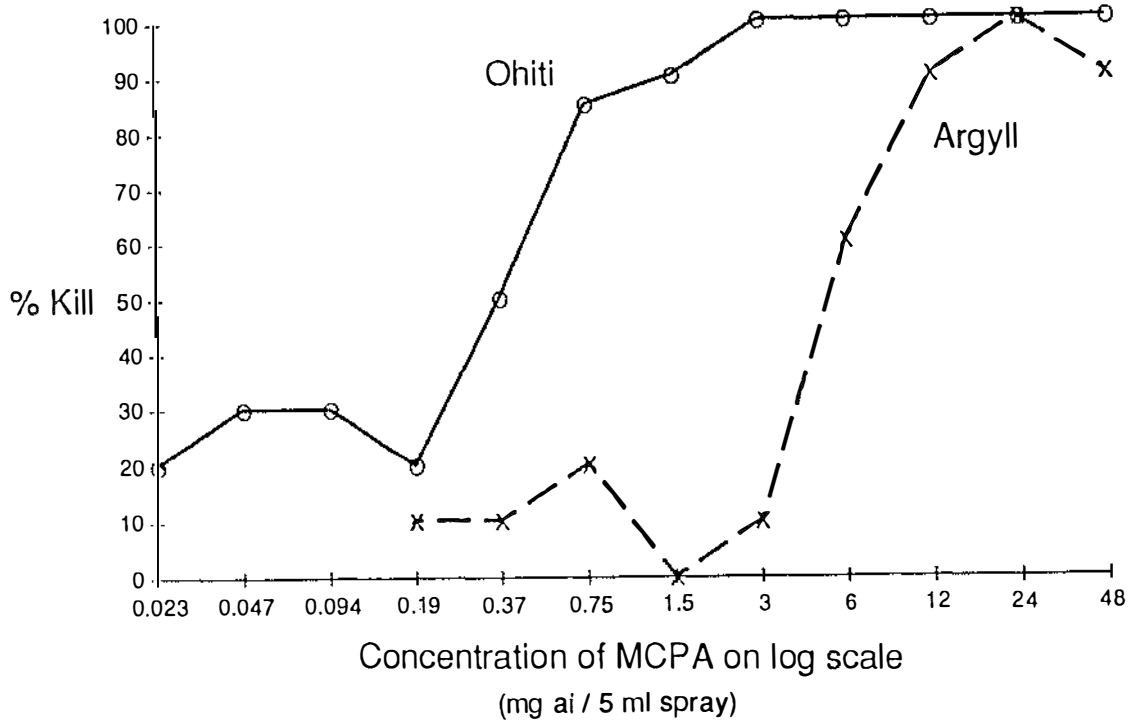


Fig 3.3: Percentage of nodding thistle plants killed from Argyll (x) and Ohiti (o) populations grown and treated with MCPA in a glasshouse in February 1987.

"Fungus gnat" is a term applied to two families of small flies (*Mycetophilidae* and *Sciaridae*) which thrive in glasshouses and feed in the larval stage mainly on fungal growths but also on the roots and stem base of plants (Fenemore 1977).

MCPA was probably not the primary cause of death for Ohiti plants receiving less than 0.19 mg ai / 5 ml or Argyll plants receiving less than 1.5 mg ai / 5 ml. The herbicide may have caused slight damage to the root system which allowed fungal organisms and then fungus gnats to establish themselves in the root. Wet potting media resulting from over-watering may have also contributed to this root damage. The etridiazole applied at planting would have been ineffective at controlling fungi by the time of herbicide application as it only persists for 6-10 weeks (O'Connor 1989). Whatever the reason for plant death at these lower concentrations, MCPA was probably not responsible so these data were excluded from probit analysis of the dose response curve.

Probit analysis estimated LD50 values (with 95% confidence limits) of 6.3 (3.4 - 11.3) mg ai / 5 ml for the Argyll population and 0.38 (0.21 - 0.58) mg ai / 5 ml for the Ohiti population. Thus the Argyll plants appeared 16.7 times more tolerant of MCPA than Ohiti plants.

3.5 CONCLUDING DISCUSSION

The LD50 values estimated in the three glasshouse experiments for the Argyll populations were almost the same (Table 3.1). In contrast, the Ohiti plants varied significantly in tolerance to MCPA between experiments, which made estimation of the magnitude of herbicide tolerance in the Argyll population difficult.

The Ohiti plants became more susceptible to MCPA as they became older and larger. Nodding thistle generally becomes more tolerant with increase in size and age (Popay *et al* 1989). The extremely limited rooting zone in the small pots could be important in explaining this phenomenon, weakening plants and so increasing their susceptibility to MCPA. Over-watering, fungal attack and fungus gnats may have also contributed to abnormal susceptibility to MCPA by these Ohiti plants. In the two experiments where plants were treated while young, etridiazole may have still been active in the potting medium after herbicide application and protected damaged roots from subsequent fungal attack. The tolerance of the Argyll plants to the factors which increased Ohiti plant susceptibility with age was obviously of interest.

Although all three experiments showed Argyll plants differed significantly to Ohiti plants in tolerance to MCPA, the magnitude of this tolerance difference remained unclear. Field trials now appeared potentially more productive for estimating tolerance differences between these populations than further glasshouse experiments in which the artificial growing conditions were probably influencing the tolerance of plants.

Table 3.1: A summary of the four glasshouse experiments conducted to assess the tolerance of Ohiti and Argyll nodding thistle populations to MCPA.

Treatment Date	Plant Age When Treated (weeks)	Average Root Size When Treated (mg DW)	LD50 (mg ai / 5 ml)		Relative Difference in Tolerance
			Ohiti	Argyll	
4 Dec 1985	7	270	2.2	-	-
17 Sept 1986	18	2100	<0.75	6.3	>8.4
22 Dec 1986	5	54	1.1	5.3	4.8
23 Feb 1987	12	1900	0.38	6.3	16.7

CHAPTER 4: FIELD COMPARISON OF POPULATIONS

4.1 INTRODUCTION

The glasshouse experiments had shown conclusively that Argyll nodding thistle plants differed from Ohiti plants in susceptibility to MCPA. It was considered important to make a further comparison of these populations, this time under the same field conditions, so the difference in susceptibility could be measured under more natural conditions. If the difference was only small and had merely been accentuated by glasshouse growing conditions, the phenomenon might not merit further research.

Seeds from one population could have been sown among naturally occurring plants at the site of the other population in Hawkes Bay to assess their susceptibility under the same field conditions. However it was more convenient to conduct the trial at Massey University as it eliminated the need to travel to Hawkes Bay. Nodding thistle does not occur naturally at Massey University, so care was needed to prevent this weed species establishing in the trial area. The dormancy characteristics of nodding thistle seed made it undesirable to establish field populations by sowing seed directly into the soil, so an alternative technique was required.

Plant age at treatment appeared to be correlated with the size of susceptibility differences between the Ohiti and Argyll populations in earlier experiments. Although pot size or presence of fungicide may have been more important than plant age in explaining this phenomenon, the field trial compared the susceptibility of Ohiti and Argyll plants at two growth stages in case plant age was important.

4.2 METHODS AND MATERIALS

Nodding thistle seeds from the Argyll and Ohiti populations were placed on moist filter paper in petri dishes on 19 November 1986. Once germinated, the seedlings were planted into 230 ml paper cups containing slow-release fertilizer, etridiazole and equal proportions of sand, peat and pumice. They were grown in a glasshouse ranging in temperature daily (with standard error) from $19 \pm 2^{o}C$ to $25 \pm 2^{o}C$ and received regular over-head watering until 4 January 1987 when they were transplanted to the field. A second batch of seeds began germination on 13 January 1987, were grown in pots as for the first batch of plants, and were transplanted to the field on 29 March 1987.

The trial site was a pasture on Tokomaru silt loam situated at the Moginie Block of Massey University's Pasture and Crop Research Unit. A steel pipe was used to stamp holes in the pasture 9 cm deep and 6 cm in diameter into which plants and potting mix were positioned. Plants from the four populations (two origins and two planting dates) were alternated 1 m apart down rows which were also 1 m apart. Plastic ear-tags were used for identifying each plant as described in Section 2.3.2, and were treated with paraquat periodically during the trial to prevent overgrowth by surrounding pasture plants. The paddock was irrigated in the weeks following transplanting to supplement rainfall. Competition from the pasture was reduced by regularly mowing both the thistles and the surrounding pasture. Mowing was preferred to grazing because the plants growing in potting mix would have been vulnerable to damage by treading or pulling during defoliation by livestock. Herbicide treatments were not applied until two months after the second transplanting date, allowing plants to adapt fully to field conditions. Of the 911 plants transferred to the field, 905 (99.3%) survived the transplanting process.

Immediately prior to treatment, the plants were given scores according to their size and vigour. Plants from the four populations were allocated to blocks based on their score, and herbicide treatments were randomly allocated within each block. Ten plants with various size scores from each of the four populations were selected and dissected to characterize plant size at time of treatment. Argyll and Matapiro plants were similar in size, but those transplanted in January had average (with range) shoot and root masses of 5.8 (1.8 - 14.8) g DW and 1.4 (0.7 - 3.3) g DW respectively, compared with 2.9 (1.0 - 5.7) and 0.69 (0.42 - 1.4) g DW respectively for the March transplants. The plants were not mown for several weeks before or after herbicide application to optimize interception and absorption of the MCPA (Plates 4.1 and 4.2).

Treatments were applied between 23 - 28 May 1987 when the older two populations were 6 months old and the rest were 4 months old. Each population received 11 rates of MCPA (potassium salt) ranging in two-fold increments from 0.125 to 128 mg ai / 5 ml of solution applied per plant (Fig 4.1), and there were also untreated control plants. Replication varied depending on availability of plants, ranging from 13 plants per treatment for the 4 month old Ohiti population to 22 plants per treatment for the 6 month old Argyll population. The treatments took several days to apply because plants were sprayed individually using the sprayer discussed in Chapter 2. Temperature and relative humidity during treatment ranged between 13 - 16^{o}C and 60 - 88% respectively. Plant mortality was assessed on 26 August 1987.

Plate 4.1: View of the field trial site at the time of treatment.

Plate 4.2: A typical nodding thistle plant at the time of treatment in the field comparison of the Argyll and Ohiti populations.

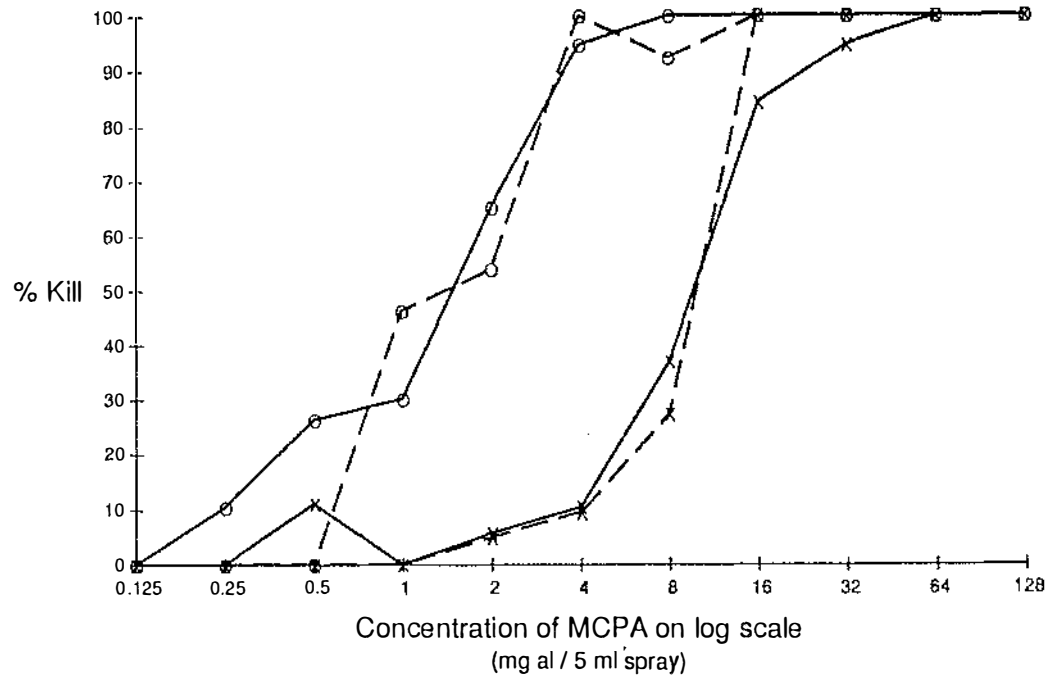


Fig 4.1: The percentage of Argyll (x) and Ohiti (o) nodding thistle plants killed by various concentrations of MCPA when grown in a pasture at Massey University and treated when four (dashed line) and six (solid line) months old.

4.3 RESULTS AND DISCUSSION

4.3.1 Influence of Plant Age

There was almost no difference between the 4 month old and 6 month old plants in susceptibility to MCPA (Fig 4.1). Allowance was made for the 2.8% natural death of untreated plants when fitting these data to dose response curves using probit analysis (Figs 4.2 and 4.3). LD50 values (with 95% confidence intervals) for the younger and older Ohiti populations were 1.6 (1.1 - 2.3) mg / 5 ml and 1.3 (0.96 - 1.7) mg / 5 ml respectively, and for the younger and older Argyll populations 8.7 (6.5 - 11.5) mg / 5 ml and 9.6 (7.1 - 13.0) mg / 5 ml. The Argyll population was estimated to be 5.5 times more tolerant of MCPA than the Ohiti population for the 4 month old plants, and 7.4 times more tolerant for the 6 month old plants. These two estimates were not significantly different ($p = 0.05$).

The difference between the Argyll and Ohiti populations in susceptibility to MCPA was larger in the glasshouse experiments if plants were treated when they were older. The similarity in susceptibility of the younger and older plants under field conditions

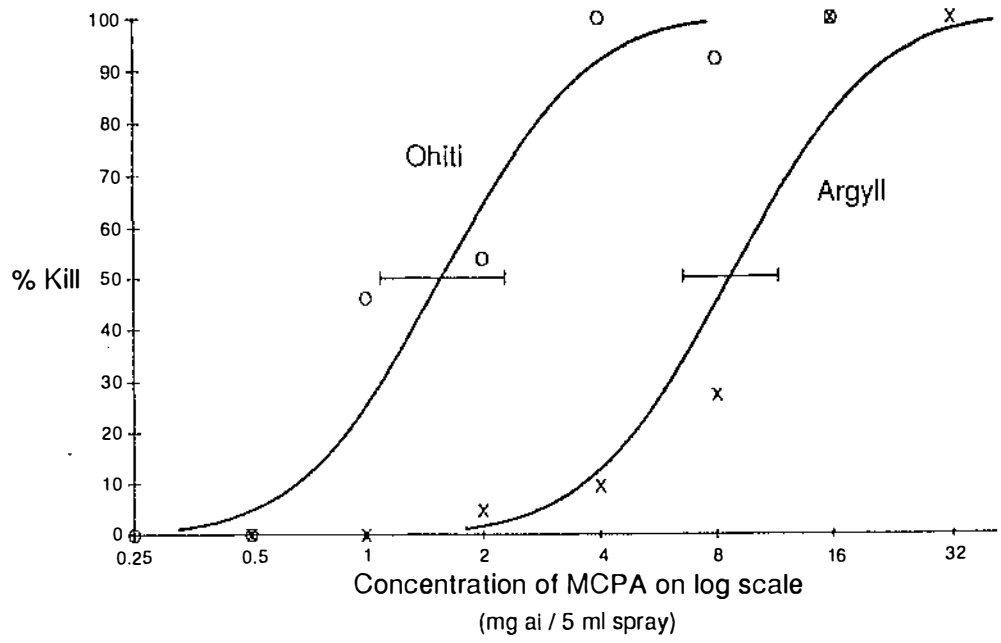


Fig 4.2: Dose response curves fitted using probit analysis for the Argyll (x) and Ohitl (o) nodding thistle populations grown in the field at Massey University and treated when four months old. The LD50 values are shown with 95% confidence limits.

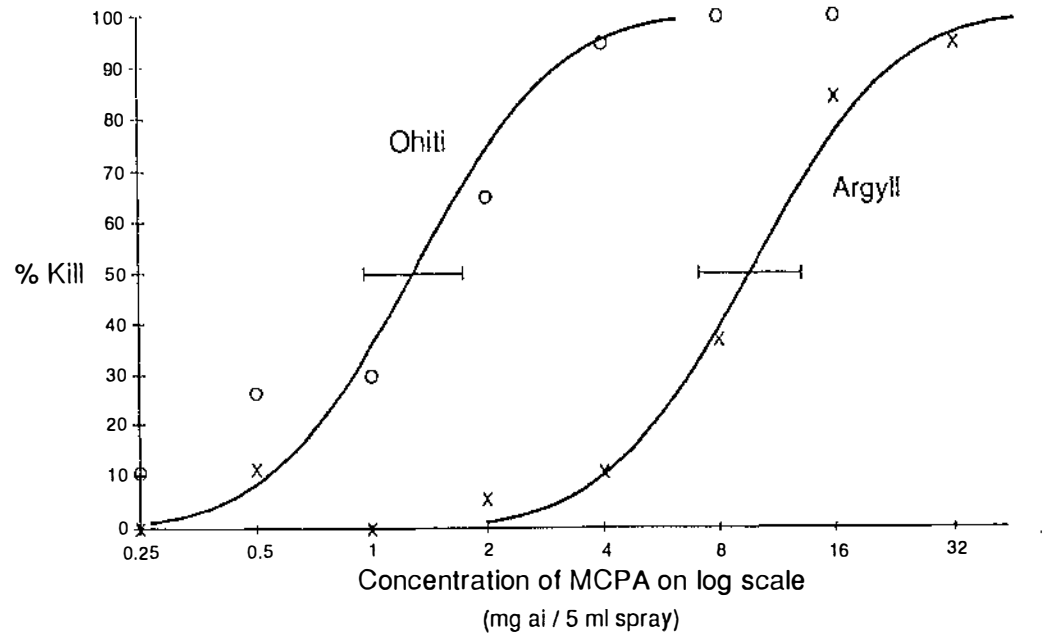


Fig 4.3: Dose response curves fitted using probit analysis for the Argyll (x) and Ohiti (o) nodding thistle populations grown in the field at Massey University and treated when six months old. The LD50 values are shown with 95% confidence limits.

may have been due to the relative difference in size and age between plants from the two sowing dates not being large by the time treatments were applied. Although another interpretation of the results is that young and old plants do not differ in susceptibility under field conditions, the young plants were 4 months old when sprayed compared with 5-7 weeks old for the youngest plants treated in the glasshouse experiments.

A comparison of LD50 values for the glasshouse and field experiments assists with interpretation of the data (Table 4.1). The field populations of Argyll plants had tolerance levels of MCPA similar to the three glasshouse populations assessed. Likewise the field-treated Ohiti plants were comparable in susceptibility to the Ohiti population treated in the glasshouse when 5 weeks old. Thus the field results further indicated that the anomalous results in the experiments conducted to date were the low LD50 values obtained for the two older glasshouse Ohiti populations.

Fungal attack and over-watering were suggested in Chapter 3 as factors contributing to the sensitivity of the two glasshouse Ohiti populations. However soil conditions were wet in the months following the herbicide application in the field experiment, and the

Table 4.1: Summary of results from the three glasshouse experiments and one field experiment comparing the susceptibility of Argyll and Ohiti nodding thistle populations to MCPA.

Treatment Date	Plant Age When Treated (weeks)	Average Root Size When Treated (mg DW)	LD50 (mg ai / 5 ml)		Relative Difference in Tolerance
			Ohiti	Argyll	
17 Sept 1986	18	2100	< 0.75	6.3	> 8.4
22 Dec 1986	5	54	1.1	5.3	4.8
23 Feb 1987	12	1900	0.38	6.3	16.7
25 May 1987	18	690	1.6	8.7	5.5
25 May 1987	26	1400	1.3	9.6	7.4

etridiazole applied initially in the potting mixture used for establishing the field plants would have dissipated. If Ohiti plants became more susceptible to MCPA under these conditions, a greater sensitivity could have been expected in the field experiment.

Fungus gnats and pot size are two factors discussed in Chapter 3 which cannot be discounted on the basis of the field results as possible reasons for variability in Ohiti plant susceptibility. Fungus gnats generally cause problems only in glasshouses (P.G. Fenemore, pers comm) and so were unlikely to have attacked dying nodding thistle root systems in the field where many other sources of decaying material were available. Similarly, limitations on the size of the root zone imposed by pots in the glasshouse experiments were absent in the field experiment. Note however that pot size did not appear to limit root growth substantially as the roots of glasshouse plants were heavier than those of the field plants despite being younger (Table 4.2). The glasshouse plants had less shoot material per gram of roots than the field plants. Deficiencies in nutrients within the glasshouse pots and competition for light with surrounding unmown pasture plants by the field plants probably both contributed to this difference (Scott-Russell 1977). Although nutrients were probably depleted in the sand potting mixture following many weeks of plant growth and irrigation water leaching through the pots, it remained unclear how this or fungus gnat attack could have affected the susceptibility of Ohiti plants but not Argyll plants.

Table 4.2: Summary of average plant size measurements taken at time of treatment for the glasshouse and field experiments conducted between September 1986 and May 1987.

Site of treatment	Age (weeks)	Longest leaf (cm)	Number of leaves (>2 cm)	Shoot mass (mg)	Root mass (mg)	Shoot: root ratio
glasshouse	5	5.3	-	121	54	2.2
glasshouse	7	9.2	7.8	457	268	1.7
glasshouse	12	8.7	17.7	1850	1900	0.97
glasshouse	18	11.3	15.5	2490	2100	1.2
field	18	23.8	18.0	2900	690	4.2
field	26	26.0	24.6	5800	1400	4.1

4.3.2 Tolerance of the Argyll Population

Disregarding the two anomalous glasshouse experiments, the Argyll nodding thistle population had been consistently shown to tolerate five to seven times more MCPA than the Ohiti population.

Since the advent of the phenoxy herbicides in the 1940s, several weed species have been discovered with intraspecific variability in tolerance of these compounds (Bandeen *et al* 1982; Gressel *et al* 1982). Comparison of our results with these reports was difficult in some cases where sufficient research had not been conducted to accurately determine the magnitude of tolerance.

One of the first documented cases of enhanced tolerance to MCPA was that reported for *Taraxacum officinale* Weber, *Ranunculus* spp and *Trifolium repens* L. in Belgian pastures in 1950 (Stryckers 1958). These species became difficult to control with either MCPA or 2,4-D following up to nine applications of these herbicides, and 250 g/ha gave tolerance levels from 20 to 100%. This work was not followed up so the magnitude of tolerance remains uncertain (Gressel *et al* 1982).

Ellis and Kay (1975) screened *Matricaria perforata* Merat populations from 43 sites in England and Wales and five sites in France for tolerance to MCPA. Although this species is generally quite tolerant of MCPA anyway, some populations were found to be 2.1 times more tolerant of MCPA than the most susceptible populations.

Variability between *Cirsium arvense* (L.)Scop. populations in susceptibility to MCPA in Sweden was noted in the 1950s (Abel 1954). Subsequent screening in 1976 of 60 Swedish *C. arvense* clones confirmed this variability, and a 3-fold difference was measured between clones in the rate of MCPA required to cause 50% reduction in growth (Gressel *et al* 1982).

Stellaria media (L.) Vill. populations were discovered in England with ED50 (equivalent dose for 50% response) values approximately 25 times higher than other populations for mecoprop (Lutman and Lovegrove 1985). There was cross-resistance to MCPA in these populations, though only four-fold differences in susceptibility were measured for this herbicide.

Following publication of results from the first two glasshouse comparisons of the Argyll and Ohiti populations (Harrington 1987; Harrington and Popay 1987), giant buttercup (*Ranunculus acris* L.) populations from Takaka (near Nelson, New Zealand) were tested for tolerance of MCPA in September 1987 (Bourdot and Hurrell 1988). A

4.8-fold difference in tolerance was detected.

Although work on the nodding thistle tolerance to date had involved only MCPA, the 1981 trial discussed in Chapter 1 indicated resistance also existed to 2,4-D. Therefore reported cases of 2,4-D tolerance are relevant to this project as well.

Two recognizable strains of *Commelina diffusa* Burm. f. were found to differ in susceptibility to 2,4-D when sprayed in Hawaiian sugar cane crops in 1950 (Bandeen *et al* 1982). One strain was easily controlled at 1.1 kg/ha, yet 5.6 kg/ha were required to kill the other, suggesting a 5-fold tolerance difference.

A similar rough estimation of tolerance to 2,4-D could be obtained for the *Cardaria chalepensis* (L.)Hand.-Maz. populations in USA found to vary in sensitivity in 1951. A 75% reduction in growth occurred at 1.1 kg/ha for the most sensitive strain and at 5 times this rate for the most resistant strains (Sexsmith 1964).

Differences between biotypes of *Daucus carota* L. in susceptibility to 2,4-D were discovered in Canada in 1957, but the limited research conducted on these biotypes never determined the magnitude of tolerance. Mortality obtained in resistant and susceptible seedlings was 7% and 49% respectively at 100 ppm of 2,4-D, while 200 ppm caused 27% and 98% mortality respectively (Whitehead and Switzer 1963).

Marked differences in *Cirsium arvense* tolerance of 2,4-D were discovered in USA in 1953, with 1.7 kg/ha causing 78% mortality in some populations and 25% mortality in others when grown at the same site (Hodgson 1970).

Convolvulus arvensis L. populations collected from 19 states in USA and one province in Canada were also found to vary in tolerance to 2,4-D, with 0.56 kg/ha causing a range in response from an 87% decrease in weight after 1 month to an 83% increase (Whitworth 1964).

Variability was also found in the susceptibility to 2,4-D of 13 *Kochia scoparia* L. selections taken from several parts of USA in 1968 (Bell *et al* 1972). The most susceptible selection was injured more by 0.35 kg/ha of 2,4-D than the most tolerant by 0.70 kg/ha based on visual injury rating, growth in plant diameter relative to untreated controls, and seed production.

Although the magnitude of tolerance remains uncertain for many of these reported cases, the five to seven-fold difference in tolerance detected for nodding thistle appears either comparable to or greater than that found in other species.

LeBaron and Gressel (1982) discussed how the terms "resistance" and "tolerance" are sometimes misused or used interchangeably. They defined tolerance as the natural and normal variability to pesticides and other agents which exists within a species and can easily and quickly evolve. In contrast, a resistant weed was defined as one that survives and grows normally at the usually effective dose of a herbicide. Resistant individuals are usually found in much lower frequencies than tolerant ones in natural untreated populations.

If these definitions were applied to the cases discussed above, tolerance was probably the more appropriate term for species such as *Matricaria perforata* and *Kochia scoparia* where differences between biotypes appeared relatively small. However the difference in susceptibility discovered with nodding thistle appeared large enough to be called resistance. Seven times more MCPA was required to obtain the same level of control in the Argyll population as the Ohiti population. Concentrations of MCPA which killed all Ohiti plants resulted in almost no death of Argyll plants. Although Argyll plants might have received a temporary check in growth, they soon recovered and eventually became indistinguishable from untreated plants.

4.4 CONCLUSION

The Argyll nodding thistle population originally came from a property in Hawkes Bay where difficulties were experienced in obtaining effective control of this species with phenoxy herbicides. Poor control was obtained because herbicide resistance has developed. A seven-fold increase in MCPA application rate is required to reach the level of control normally obtained for nodding thistle.

CHAPTER 5: TESTING FOR CROSS-RESISTANCE

5.1 INTRODUCTION

As discussed in Chapter 1, nodding thistle can reduce animal production considerably if left uncontrolled in pastures. Results from trials discussed in Chapters 3 and 4 indicated that applying MCPA was not a practical technique for controlling nodding thistle at Argyll. Increasing application rates to allow for herbicide resistance would cause unacceptable damage to pasture legumes and increase the cost of spraying to an uneconomic level. To control Argyll nodding thistle chemically, either the resistance mechanism must be overcome or an alternative herbicide would be required.

Several experiments were conducted to establish whether the Argyll nodding thistle population was resistant to other herbicides. In addition to exploring alternative control strategies, it was expected these experiments would provide information on the mechanism of resistance. If poor foliar penetration was causing resistance, other foliar-applied herbicides would probably also be affected. However if increased degradation of the MCPA molecule within the plant was involved, herbicides differing greatly in molecular structure would probably not be affected.

5.2 CLOPYRALID EXPERIMENT

5.2.1 Introduction

The Argyll nodding thistle population was resistant to MCPA, and the 1981 trial suggested it was also resistant to 2,4-D (Section 1.2.4). The similarity between MCPA and MCPB in structure and action (Loos 1975) meant MCPB would probably also be ineffective. As discussed in Section 1.2.4, clopyralid is another herbicide used to control nodding thistle in pastures, though usually in combination with MCPB ester.

A number of Argyll and Ohiti nodding thistle plants received little or no MCPA in the field trial discussed in Chapter 4. Plants left from that trial were used to test if there was cross-resistance to clopyralid as this appeared potentially the best herbicide for controlling Argyll nodding thistle. It was not initially tested in combination with MCPB to avoid problems interpreting data if there was resistance to MCPB and not clopyralid.

5.2.2 Methods and Materials

Plants were used from the May 1987 field trial only from treatments where no death had occurred to ensure susceptible individuals had not been selectively removed. None of these plants displayed any symptoms of past herbicide application. Treatments in the present trial were applied 20 weeks after those applied in the first trial, probably allowing ample time for plants to have recovered from sub-toxic effects of MCPA. Plants established in November 1986 were not differentiated from those established in January 1987 because of the results obtained in the first trial.

Plants were blocked based on size as determined by maximum leaf length prior to herbicide application, and treatments were randomly allocated within blocks. Ohiti plants received clopyralid (amine salt) at 0.15, 0.3, 0.6 or 1.2 mg ai / 5 ml of spray solution, and Argyll plants received these plus one additional treatment of 2.4 mg ai / 5 ml. Each treatment was replicated 10 times, with untreated controls for each population. Ten plants were harvested and measured to characterize plant size at treatment. Average (with range) shoot and root masses were 6.77 (2.86 - 17.33) g DW and 1.13 (0.62 - 1.70) g DW respectively. The longest leaf was 24.1 (17.0 - 34.0) cm and crown diameter was 1.8 (1.3 - 2.3) cm. Most rosettes bolted and began flowering within 4 to 6 weeks of treatment.

The 0.15 mg / 5 ml treatment and half of the 0.3 mg / 5 ml treatment were applied on 9 October 1987. Applications were stopped when light drizzle began falling, and the rainfall intensified 20 minutes later, with 20 mm falling during the following 9 hours. The remaining plants were treated on 12 October 1987, with the lower concentrations applied before higher concentrations. Within 1 hour of the 2.4 mg / 5 ml treatment being applied, it began raining again, with 9 mm falling in the following 6 hours. The temperature fluctuated between 7^{o}C and 15^{o}C from 8-14 October 1987. Most plants not killed by the herbicide treatments bolted and flowered several weeks later, and were destroyed before setting seed. The percentage of plants killed by clopyralid was recorded.

5.2.3 Results and Discussion

Rain falling within 3 hours of clopyralid application may reduce effectiveness of the herbicide (O'Connor 1989). Therefore rainfall had been expected to influence results obtained in this trial, though Argyll and Ohiti plants were treated alternately so both populations would have been influenced similarly. However the results appeared unaffected by the rainfall. The two populations did not differ in susceptiblity to

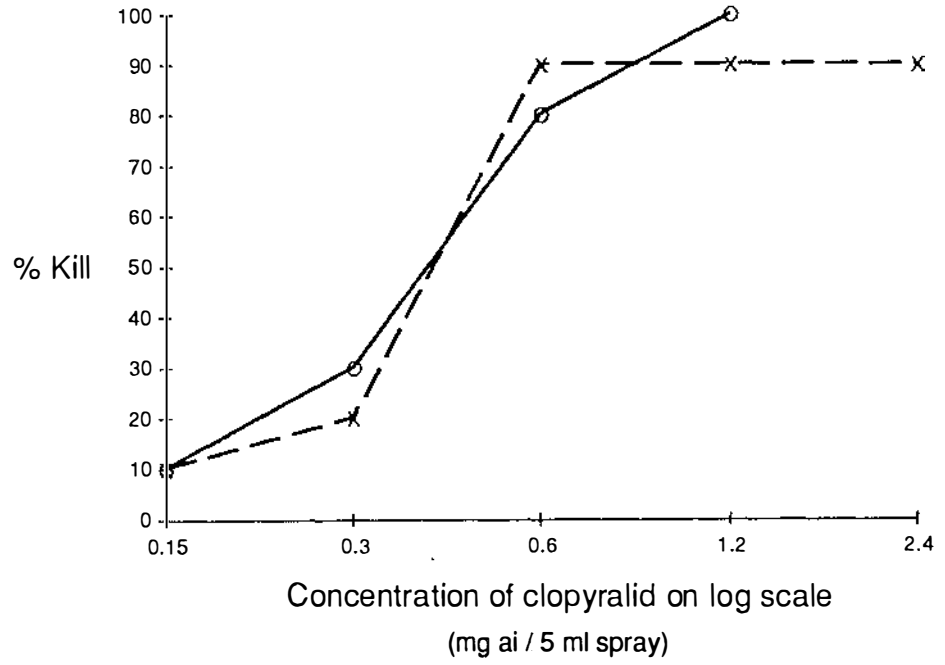


Fig 5.1: The effect of clopyralid on Argyll (x) and Ohiti (o) nodding thistle populations treated in October 1987 when 9-11 months old.

clopyralid (Fig 5.1), indicating no cross-resistance of this herbicide in the Argyll population.

One Argyll plant survived at each of the three highest application rates. This may indicate some resistance to clopyralid within the population. However the plants which survived 0.6 and 1.2 mg / 5 ml were the largest plants receiving the treatment and so would be expected to tolerate more of the herbicide than smaller plants. The plant which survived 2.4 mg / 5 ml was only of average size, but the rain that fell an hour following application may have influenced the survival of this plant.

The 0.3 mg / 5 ml treatment was one of the most important for differentiating between the susceptibility of the two populations to clopyralid as it resulted in population mortality levels near 50%, thus influencing the probit analysis more than results from the other concentrations (Finney 1971). The results were therefore not conclusive as this was also the treatment applied closest to the onset of rain. However half of this treatment was applied on 12 October when rain did not fall until 3 hours after the last plant was sprayed. Two of the three Argyll plants and two of the six Ohiti plants treated on 12 October died, suggesting rainfall was not masking any tolerance within the Argyll population.

5.3 HERBICIDE SCREENING TRIAL

5.3.1 Introduction

Although comparing dose-response curves of populations was a good technique for detecting herbicide resistance, large numbers of plants were required to ensure adequate replication across sufficient application rates to produce complete curves. Field-grown plants appeared preferable to glasshouse plants following the anomalous results obtained with MCPA. Comparison of two populations in the same environment required populations to be established artificially. To screen as many herbicides as possible efficiently, the next trial compared the Argyll and Ohiti populations at one application rate only. The rate recommended for nodding thistle control was generally used because resistance should be most evident at this rate.

A disadvantage of the metal spray-box used in all previous experiments was the difficulty in relating its application rates to those normally used in the field with boom-sprayers because of the uneven distribution of spray droplets (see Section 2.2.4). To ensure application rates in this trial were comparable with normal recommended rates, a precision plot-sprayer was used instead of the spray-box.

Although nodding thistle plants in the 1987 field trials were more "natural" than those in glasshouse experiments, the constant mowing and competition with unmown vegetation when treatments were applied may have affected the susceptibility of plants to herbicides. These pressures were probably responsible for average root mass not increasing between May and October. Nodding thistle normally grows in pastures grazed by sheep, but grazing was avoided to prevent plants being pulled from the soft potting mixture used for transplanting and to stop the selective grazing of thistles following spraying discussed in Section 1.2.3.1. To eliminate mowing and competition, glyphosate was used in the next field trial to remove all vegetation immediately prior to transplanting.

5.3.2 Methods and Materials

Argyll and Ohiti plants were initially established in a glasshouse as described in Section 4.2, with germination beginning on 25 February 1988. The site used for the 1987 trials was sprayed on 22 March 1988 with glyphosate at 2.7 kg ai/ha in 150 l/ha of water. Seedlings were transplanted into the dead turf and tagged on 15-20 April 1988 as described in Section 4.2.

The 12 herbicide treatments listed in Table 5.1 were applied to the plants when 12 weeks old on 23-26 May 1988. Average (with range) shoot and root masses of 12 plants removed immediately prior to treatment were 1520 (730 - 2560) mg DW and 330 (51 - 730) mg DW respectively. The average longest leaf was 10.2 (7 - 14) cm and crown diameter was 9.9 (6 - 12) mm. Each herbicide was applied to three different plots within the paddock, resulting in a total of 28-30 plants from each population being treated. Most of the herbicides were applied using a propane-powered precision plot sprayer with muslin screens around the sprayed plots to prevent drift on to neighbouring plots (Plate 5.1). Picloram was applied as granules and tribenuron-methyl was applied in 5 ml of water per plant using the sprayer from past trials to simulate spot-application. All other herbicides were applied with 200 l/ha of water except the paraquat/diquat which was applied in 400 l/ha. In accordance with manufacturers' instructions (O'Connor 1989), rosettes were bruised prior to application of picloram, and tribenuron-methyl was applied with 0.05% non-ionic surfactant (ICI Contact).

Table 5.1: Herbicide treatments applied to Argyll and Ohiti nodding thistle populations at Massey University in May 1988.

Herbicide	Formulation	Application Rate (ai)
MCPA	potassium salt	1.1 kg/ha
2,4-D	butyl ester	1.1 kg/ha
mecoprop	diethanolamine salt	2.8 kg/ha
MCPB	sodium salt	2.4 kg/ha
MCPB	iso-octyl ester	2.4 kg/ha
MCPB + clopyralid	butyl ester + amine salt	500 g/ha + 14 g/ha
clopyralid	amine salt	150 g/ha
picloram	amine salt	40 mg/plant
dicamba	dimethylamine salt	140 g/ha
glyphosate	isopropylamine salt	360 g/ha
paraquat + diquat	dichloride salt + dibromide salt	360 g/ha + 180 g/ha
tribenuron-methyl	water dispersable grain	0.75 mg/plant

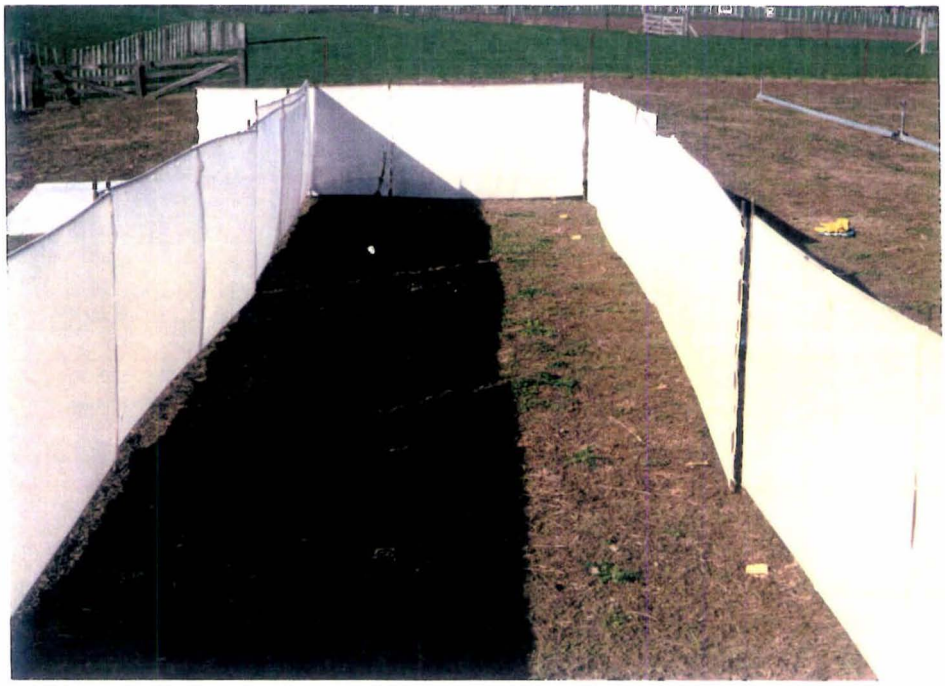


Plate 5.1: The screens used to enclose each plot during boom-application of the various herbicide treatments with a precision plot sprayer.

Plate 5.2: An Argyll nodding thistle plant 7 weeks after being treated with the MCPB ester and clopyralid mixture. The plant was dead at the final assessment 5 months after application.

Only paraquat/diquat was applied on 23 May, and 0.1 mm of rain fell 30 minutes afterwards, then a further 7.0 mm fell 6-11 hours later. No rain fell for several days after all other treatments. Average (with range) daily minimum and maximum air temperatures for 23-31 May 1988 were 5.7 (0.5 - 10.3)^{o}C and 13.6 (10.4 - 16.7)^{o}C respectively.

The number of plants killed was recorded after 5 months. Differences between the Argyll and Ohiti populations were compared for each herbicide using an adjusted chi-square analysis.

5.3.3 Results and Discussion

5.3.3.1 **MCPA**

The recommended application rate of MCPA killed 93% of Ohiti plants but only 21% of Argyll plants (Fig 5.2). Curves produced in Chapter 4 were difficult to relate to normal spraying practices because of the application technique used. However the present result allowed this comparison to be made because the concentration of MCPA in Fig 4.2 causing 21% mortality of 4 month old Argyll plants was very similar to that

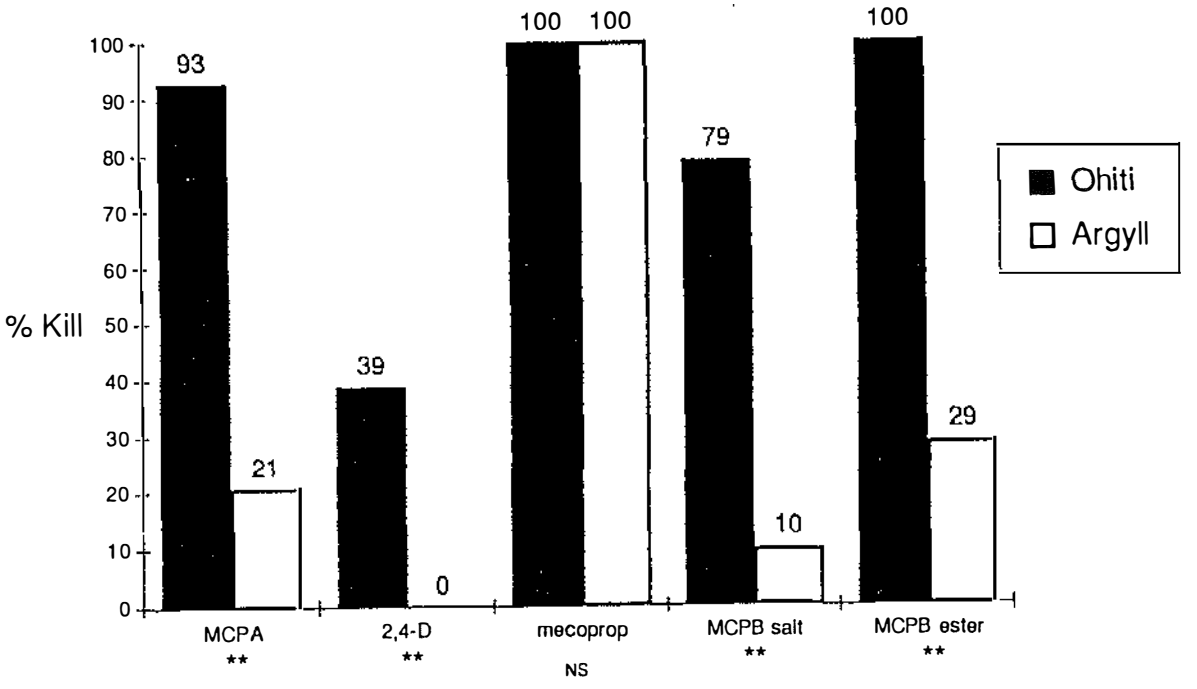


Fig 5.2: The control obtained of Argyll and Ohiti nodding thistle populations when sprayed with phenoxy herbicides at recommended rates (see Table 5.1) in May 1988.
NS = differences between populations not significant at p = 0.05.
** = differences between populations significant at p = 0.01.

causing 93% mortality of Ohiti plants, suggesting the relative difference between the Argyll and Ohiti populations in susceptibility to MCPA was the same in both trials. As the trial site, method of planting, plant age and time of application were all similar for the two trials, comparable results were expected.

The dose response curves from Fig 4.2 have been superimposed on a scale of comparable field application rates in Fig 5.3 by assuming the herbicide concentration which caused 21% and 93% mortality of Argyll and Ohiti populations respectively in 1987 was the same as the application rate causing these levels of mortality in 1988. This calibration of the 1987 dose response curves indicates that applying twice the recommended application rate could give 50-60% kill of the Argyll population, but at least five times the recommended rate is required for control levels above 95%.

The dashed line in Fig 5.3 represents MCPA concentrations of 27 mg ai / 5 ml for boom-application and 4.5 mg ai / 5 ml for application from the sprayer-box. This 6-fold difference in concentration required to give similar levels of mortality illustrated the difficulty prior to this trial in comparing results between experiments using the two

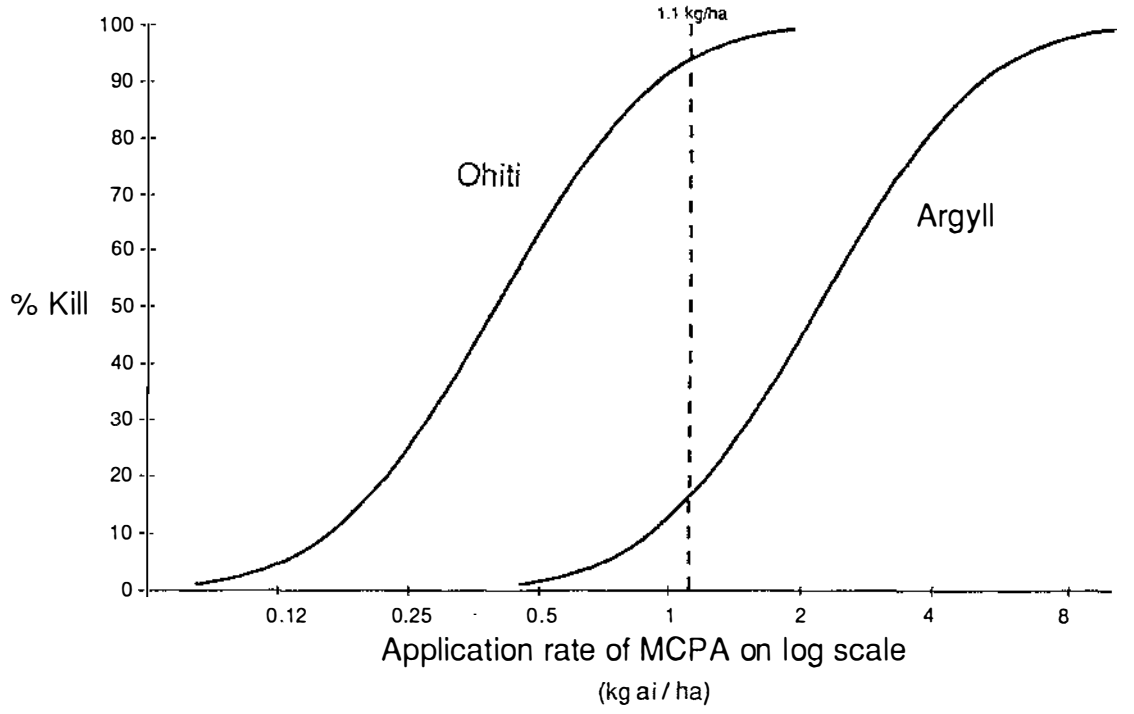


Fig 5.3: The hypothesized relationship between results obtained from applying MCPA to Argyll and Ohiti nodding thistle populations in May 1987 (see Fig 4.2) and May 1988 (see Fig 5.2).

techniques. Presumably the sprayer-box applied six times more solution to each plant than the spray-boom as this would result in the same amount of active ingredient being intercepted by the plant. However this had been difficult to calculate accurately using other methods because of the uneven distribution of droplets from the nozzle of the spray-box (see Section 2.2.4).

5.3.3.2 **2,4-D**

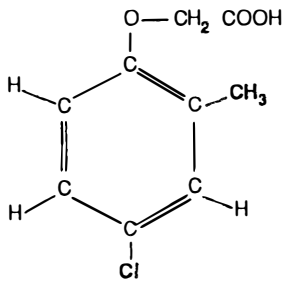
The 39% mortality of Ohiti plants by 2,4-D was unexpected and difficult to explain. Although Popay *et al* (1989) found 1.0 kg ai/ha 2,4-D was less active on nodding thistle than 1.0 kg ai/ha MCPA, the 1.1 kg ai/ha used in this trial was the recommended application rate for 2,4-D (O'Connor 1989).

However, as Ohiti and Argyll plants were positioned alternately within the trial site and sprayed simultaneously, the relative susceptibility of the two populations could still be assessed. The Argyll plants tolerated the 2,4-D significantly better than the Ohiti plants (Fig 5.2), indicating cross-resistance of this herbicide. This confirmed the results obtained in the 1981 trial (Section 1.2.4). As discussed in Section 1.3, MCPA and 2,4-D are very similar in structure and action so, regardless of the mechanism of resistance involved, cross-resistance was to be expected.

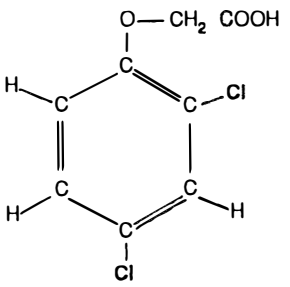
5.3.3.3 **Mecoprop**

Mecoprop is also very similar in structure to MCPA, differing only in an extra methyl group on the carboxyl chain of the molecule (Fig 5.4). Mecoprop is used in combination with MCPA in cereals and turf for control of species such as the mouse-ear chickweeds (*Cerastium* spp.) which tolerate MCPA (Matthews 1975). Cross-resistance to mecoprop within the Argyll nodding thistle population appeared possible as cross-resistance to MCPA and mecoprop had been found for chickweed (*Stellaria media*) populations in England (Lutman and Lovegrove 1985).

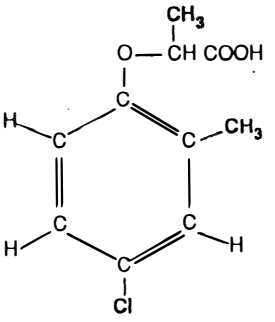
All Argyll and Ohiti nodding thistle plants were killed by mecoprop in the trial (Fig 5.2). This did not prove conclusively that cross-resistance to mecoprop does not exist. Ohiti plants may have been more sensitive than Argyll plants but the single application rate used in the trial could have been too high to show this difference (*ie* above **y** in Fig 5.5). However if resistance to mecoprop had been of the magnitude found for MCPA in Chapter 4, the application rate required to kill all resistant plants (**y**) needed to be several times larger than the application rate which normally controls nodding



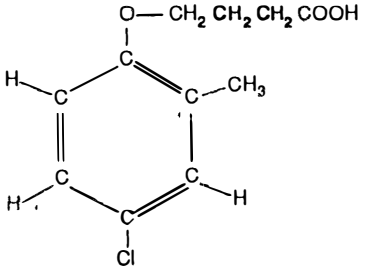
MCPA



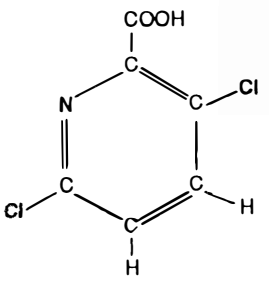
2,4-D



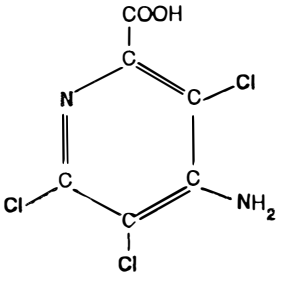
mecoprop



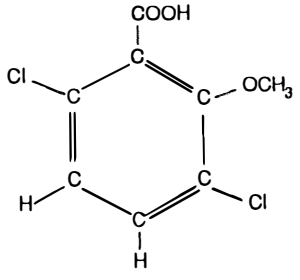
MCPB



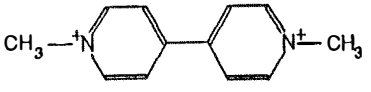
clopyralid



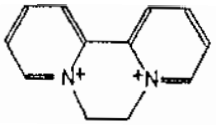
picloram



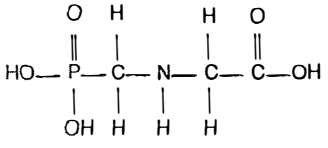
dicamba



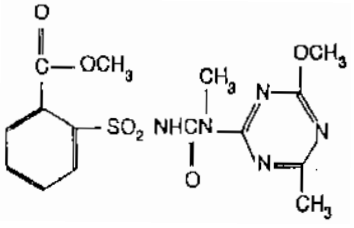
paraquat



diquat



glyphosate



tribenuron-methyl

Fig 5.4: Molecular structures of herbicides tested for cross-resistance in May 1988 (Worthing and Walker 1983; Ferguson *et al* 1985).

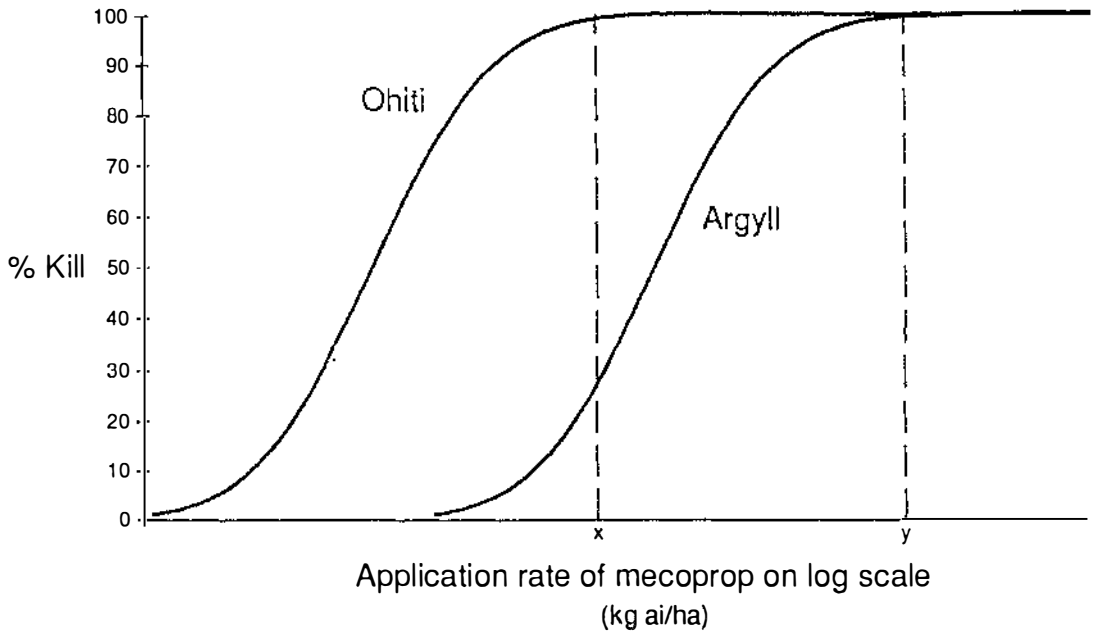


Fig 5.5: A diagram relating the application rate (x) at which nodding thistle is normally controlled by mecoprop to the lowest application rate (y) at which the trial result could have occurred if Argyll nodding thistles were resistant to this herbicide.

thistle (**x**). There was some uncertainty over the value of **x** for mecoprop as label recommendations stated 2.8 kg ai/ha were required for young weeds past the 3 true leaf stage without stating a specific recommendation for nodding thistle (O'Connor 1984). Although 2.8 kg ai/ha appears substantially higher than the 1.1 kg ai/ha recommended for controlling nodding thistle with MCPA, the formulation of mecoprop used in this trial was a racemic mixture of an active dextrorotatory isomer and an inactive laevorotatory isomer (Matthews 1975). An application rate of 2.8 kg ai/ha of mecoprop was recommended for Scotch thistle (*Cirsium vulgare*) control in British grasslands (Fryer and Makepeace 1978), and Scotch thistle is generally considered more susceptible to phenoxy herbicides than nodding thistle (Matthews 1975). Therefore x in Fig 5.5 should have been quite close to the 2.8 kg ai/ha application rate used in the present trial, which suggests the Argyll population was not substantially more tolerant of mecoprop than "normal" nodding thistle populations. However conclusive proof of this hypothesis would require the experiment to be repeated using more application rates.

5.3.3.4 **MCPB**

The MCPB molecule is identical to MCPA apart from two extra -CH_2 groups in the carboxyl chain (Fig 5.4). Although not phytotoxic in this form, MCPB undergoes beta-oxidation in susceptible plants and is converted to MCPA (Loos 1975). Therefore the cross-resistance of the Argyll population detected in this trial to MCPB was expected.

As MCPB is sold in New Zealand as a salt formulation and combined with clopyralid as an ester formulation (O'Connor 1989), two formulations were tested in this trial. Ester formulations of herbicides are more effective than salt formulations because they penetrate leaf cuticles more effectively (Klingman and Ashton 1982). The superior effectiveness of the ester formulation was apparent in the trial both for the Ohiti and Argyll populations (Fig 5.2). If resistance was caused by poor cuticular penetration, the difference between populations in susceptibility to MCPB should have become smaller using the ester formulation compared with the salt formulation. However the differences between the populations appeared unchanged.

5.3.3.5 **Clopyralid**

As with the earlier trial using clopyralid, no differences in tolerance were detected between the Ohiti and Argyll populations to this herbicide (Fig 5.6). Although 10% survival of Argyll plants was obtained at high concentrations of clopyralid in the earlier trial, this was not evident here. However, as discussed for mecoprop, the single application rate used may have been too high to detect small tolerance differences between populations, though it was only half the recommended rate (O'Connor 1989).

As discussed by Popay *et al* (1989), a small amount of clopyralid is added to MCPB ester to form a commercial herbicide mixture commonly used for nodding thistle control in New Zealand. The recommended application rate of this mixture killed all Ohiti plants, but the 29% survival of Argyll plants represented a significant ($p = 0.01$) difference in susceptibility between populations (Fig 5.6). The low concentration of clopyralid appeared to kill some (Plate 5.2) but not all of the Argyll plants resistant to MCPB. Doubling this application rate would probably control over 90% of Argyll plants, though this would suppress white clover production for up to 6 months and kill annual clover species (O'Connor 1989).

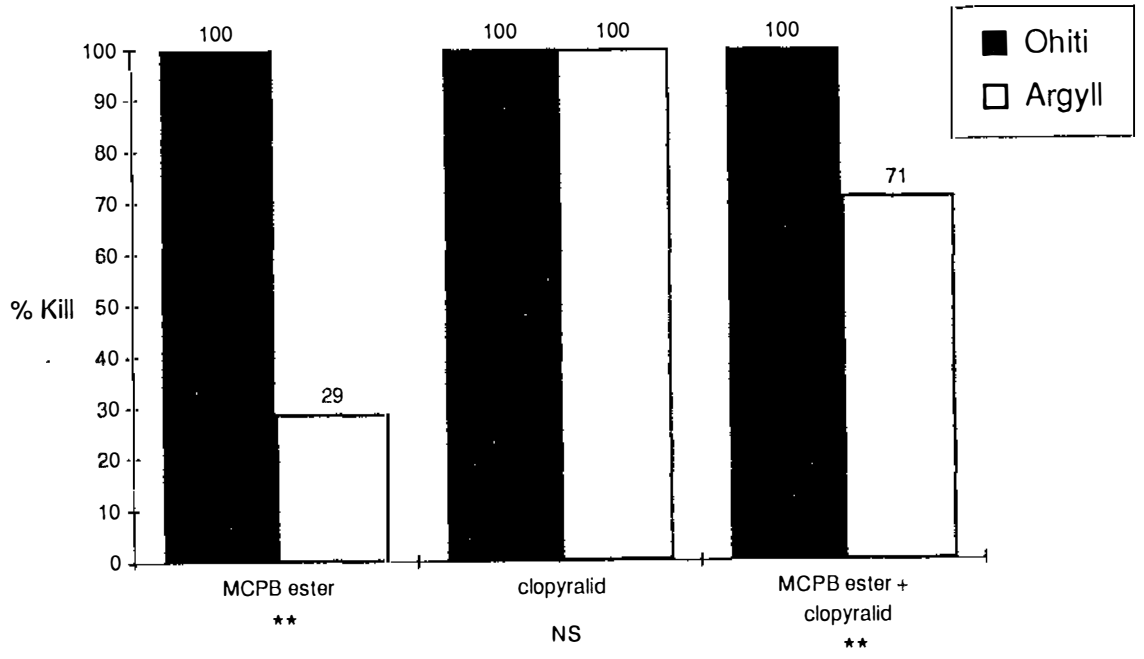


Fig 5.6: The control obtained of Argyll and Ohiti nodding thistle populations when sprayed in May 1988 with 150 g ai/ha of clopyralid, 2.4 kg ai/ha of MCPB (iso-octyl ester) and a mixture of 14 g ai/ha of clopyralid and 500 g ai/ha of MCPB (butyl ester).

NS = differences between populations not significant at p = 0.05.

** = differences between populations significant at p = 0.01.

5.3.3.6 **Picloram and dicamba**

The other two hormone herbicides tested for resistance were picloram and dicamba, both which have similarities in structure (Fig 5.4) and action to clopyralid, and were discussed in Section 1.2.4.

The recommended rate of picloram gave complete control of both populations (Fig 5.7), suggesting no resistance to this herbicide within the Argyll population, but not proving it conclusively as discussed with mecoprop.

Dicamba is usually applied to nodding thistle only as a spot-application in combination with 2,4-D to reduce clover damage. Therefore the recommendation for boom-application of dicamba by itself was difficult to obtain for nodding thistle, so the standard rate for selective application in crops such as forage brassicas, linseed and oil-seed rape was used (O'Connor 1989). Although below the level required for 100% control of nodding thistle (Fig 5.7), it gave a good comparison of susceptibility for the two populations. Argyll plants tolerated dicamba marginally better than Matapiro plants, but this difference in tolerance was not significant (p = 0.05).

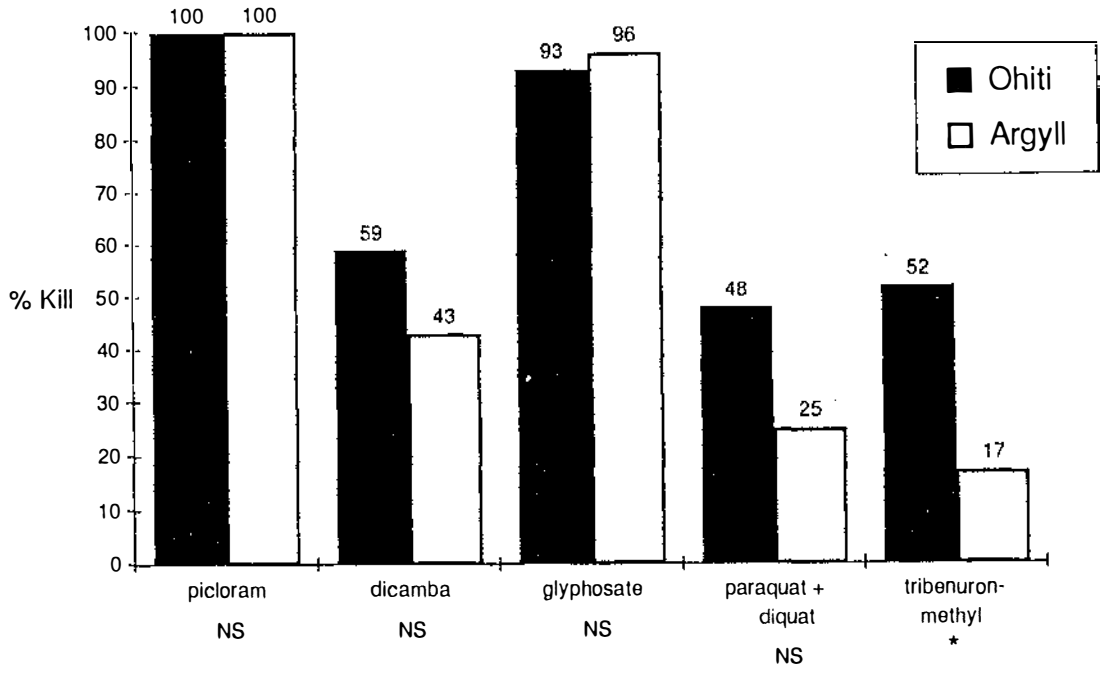


Fig 5.7: The control obtained of Argyll and Ohiti nodding thistle populations when treated with herbicides at the rates listed in Table 5.1 in May 1988.

NS = differences between populations not significant at p = 0.05.

* = differences between populations significant at p = 0.05 but not p = 0.01.

5.3.3.7 **Herbicides with other modes of action**

Although the herbicides discussed so far differ from each other in such traits as the range of weed species controlled and their relative mobility within plants, they all have similar mechanisms of action based on their similarity to auxins (Ashton and Crafts 1981). Several herbicides with totally different structures (Fig 5.4) and modes of action were also tested for cross-resistance.

Glyphosate is a non-selective, translocated herbicide which inhibits the production of aromatic amino acids within plants (Grossbard and Atkinson 1985). The lowest recommended application rate (O'Connor 1989) was used to compare the susceptibility of the Argyll and Matapiro nodding thistle populations. Therefore there were some plants which survived the glyphosate treatment (Fig 5.7) but, as similar numbers of plants survived from both populations, resistance within the Argyll population appears unlikely.

Paraquat and diquat are both broad-spectrum contact herbicides which defoliate plants by damaging cell membranes in treated tissues (Ashton and Crafts 1981). Although effective on nodding thistle seedlings, established rosettes are not adequately

controlled by these herbicides (Matthews 1975). Paraquat and diquat penetrate foliage very rapidly and so their activity is not seriously affected by rain falling soon after application (Muzik 1976). Therefore plant age rather than rainfall was probably responsible for the poor control of nodding thistle by the paraquat/diquat mixture in this trial (Fig 5.7). The foliage of all treated plants became necrotic within days of application, but surviving plants had begun regrowing within a week. Although more Argyll plants survived the paraquat/diquat treatment than Matapiro plants, the difference in tolerance was not as large as with the phenoxy herbicides and was not statistically significant ($p = 0.05$).

Tribenuron-methyl (formerly DPX-L5300) is a sulphonylurea herbicide which has undergone evaluation since 1982 for selective weed control in cereals (Ferguson *et al* 1985). It was first registered in New Zealand in the late 1980s for cereal weed control, clover control in direct drilling, and spot-control of some pasture weeds (O'Connor 1989). Tribenuron-methyl translocates throughout treated plants, inhibiting the acetolactate synthase enzyme, thereby indirectly disrupting cell division (Ferguson *et al* 1985). This herbicide is less persistent in soils than other sulphonylurea herbicides such as chlorsulfuron or metsulfuron because it undergoes very rapid chemical hydrolysis (Beyer *et al* 1987). Although registered in New Zealand for control of winged thistles (*Carduus tenuiflorus* and *Carduus pycnocephalus*) and variegated thistle (*Silybum marianum* (L.)Gaertn.), tribenuron-methyl is not yet registered for nodding thistle (O'Connor 1989). However Martin *et al* (1988) killed over 90% of nodding thistle plants with an average diameter of 30 cm using tribenuron-methyl in Waikato in July 1987.

Although tribenuron-methyl was less effective on nodding thistle in our trial, sufficient plants died to show a significant ($p = 0.05$) difference between Argyll and Ohiti plants in their tolerance of this herbicide (Fig 5.7). Treated plants became necrotic several weeks after application, then 48% of the Ohiti plants and 83% of the Argyll plants regrew from the crown. This was similar to the paraquat/diquat treatment in that both populations appeared equally affected initially, then differences occurred in regrowth of damaged plants. Plant size might have influenced the subsequent regrowth of plants following treatment, so the relationship between plant size when treated with tribenuron-methyl and subsequent plant mortality was studied in case this explained the difference between populations. A histogram of plant size showed that many of the Ohiti plants were larger than Argyll plants when treated (Fig 5.8), and a second superimposed histogram showed that plant size did not appear to influence which of the treated plants subsequently died. Therefore differences between Ohiti and Argyll plants in tolerance of tribenuron-methyl probably resulted from differences in their physiology rather than their size.

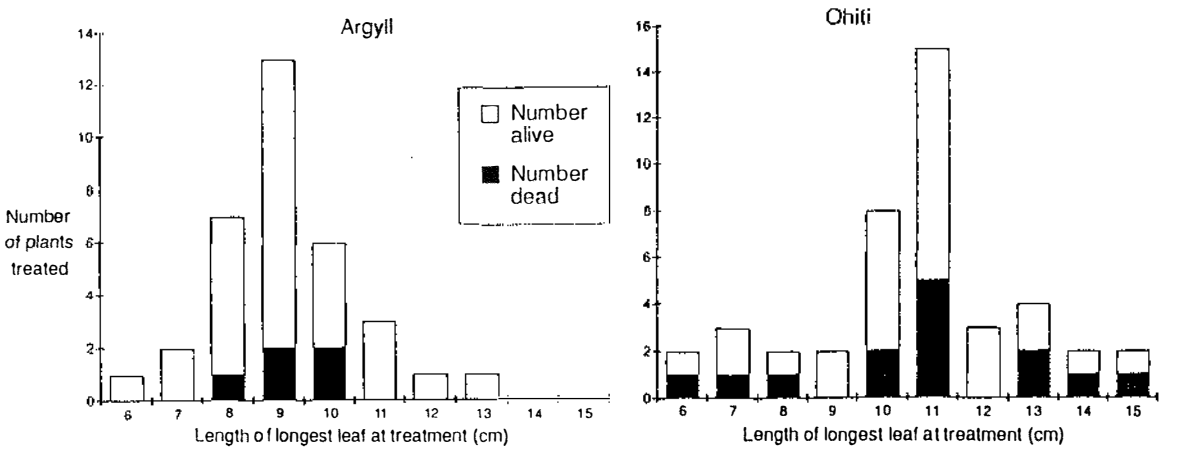


Fig 5.8: Histograms of nodding thistle plant size at treatment and the subsequent fate of these plants when sprayed with tribenuron-methyl at 150 mg ai/litre in May 1988 for the Argyll and Ohiti populations.

5.4 SECOND TRIBENURON-METHYL TRIAL

5.4.1 Introduction

Tribenuron-methyl was the only herbicide with a molecular structure and mode of action completely different to MCPA that was tolerated by the Argyll nodding thistle population. Cross-resistance to both phenoxy herbicides and tribenuron-methyl could help in elucidating the mechanism of resistance if it did exist. It could also have implications for future herbicide resistance problems in cereals if a weed species can develop resistance to both phenoxy and sulphonylurea herbicides simultaneously, as postulated by Gressel (1988).

However the difference between the Argyll and Ohiti populations in tolerance to tribenuron-methyl was smaller than with MCPA, 2,4-D and MCPB, being significant only at $p = 0.05$ compared with $p = 0.01$ for the three phenoxys (Figs 5.2 and 5.7). Another trial was conducted in 1990 to confirm the tolerance of tribenuron-methyl by the Argyll plants, this time comparing dose response curves for two populations.

Seed supplies from the original Argyll and Ohiti populations were low by 1990 and germination of remaining seeds was poor. Therefore surplus seed from populations confirmed in 1988 as susceptible (Ohutu) and resistant (Waotu) to MCPA (see Chapter 6) were used in this trial.

Although some problems were encountered when growing nodding thistle populations in the glasshouse in past trials (see Chapter 3), differences between populations in tolerance to herbicides had always been detected satisfactorily. The second tribenuron-methyl trial was conducted in a glasshouse to minimize the time required to complete the comparison. In an attempt to overcome problems encountered in earlier glasshouse trials, larger potting containers were used, aeration of the potting media was increased by including pumice and peat, and the plants were treated while they were young.

5.4.2 Methods and Materials

Seeds from the Ohutu and Waotu populations began germinating in petri dishes on 6 September 1990. Seedlings were transferred into 550 ml planter bags on 17-21 September 1990. The potting media comprised equal parts of peat, sand and pumice, contained slow-release fertilizer and was treated with etridiazole. Overhead irrigation four times daily was used for the first month while seedlings established, then the bags were transferred into trays and sub-irrigated twice daily.

Plants were treated with 2.5, 10, 40 or 160 mg ai / litre of tribenuron-methyl on 25 October 1990. Fifteen plants from each population were treated with the lower three rates, and 11 plants per population received the highest concentration. Plants were sprayed individually with 5 ml of herbicide solution using the sprayer discussed in Chapter 2. The organosilicone surfactant Pulse (Silwet L-77 or polyalkyloxylated dimethylpolysiloxane) was added to all solutions at 0.1% v/v. The temperature and relative humidity during spraying were 23^{o}C and 64% respectively. Eight plants harvested to characterise plant size had average (with range) shoot and root masses of 1310 (480 - 2410) mg and 585 (135 - 1130) mg respectively. The average longest leaf was 14.4 (9.8 - 17.8) cm and crown diameter was 8.1 (6 - 10) mm. Plant survival was assessed four months later.

5.4.3 Results and Discussion

There was no difference ($p = 0.05$) between the Ohutu and Waotu populations in susceptibility to tribenuron-methyl (Fig 5.9). This result cast doubt on whether the difference between the susceptibility of the Ohiti and Argyll populations to tribenuron-methyl in 1988 was real.

Although the mechanism of resistance in the Waotu population may be different to that

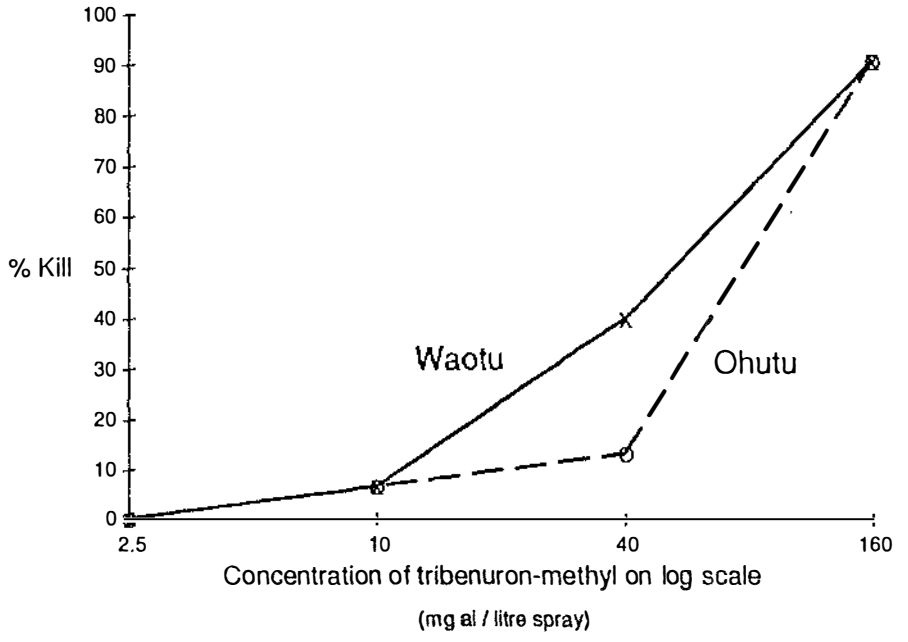


Fig 5.9: The effect of tribenuron-methyl on phenoxy-resistant (Waotu) and phenoxy-susceptible (Ohutu) nodding thistle populations treated in a glasshouse in October 1990.

in the Argyll population, this appears to be an unlikely reason for the difference in results. Organosilicone surfactants such as Pulse are considered superior to surfactants such as Contact which was used with tribenuron-methyl in the 1988 trial (Stevens *et al* 1988), so this could have explained the results if the mechanism of resistance involved cuticular penetration. However results discussed in later chapters suggest this is not the mechanism of resistance for the phenoxy herbicides. Therefore if the mechanism of resistance was the same for tribenuron-methyl as the phenoxys, the type of surfactant used should not have affected results because penetration would have been improved by the same amount in both ecotypes. Plant size at treatment was similar for the two trials so can also be discounted as a factor affecting results.

Temperatures were warmer for the 1990 trial conducted in a glasshouse in summer than in 1988 which was conducted in the field in winter. The average (with range) daily temperature for the 6 weeks following herbicide application was 9.4 (3.9 - 13.5)^{o}C for the 1988 trial and 21.0 (18.2 - 24.1)^{o}C for the 1990 trial. Muntan and Bencivelli (1987) have found tribenuron-methyl activity is enhanced by warmer conditions. This may explain why the 20-50% mortality obtained at a tribenuron-methyl concentration of 150 mg ai/litre in the first trial was similar to the mortality

levels obtained at the much lower concentration of 40 mg ai/litre in the second trial. Nodding thistle plants were also affected more rapidly by tribenuron-methyl in 1990 than in 1988, so herbicide molecules may have been exposed to degradation processes for less time prior to reaching the site of action under these warmer conditions. If resistance is caused by increased degradation rates, higher temperatures may have reduced this effect.

Whatever the reason for results differing between the two trials, it appears that phenoxy-resistant nodding thistle populations are either no more tolerant of tribenuron-methyl than other populations, or are more tolerant only under certain conditions, *eg* when environmental conditions reduce the rate of herbicide activity.

5.5 CONCLUDING DISCUSSION

5.5.1 Alternative Chemical Control Strategies

The Argyll nodding thistle population was resistant to MCPA, 2,4-D and MCPB. These are the three main herbicides available for selectively controlling nodding thistle in legume-based pastures. All herbicides tested which were not tolerated by the Argyll nodding thistle population are also not tolerated by pasture species, especially clovers.

Some of the results have since been confirmed by a Dow-Elanco field trial at Argyll in June 1989 (B. Harris, pers comm). Only 32% control of nodding thistle was obtained using 11.5 kg ai/ha of 2,4-D (butyl ester), an application rate ten times that recommended for nodding thistle control (O'Connor 1989). The recommended rate of MCPA killed no thistles. The MCPB + clopyralid mixture was applied at 1 kg + 28 g ai/ ha, twice the rate used in our trial, and this gave 97% control of nodding thistle but caused 63% suppression of clovers as assessed 17 weeks after application.

The trial also tested whether low rates of clopyralid will control nodding thistle selectively in a clover-based pasture. Fig 5.10 shows adequate thistle control was not possible without substantial clover damage. The present Dow-Elanco recommendation for farmers with phenoxy-resistant nodding thistle is to add 30 g ai/ha of clopyralid to the standard rate of MCPA or 2,4-D, with the warning that severe suppression of perennial clovers and removal of annual clovers from the sward may occur (O'Connor 1989). Addition of 30 g ai/ha of clopyralid in the June 1989 Dow-Elanco trial at Argyll to 2,4-D and MCPA gave 90% and 85% control respectively of nodding thistle, and 52% and 37% mortality respectively of clovers (B. Harris, pers comm).

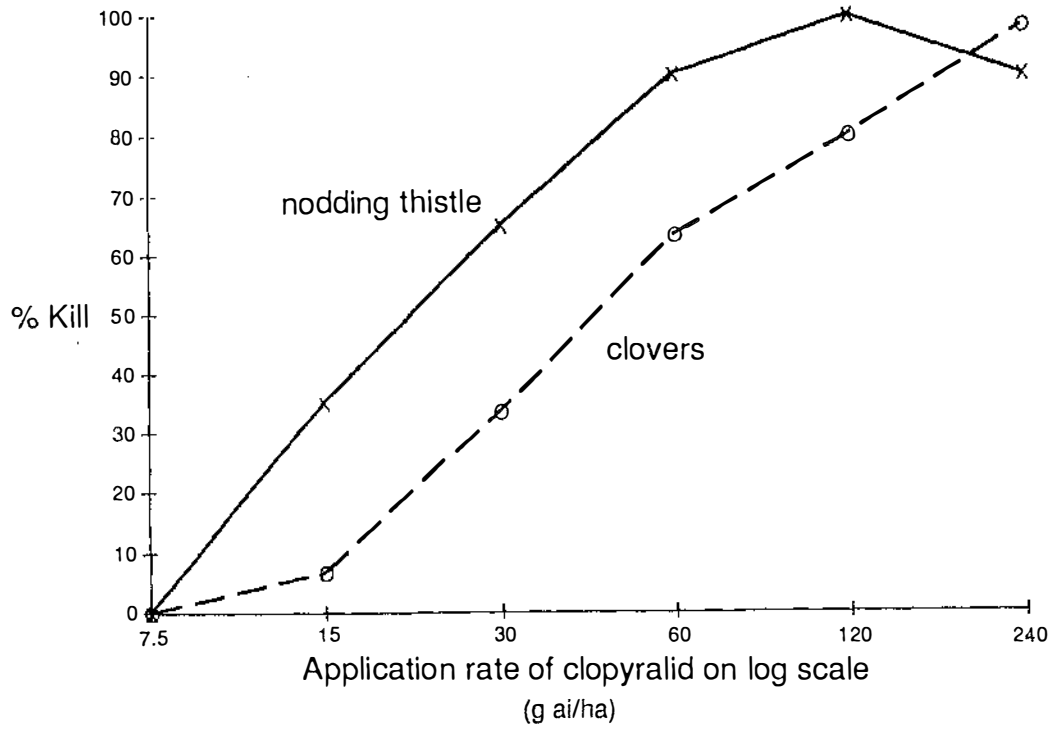


Fig 5.10: The effect of clopyralid on nodding thistle and clovers at Argyll applied by Dow-Elanco staff in June 1989 (B. Harris, unpublished data).

Another possibility is to wipe translocated herbicides such as glyphosate, picloram or clopyralid on to phenoxy-resistant nodding thistles once they have bolted. This can be achieved using rope-wick applicators (Thompson 1983) or rotary weed wipers (Martin *et al* 1990). The main drawback with this method is that thistles have already been interfering with pasture growth and livestock grazing for many months before control is possible. Infested paddocks would also need treating several times each year as plants do not all bolt at the same time.

Thus resistance to phenoxy herbicides by nodding thistle makes satisfactory selective control of this species in legume-based pastures almost impossible using presently available chemicals.

5.5.2 Mechanism of Resistance

The results obtained from the series of experiments outlined in this chapter gave some clues about the mechanism of resistance. If resistance was caused by poor cuticular penetration, cross-resistance would have been expected to herbicides with different modes of action. However only herbicides very similar in structure and action to

MCPA were tolerated. Problems with cuticular penetration should also have been overcome at least partially by ester formulations of herbicides, yet the Argyll plants appeared as resistant to the ester formulations of 2,4-D and MCPB as they did to the salt formulations of MCPA and MCPB.

As significant levels of resistance only occurred for MCPA, MCPB and 2,4-D, the mechanism of resistance probably involves a deactivation or immobilization process specific to the molecular structure these three chemicals have in common.

CHAPTER 6: OTHER SITES WITH PHENOXY-RESISTANT NODDING THISTLE

6.1 INTRODUCTION

As resistance to phenoxy herbicides had developed in nodding thistle at Argyll, it had probably developed also in other parts of New Zealand. Trials were conducted to establish whether other sites were affected, though it was considered outside the scope of this project to estimate the overall proportion of nodding thistle populations with resistance.

In addition to showing herbicide resistance in nodding thistle at sites other than Argyll, identification of other resistant sites would be useful in determining how much selection pressure was required for resistance to develop. Such information could aid prediction of future problems with herbicide resistance and assist in formulation of strategies to prevent further resistance from developing.

Resistant and susceptible populations of nodding thistle appeared morphologically indistinguishable. Therefore populations could only be distinguished as herbicide resistant by applying herbicide to plants grown in a standard environment. Glasshouse trials differentiated between resistant and susceptible populations despite problems with defining the magnitude of resistance. In the trials described in Chapter 3, 3.0 mg ai MCPA / 5 ml of spray solution consistently allowed good differentiation between Argyll and Ohiti plants (Figs 3.1, 3.2 and 3.3). Although production of dose response curves for each population screened would have better quantified the extent of resistance within the populations, a single application rate was used thus reducing plant numbers required from each population and allowing more populations to be tested.

6.2 FIRST POPULATION SCREENING TRIAL

6.2.1 Introduction

The first trial in which several populations were screened for resistance to MCPA was conducted mainly to test whether application of a single concentration could adequately differentiate between resistant and susceptible populations. Stocks of seed from the standard phenoxy-susceptible population (Ohiti) were almost depleted and the remaining seed was losing viability because of age. Therefore a secondary objective

was to identify other populations from which seed had been collected more recently that were similar to the Ohiti population in herbicide susceptibility and thus could be used in future trials.

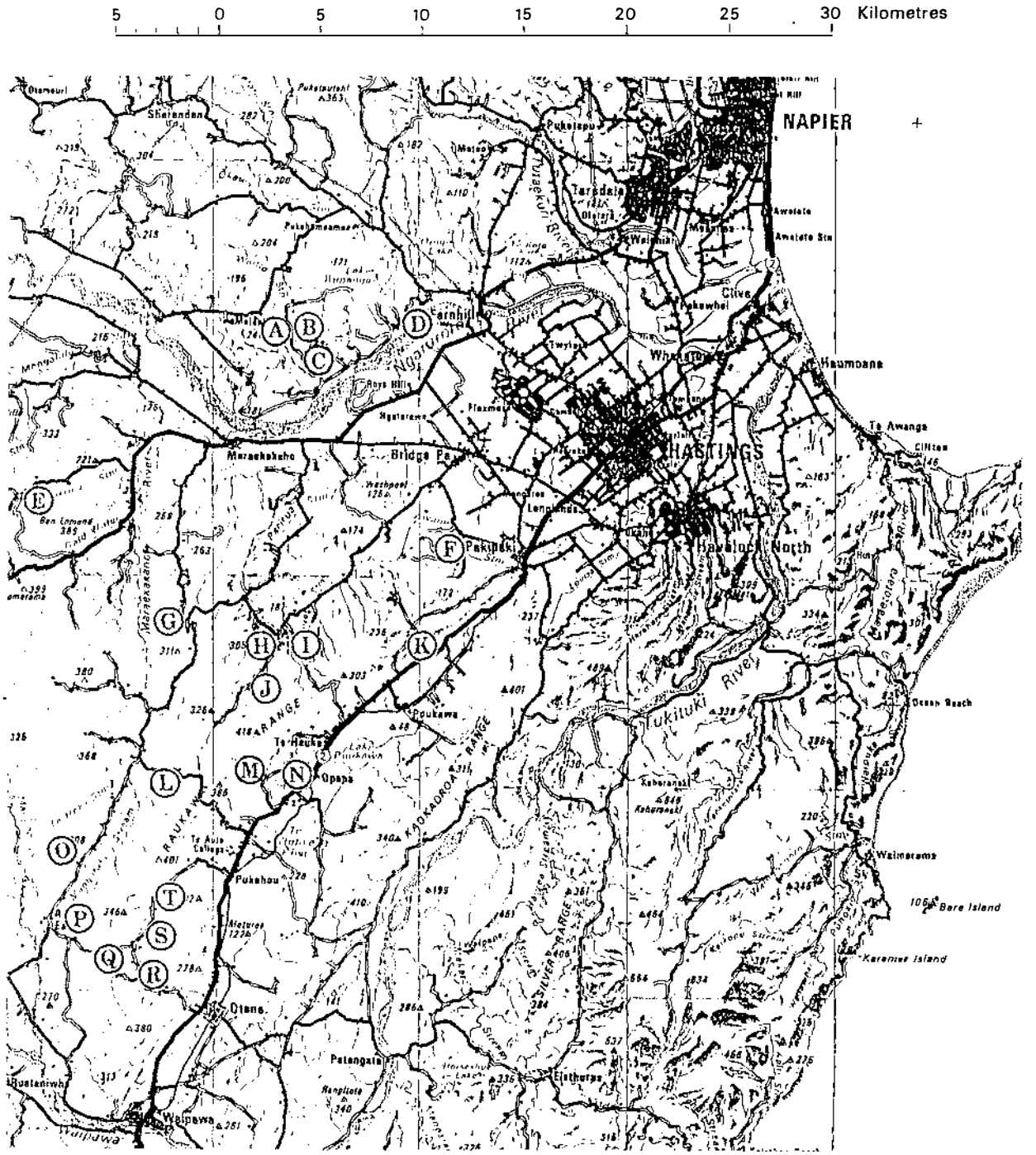
6.2.2 Methods and Materials

Seed from seven nodding thistle populations were placed in petri dishes on 8 January 1988 to begin germination. Two of the populations were Argyll and Ohiti, included to relate results to past experiments. Two populations were taken from the Maraekakaho (Site 3) and Colyton sites investigated in earlier work (Section 2.4). The origins of the other three populations are shown as Poukawa, Opapa and Hickey Road in Fig 6.1. Seedlings were planted 12-13 days later into 230 ml plastic pots containing equal proportions of peat, sand and pumice, and also slow-release fertilizer and etridiazole. The plants, grown in a glasshouse whose temperature range was between 18.4 $\pm$ 1.5^{o}C (standard deviation) and 23.0 $\pm$ 2.5^{o}C, received overhead irrigation.

The number of plants treated from each population varied from 9 to 34, with low numbers for some populations due to poor germination, natural seedling mortality and discard of plants significantly smaller and less thrifty than the rest. MCPA was applied at 3.0 mg ai / 5 ml on 19 February 1988 when temperature and relative humidity were 22^{o}C and 58% respectively. Average (with range) interception of the 5 ml of spray solution for 55 plants weighed immediately before and after spraying was 1.2 (0.8 - 1.6) ml per plant. Eight plants harvested on the day of treatment to characterize plant size had average (with range) shoot and root masses of 238 (17 - 560) mg and 58 (3 - 140) mg respectively. Plant survival was assessed four months later, and differences between populations were compared using chi-square analysis with Yates correction for continuity compensating for the low degrees of freedom (Little and Hills 1978).

6.2.3 Results and Discussion

Five of the populations were as severely affected by the MCPA as the Ohiti population ($p = 0.05$), and only the Argyll population showed a significant degree of resistance (Fig 6.2). Thus the technique successfully differentiated between resistant and susceptible populations, though no new resistant sites had been identified. However several new sources of seed had been confirmed as suitable replacements for the Ohiti population in future experiments.



KEY TO LOCATION SYMBOLS:

A Matapiro
B Tauhara
C Ohiti
D Runanga
E Maraekakaho
F Awanui
G Mason Ridge
H Raukawa
I Glenalvon
J Rotoma
K Poukawa
L Te Onepu
M Waikareao
N Opapa
O Mananui
P Kia Ora
Q Argyll
R Hickey Rd
S Ohutu
T College Rd

Fig 6.1: Location of sites in Hawkes Bay from which nodding thistle populations were tested for resistance to MCPA.

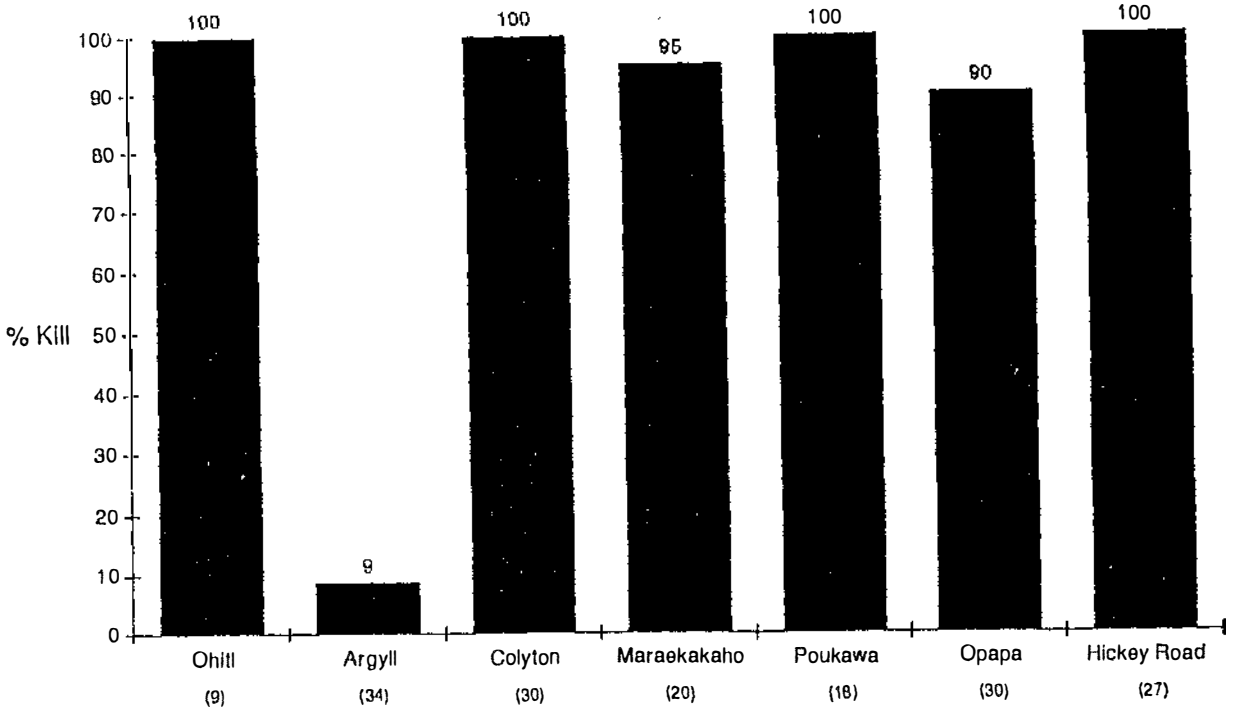
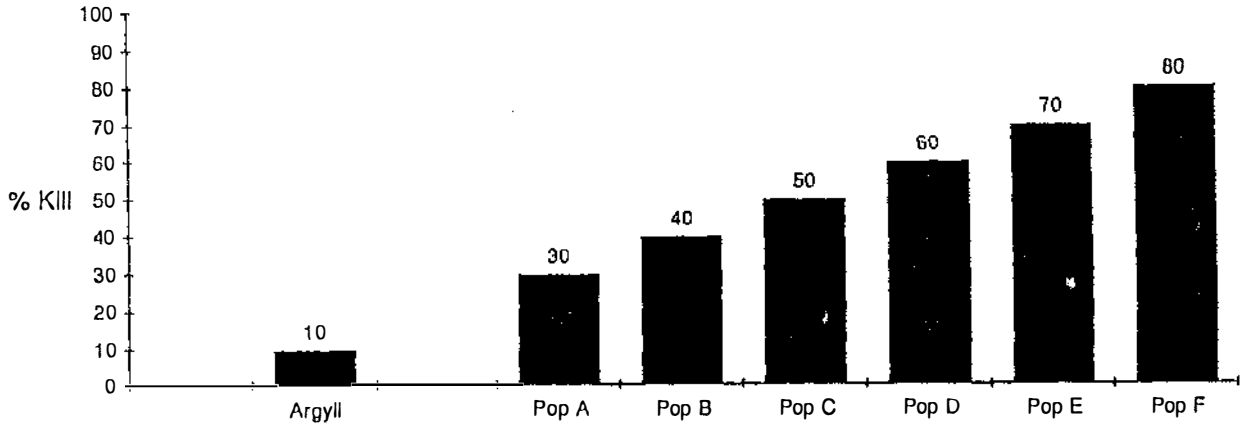


Fig 6.2: The percentage of plants killed by 3.0 mg ai MCPA / 5 ml for seven nodding thistle populations grown and treated in a glasshouse in February 1988. The total number of plants treated is shown in brackets for each population.

The Colyton and Maraekakaho populations were from the two sites showing most tolerance of MCPA in an earlier field trial (Fig 2.2) but no significant tolerance was shown here. This result confirmed a previous glasshouse experiment with the Colyton population (Fig 3.1), and suggested that tolerance detected in the field trial resulted from environmental rather than genetic factors.

The other interesting result from this experiment was the susceptibility of the Hickey Road nodding thistle population which was located only 2 km from the Argyll site. This indicated that herbicide resistance can be very localised.

The adjusted chi-square analysis showed the Matapiro population to be significantly different from the Argyll population despite only nine Ohiti plants being treated. This suggested only small numbers of plants are needed for each population if differences between resistant and susceptible populations are as marked as in Fig 6.2. Caution is required when using adjusted chi-square analysis with small numbers of plants, but only because estimates of whether differences are significant become conservative (Steel and Torrie 1981). Fig 6.3 shows the magnitude of differences in population mortality required to be statistically significant when 10, 20 or 30 plants are treated per



If 10 plants / population:	NS	NS	NS	NS	*	**
If 20 plants / population:	NS	NS	*	**	**	**
If 30 plants / population:	NS	*	**	**	**	**

Fig 6.3: The statistical significance of differences in mortality between Argyll (if mortality is 10%) and six hypothetical populations if 10, 20 or 30 plants were tested per population.

NS = population not significantly different to Argyll population

* = population significantly different to Argyll population at p = 0.05

** = population significantly different to Argyll population at p = 0.01

population. Treating only 10 plants per population should distinguish adequately between resistant and susceptible populations, though differences between populations with intermediate levels of tolerance would require greater replication.

6.3 SECOND POPULATION SCREENING TRIAL

6.3.1 Methods and Materials

Chemical company staff throughout New Zealand were asked to locate farms where problems had been experienced in obtaining satisfactory control of nodding thistle with MCPA or 2,4-D. No problem areas were reported in the South Island, but many properties in Hawkes Bay and Waikato were listed. These sites were visited while nodding thistle was flowering in January 1988 and seed was collected from the flower heads of randomly selected plants (Plate 6.1). Seed was also taken from some sites where nodding thistle was described as easily controlled so that their spraying histories could be compared with those for resistant sites.

Plate 6.1: The paddock from which seed of the Raukawa population was collected in January 1988. It had been sprayed with 2,4-D while the plants were vegetative, so all plants visible had survived that application.

Plate 6.2: The plants from the Waotu and Matapiro populations 4 months after application of MCPA.

Nodding thistle seed from 16 Hawkes Bay sites and seven Waikato sites were placed in petri dishes with 0.2% solution of potassium nitrate on 9 May 1988 to begin germination. The delay of 4 months between collection and germination of seed was to allow for the innate dormancy described by Popay *et al* (1987); potassium nitrate was used to increase germination (Medd and Lovett 1978a). The names of these sites are listed in Fig 6.5 and their locations are shown in Figs 6.1 and 6.4. Two populations, Argyll and Poukawa, were included from the first experiment for comparison.

Slender winged thistle (*Carduus pycnocephalus*) seed was collected from the Rotoma site in addition to nodding thistle seed as the property owner was more concerned with the poor control of the former species. This seed also began germination on 9 May 1988.

The healthiest seedlings were planted into individual 230 ml plastic pots as described in Section 6.2.2 and grown in a glasshouse ranging in temperature daily from 15.3 $\pm$ 1.3^{o}C (standard deviation) to 18.4 $\pm$ 1.2^{o}C. Overhead watering was used initially until seedlings were established, then plants were placed into trays and received sub-irrigation.

Thirty plants from each population were sprayed individually with 3.0 mg ai MCPA / 5 ml on 15-17 August 1988 with the temperature and relative humidity ranging between 17-19^{o}C and 63-85% respectively. Other plants were harvested and measured to characterize the size of treated plants (Table 6.1). Plant mortality was assessed four months later and differences between populations compared using an adjusted chi-square analysis.

6.3.2 Results and Discussion

Fourteen of the sites had nodding thistle populations which were significantly ($p = 0.05$) more tolerant of MCPA than the most susceptible populations (Fig 6.5 and Plate 6.2). Mortality for these 14 populations varied from 0 to 73%, and some of these differences in mortality were also statistically significant. As the adjusted chi-square analysis gives conservative estimates of the significance of differences when mortality is near 0% or 100% (Steel and Torrie 1981), more of these differences may have been significant.

However caution is required in interpreting relative differences in mortality between the resistant populations. Most resistant sites had been sprayed prior to flowering, so

KEY TO LOCATION SYMBOLS:

A	Limestone Downs	E	Arohena
B	Buckland	F	Mangatutu
C	Maungatautari	G	Rangitoto
D	Waotu		

Fig 6.4: Location of sites in Waikato from which nodding thistle populations were tested for resistance to MCPA.

Table 6.1: Measurements of plants harvested on 17 August 1988 to characterize the size of treated plants. The same measurements taken for plants from the first population screening trial (Section 6.2) are included for comparison.

Measurement	1st Screening Trial	2nd Screening Trial	
	7 nodding thistle populations	23 nodding thistle populations	slender winged thistle
Age when treated	6 weeks	14 weeks	14 weeks
Number of plants harvested	8	25	10
Shoot mass (mg DW):			
- mean	238	2000	2990
- range	17 - 560	930 - 3760	1420 -4060
Root mass (mg DW):			
- mean	58	494	1120
- range	3 - 140	117 - 831	550 - 1650
Longest leaf (cm):			
- mean	7.9	17.6	26.7
- range	2.4 - 13.5	11 - 26	24 - 30
Crown diameter (mm):			
- mean	-	8.7	9.2
- range	-	7 - 10	8 - 10

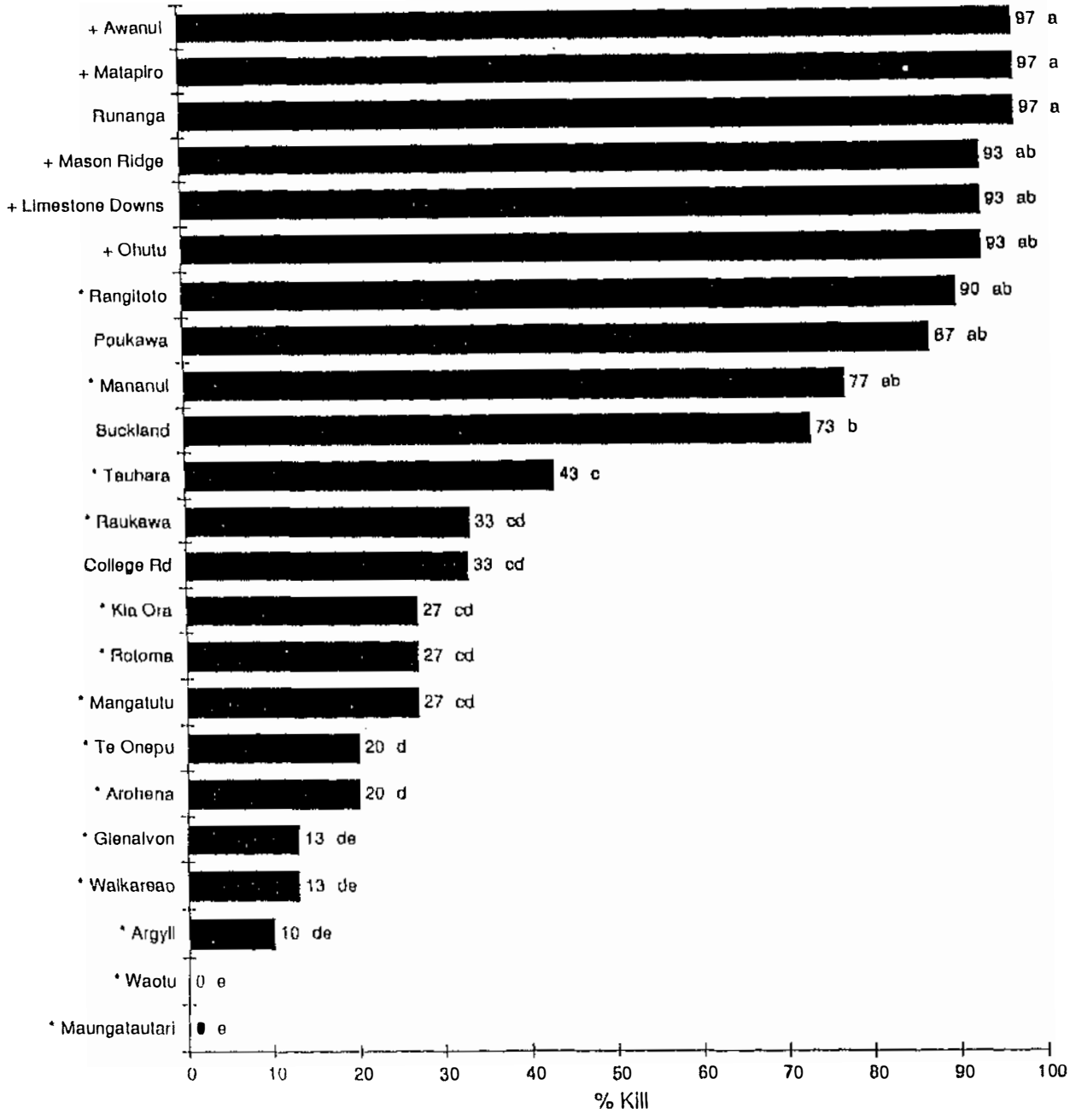


Fig 6.5: The percentage of plants killed by 3.0 mg ai MCPA / 5 ml for 23 nodding thistle populations grown and treated in a glasshouse in August 1988. Mortality levels sharing the same letter are not significantly different at p = 0.05. Problems had been experienced by property owners controlling nodding thistle at some of these sites (*), no problems had been experienced at other sites (+), and no information was available for a few sites (unmarked).

plants flowering when seed was collected would have been plants either missed by the spraying operation or resistant to the herbicide. Therefore the higher proportion of susceptible plants in glasshouse populations such as Buckland compared with Maungatautari may have resulted from greater numbers of susceptible plants missed by spraying operations being present at the former site than the latter site when seed was collected.

Seed of populations with mortality levels greater than 77% was generally collected from sites which had not been sprayed prior to seed collection. Thus some resistant plants may have been present and were outnumbered by susceptible plants. The results depicted in Fig 6.5 could therefore be interpreted as reflecting the immediate past spraying history of sites. However plant density was generally similar or higher at the resistant sites than at susceptible sites, so large numbers of plants with resistant seeds were present at sites such as Maungatautari. However if susceptible plants had been removed prior to seed collection at sites such as Matapiro, the number of resistant plants, if any, would presumably have been very low.

The populations from sites where property owners had difficulty controlling nodding thistle with phenoxy herbicides were generally also those with low mortality levels in the trial (Fig 6.5). The Mananui site had not been sprayed prior to seed collection, leaving Rangitoto as the only population where resistance had been expected but not detected. All populations from properties where good control of nodding thistle was reported had high mortality levels in the trial. Therefore trial results indicated that herbicide resistance was the main reason for the poor nodding thistle control reported in many parts of Hawkes Bay and Waikato.

Subsequent field trials in 1989 by a chemical company confirmed resistance at three of these sites. Application of 2,4-D at 10 times the recommended rate only gave 60% and 57% control of nodding thistle at Waotu and Maungatautari respectively (G. Saville, pers comm), and 65% control at Raukawa (B. Harris, pers comm).

Plants were significantly older and larger when treated in the second trial than the first trial (Table 6.1), which was probably why the susceptible populations were less affected by 3.0 mg ai / 5 ml of MCPA in the later trial. Although no plants survived this treatment for four of the six susceptible populations in the first trial, all populations in the second trial had at least one plant survive. All Poukawa plants died in the earlier trial, yet only 87% mortality was recorded in the second trial. However the relative difference between susceptible and resistant populations was readily detected in both trials despite the difference in plant size.

Only 13% of the slender winged thistle population died. Although these plants were larger than the nodding thistle plants when treated (Table 6.1) because of faster growth rates, herbicide resistance was probably the reason for this low mortality. The nodding thistle population from Rotoma was also herbicide resistant. Slender winged thistle is claimed to be more tolerant of phenoxy herbicides than winged thistle (*Carduus tenuiflorus*) (Taylor 1977), so this population needs comparing with other slender winged thistle populations to ensure the resistance measured is not typical for the species. However field trials by a chemical company in 1989 indicated that Rotoma slender winged thistle required eight times more 2,4-D for adequate control than the recommended rate (B. Harris, pers comm).

6.4 SPRAYING HISTORIES

6.4.1 Introduction

Information on past herbicide application practices at sites where resistant weed biotypes are located is useful for deciding whether that resistance has evolved due to selection pressure from herbicide use, and also in determining how much pressure is required to develop resistant biotypes. Historical data is scant for most cases of phenoxy resistance discussed in Section 4.3.2. Natural selection for MCPA resistance was implicated by differences in spraying history of *Matricaria perforata* biotypes studied by Ellis and Kay (1975). Roadside populations of *Daucus carota* resistant to 2,4-D had been sprayed with 2,4-D repeatedly for at least five seasons (Bandeen *et al* 1982). *Cirsium arvense* clones tolerant of MCPA in Sweden were more common in areas frequently sprayed with MCPA than in areas where herbicides were never used (Gressel *et al* 1982). The *Ranunculus acris* populations most resistant to MCPA in trials conducted by Bourdot *et al* (1990) had been sprayed more regularly than the susceptible populations.

Differences in spraying history between resistant and susceptible nodding thistle sites were investigated once plant susceptibility had been determined for these sites.

6.4.2 Methods and Materials

Current and past managers of farms from which seven resistant and seven susceptible populations had been obtained were interviewed either in person or by telephone. Information was obtained about past spraying practices for the paddocks from which seed had been collected. As none of the farmers had kept detailed records, accuracy of

the information gleaned depended on their memories. Most properties had been managed by several people since treatment of nodding thistle with phenoxy herbicides had begun, so spraying histories for properties were not complete where past managers could not be located or had died. All 14 properties were sheep farms located on rolling hill country. Time intervals discussed below relate back from the time seed was collected for testing.

6.4.3 Results

6.4.3.1 **Resistant Sites**

(i) *Maungatautari:* The present owner had lived on this farm since birth and managed it for the past 11 years. Nodding thistle had arrived on the property 15 years ago from a neighbouring property, and it had been sprayed every year since with 2,4-D. Despite this treatment, nodding thistle had spread across the entire property. Approximately 5 years prior to seed collection, poor control with 2,4-D led to use of a clopyralid / MCPB mixture. Good thistle control was obtained for 3 years, but control levels were lower in the subsequent 2 years.

(ii) *Waotu:* The owner had begun developing the property in 1953, and nodding thistle established about 20 years prior to our seed collection. The thistle population had been sprayed for the past 15 years, initially with MCPA but then with a clopyralid / MCPB mixture when MCPA no longer gave satisfactory control 10 years after spraying commenced. In 1988, the highest recommended rate of clopyralid / MCPB (28 g + 1 kg ai/ha respectively) was claimed to control only young nodding thistle plants, and a 2,4-D / picloram mixture was necessary to kill older plants.

(iii) *Argyll:* Nodding thistle had been well established on this Hawkes Bay property since at least the mid 1940s. The manager from 1964 to 1969 sprayed annually with 2,4-D and claims that plants were easily controlled. The present owner has managed the property since then, and he has continued with annual applications of 2,4-D. A reduction in the effectiveness of the control programme was noticed in the late 1970s, and this resulted in the trial by the Ministry of Agriculture and Fisheries outlined in Section 1.2.4.

(iv) *Glenalvon:* The owner has been on this property since nodding thistle first established in the late 1950s. Annual applications of 2,4-D have been made since the weed established, but susceptibility to 2,4-D had been poor for 5 years prior to our seed collection. Clopyralid had been added to 2,4-D in recent years to improve its

effectiveness.

(v) *Te Onepu:* The owner has lived on this property since birth and claims low densities of nodding thistle have been present for several decades. Densities began increasing 20 years ago, so herbicide applications were commenced. MCPA has been applied at least annually since then, and often applications have been made both in autumn and spring. Poor control began occurring 6 years ago, and a clopyralid / MCPB mixture is now used to improve control.

(vi) *Arohena:* This property was initially developed in 1951. Nodding thistle increased in density due to no herbicides being used, and there were high densities when the present manager arrived in 1971. The property was sprayed annually with 2,4-D from 1971, and control of nodding thistle began to decline after 9 years. A clopyralid / MCPB mixture was used from 1980, and metsulfuron is now used for spot-spraying despite damage to clovers and no registration for use for thistle control in pastures.

(vii) *Kia Ora:* MCPA or 2,4-D had been applied every year to this site since 1972, and often two applications were made each year. A previous manager claimed applications had been made annually since the late 1950s, although he had only worked there from 1972. The present owner noticed effectiveness of herbicides decreasing on nodding thistle from 5 years prior to our seed collection.

6.4.3.2 **Susceptible Sites**

(i) *Hickey Road:* Nodding thistle was sprayed with 2,4-D annually from 1970 to 1980, then only every 2-3 years for the 8 years prior to seed collection as the manager lost interest in controlling them. A neighbour commented that spraying usually occurred once flowering had begun in November so plants were usually poorly controlled. No information could be obtained on control activities prior to 1970.

(ii) *Colyton:* Annual applications of 2,4-D were made to nodding thistle from approximately 1965 to 1978, then treatment only occurred every 2 or 3 years prior to seed collection in 1988 due to a change in management and the inaccessibility of the site.

(iii) *Matapiro:* Nodding thistle had grown profusely at this site since at least the 1950s, and 2,4-D was sprayed once or twice a year from the late 1950s until 1982. The present manager reduced herbicide application considerably from 1982, and now

only sprays particularly dense patches. He did not consider there were any problems with thistle susceptibility to 2,4-D now or in 1982, and he reduced herbicide application because spraying did not appear to reduce the number of seedlings which germinated in subsequent seasons.

(iv) *Maraekakaho:* Nodding thistle arrived on the property in the late 1940s, and hand-hoeing or spot-spraying was used initially to control it. Boom-spraying with MCPA began in the late 1950s and continued annually until the early 1970s. Although plants were still susceptible to MCPA, germination of new seedlings was not decreasing despite the constant spraying. Therefore herbicide application was decreased for the 15 years prior to our seed collection, with thistle infestations only being sprayed when particularly dense.

(v) *Ohutu:* This property was part of the same sheep station as the Argyll site until 19 years ago, and it had similar nodding thistle problems and spraying regimes in the past. Annual applications of 2,4-D continued until 1976 when the present manager arrived. The collection site had only been sprayed about three times since 1976 when nodding thistle densities were high.

(vi) *Limestone Downs:* Nodding thistle had been sprayed with 2,4-D for at least 10 years prior to seed collection, but only dense patches were treated and only in years when plant numbers were high.

(vii) *Mason Ridge:* In the 1960s and 1970s, nodding thistle was mainly controlled by mowing bolted plants, though 2,4-D was occasionally applied to denser infestations. MCPA or MCPB had been used for the 10 years prior to seed collection, but still only for dense infestations.

6.4.4 Discussion

Despite the inaccuracy of using farmers' recollections of herbicide application details, some generalizations could be drawn from the information collected. All resistant sites had been sprayed annually for many years with phenoxy herbicides until the time seed was collected. All susceptible sites had been sprayed less constantly in the 10 years preceding seed collection, although some of these sites had received many years of annual herbicide applications in the past. Thus herbicide resistance was related to spraying history.

Gressel and Segel (1982) claimed that resistance was less likely to occur with

herbicides such as 2,4-D because of the low "effective kill" achieved compared with persistent herbicides like simazine. Although plants present when 2,4-D is applied may die, new plants can germinate immediately after application due to lack of residual activity. Gressel and Segel were considering mainly annual species capable of rapidly completing their life cycle when discussing this "effective kill" concept, and these plants could then produce seed regardless of whether they were resistant or susceptible to 2,4-D. Nodding thistle does not set seed as rapidly as many other weed species, and annual applications of 2,4-D could theoretically prevent plants ever seeding. However, nodding thistle can occasionally produce seed less than one year after germination (Popay and Thompson 1979). Also plants which germinate many months prior to an application of herbicide may be large enough to tolerate the herbicide (Popay *et al* 1989). Herbicide application in hilly terrain often involves aerial spraying, and some plants may be missed each year. A low effective kill of nodding thistle probably occurred on most of the surveyed farms. Evidence of this included the spread of new infestations of nodding thistle at some sites and the persistence of established populations at high densities at all sites despite many years of annual herbicide applications and prior to resistance of the herbicide becoming evident.

Despite this apparent low effective kill, the proportion of resistant individuals in nodding thistle populations had increased to a noticeable level on many properties where herbicide had been applied annually. At the Maungatautari, Waotu and Arohena sites, only 10 years of herbicide application appeared necessary for resistance to become apparent, though longer periods of time seemed necessary for the Hawkes Bay sites. The anecdotal nature of information collected prevents detailed comparison of the sites being meaningful. However a factor that could have been important in the speed of herbicide resistance development was the proportion of resistant individuals in the population prior to selection pressure being applied (Gressel and Segel 1982). The Waotu and Maungatautari sites had apparently only been invaded by nodding thistle since the late 1960s. As phenoxy herbicides were commonly used throughout the 1960s, the seeds which initially established populations at these sites probably originated from other populations which had been subjected to these herbicides. Thus the proportion of resistant individuals may have been higher in the small populations which initially colonised the Waotu and Maungatautari sites than in the original populations, so less years of selection pressure would be required to allow resistance to become noticeable. In contrast, many of the Hawkes Bay sites such as Argyll and Matapiro had nodding thistle populations which were well established prior to the 1950s when phenoxy herbicides first became common (Featherstone 1957).

Another reason the three Waikato sites apparently developed herbicide resistant

populations more rapidly than Hawkes Bay sites could relate to climate differences. The Hawkes Bay sites have average annual rainfall figures below 800 mm, compared with figures above 1200 mm in Waikato (Wards 1976). The higher rainfall in Waikato might allow pastures to compete more aggressively with nodding thistle seedlings than in Hawkes Bay and reduce the number of susceptible plants producing seed before herbicides are applied each year. This would increase the "effective kill" and therefore also increase the rate of resistance build-up (Gressel and Segel 1982).

The infrequent herbicide application at sites such as Maraekakaho and Mason Ridge presumably applied a lower selection pressure to the nodding thistle populations. The proportion of resistant individuals in the population would therefore have been increasing less rapidly than at sites with annual herbicide applications and perhaps resistance may never become evident. However sites such as Matapiro and Ohutu apparently had high selection pressures for many years prior to the 1980s, so the proportion of resistant individuals in these populations may be quite high, though below the 30% level discussed by Gressel and Segel (1982) necessary to be noticed by farmers. Once the selection pressure was reduced in the 1980s, the proportion of resistant individuals may have begun decreasing again if Gressel and Segels' hypothesis is correct concerning resistant individuals being less "fit" than susceptible individuals. This fitness hypothesis is discussed further in Chapter 7.

The Argyll and Matapiro sites apparently had similar spraying histories prior to the early 1980s, yet the trial discussed in Section 1.2.4 showed Argyll nodding thistle plants were significantly more resistant of MCPA and 2,4-D than Matapiro plants in 1981. Detailed monitoring over many years would have been necessary to explain this difference in rate of resistance build-up. The Argyll spraying programme may have been more successful than the Matapiro programme in preventing seed production from susceptible nodding thistle individuals due to better timing of application, more frequent spraying, better application techniques or use of higher application rates. The initial proportion of resistant individuals in the Argyll population may have also been higher than at Matapiro.

Although Gressel and Segel (1982) originally postulated that resistance was less likely to evolve in weeds sprayed with non-persistent foliar-applied herbicides, several examples of such resistance have since been published apart from the phenoxy resistance within *Carduus nutans* and *Ranunculus acris* in New Zealand. Examples are the resistance of *Lolium rigidum* to aryloxyphenoxypropanoate and cyclohexanedione herbicides in Australia (Heap 1991) and resistance of several weed species to paraquat in Japan (Matsunaka and Itoh 1991).

All sites from which resistant nodding thistle populations were located had other thistle species present. These included Scotch thistle (*Cirsium vulgare*), slender winged thistle, winged thistle (*Carduus tenuiflorus*), Californian thistle (*Cirsium arvense*) and variegated thistle (*Silybum marianum*). However nodding thistle was the only species which farmers had noticed becoming more difficult to control, except for the slender winged thistle population at Rotoma. Resistance may have developed in nodding thistle and not other species because of its noxious status. Noxious plants officers have compelled farmers to control nodding thistle since the 1950s (Neill 1952), whereas variegated thistle is the only other species listed above with a noxious status. The aggressive growth of nodding thistle has also encouraged farmers to spray this weed more vigilantly than other species. Although surveyed farmers had not noticed resistance developing in variegated thistle, several suspected cases of phenoxy resistance in this species were reported to Massey University from other parts of New Zealand during the project. This biennial thistle is also sprayed vigilantly in most areas.

Farmers from most of the seven resistant sites investigated in detail claimed nodding thistle could still be controlled on their properties if 2,4-D was applied while plants were very young, especially if twice the recommended application rate was used. Resistance apparently caused problems once plants were well established and older, as plants became much more resistant with age than the normal increase in tolerance discussed by Popay *et al* (1989). One possible explanation for this observation is that the relative difference between resistant and susceptible biotypes increases with age. Another possiblity is that the relative difference remains the same but very young plants are extremely sensitive to phenoxy herbicides. Therefore the recommended application rate may be five times higher than is required to kill very young "susceptible" plants and it is just high enough to kill very young "resistant" plants. Results summarised in Table 4.1 give some support to both of these possibilities. Although the resistant nodding thistle biotype apparently can be controlled with 2,4-D when very young, resistance still creates major control problems on these properties. Nodding thistle can germinate over many months during the year (Popay *et al* 1979), so many herbicide applications would be necessary each year to treat all plants within a few weeks of germination.

6.5 CONCLUSIONS

Nodding thistle populations with significant levels of resistance to phenoxy herbicides have been located at 14 sites in Hawkes Bay and Waikato. All populations studied in detail have been sprayed by phenoxy herbicides in the past, but the resistant

populations had been sprayed more regularly than susceptible populations in the 10 years prior to seed collection. Thus selection pressure from herbicide use, mainly 2,4-D, appeared responsible for development of resistance in these nodding thistle populations.

CHAPTER 7: FITNESS OF RESISTANT NODDING THISTLE

7.1 INTRODUCTION

A decrease in fitness has been measured in pesticide-resistant biotypes of bacteria, fungi, insects and rats when pesticides are absent (Gressel and Segel 1982). Conard and Radosevich (1979) have also measured reduced fitness in groundsel (*Senecio vulgaris* L.) and redroot (*Amaranthus retroflexus* L.) biotypes resistant to triazines. Dry matter production of susceptible biotypes was greater than that of susceptible biotypes under both competitive and non-competitive conditions for both species. When equal numbers of susceptible and resistant plants were grown in trays with 3 cm between each plant, dry matter production was three times greater for susceptible plants than resistant plants for both groundsel and redroot. Decreased dry matter production was probably caused by inefficiency in the photosynthetic light harvesting and electron transferring capability of resistant plants, which are processes closely linked to the mechanism of resistance (Radosevich and Holt 1982).

The development of plant biotypes resistant to heavy metals has many similarities to the development of herbicide resistance, including reduced fitness of resistant biotypes (Bradshaw 1982). In the absence of competition, the reduction in fitness of metal-resistant plants compared with normal plants in normal habitats is not great. However the fitness of resistant plants is reduced considerably when there is competition from other plants, and this reduction in fitness varies considerably between species. Zinc-resistant individuals had only 16%, 0.1% and 28% as much dry matter production as normal individuals of browntop (*Agrostis capillaris* L.), sweet vernal (*Anthoxanthum odoratum* L.) and narrow-leaved plantain (*Plantago lanceolata* L.) respectively after 1 year of competition (Hickey and McNeilly 1976). Bradshaw (1982) suggested this reduced fitness was caused by an elevated requirement in normal soils for the metal to which plants were resistant.

Although reduced fitness has been measured in plants resistant to triazines and heavy metals, a New Zealand trial found giant buttercup plants resistant to MCPA were as competitive as susceptible plants (Bourdot *et al* 1990). However reduced fitness may involve factors other than the growth rates measured by Bourdot *et al.* Gressel and Segel (1982) postulated that reduced fitness could occur at any of a number of stages in the life cycle because of the following factors: (a) the proportion of seeds germinating at a given time; (b) the rate of germination; (c) success in establishment following self-thinning; (d) any of the physiological characters resulting in differences in growth rate; (e) plasticity; and (f) the seed size and yield per flower and per plant.

A full investigation of all these factors for phenoxy-resistant nodding thistle was considered beyond the scope of this project. However a greater susceptibility of herbicide-resistant nodding thistle plants to competition by pasture species, if it existed, would be useful for the control of these plants. A competition trial was conducted with nodding thistle to determine whether sufficient competitive differences exist between resistant and susceptible plants of this species to allow more effective use of the pasture management techniques discussed in Section 1.2.3.1 once resistance occurs.

7.2 METHODS AND MATERIALS

Seed from the Argyll (resistant) and Matapiro (susceptible) sites discussed in Chapter 6 were placed into petri dishes to germinate on 14 November 1989. Seedlings of uniform size were then transplanted into trays 42 cm x 30 cm x 7 cm deep filled with 50% pumice, 25% sand and 25% peat which was treated with etridiazole but contained no fertilizer. Three trays were planted with 35 plants per tray with plants equidistant from each other and with Argyll plants alternating within and across rows with Matapiro plants. A further five trays were planted each with two Argyll plants and two Matapiro plants, equivalent to 2200 ml of potting mixture per plant, compared with 250 ml / plant at the higher density. The trays, placed in a glasshouse with the daily temperature ranging from $18.7 \pm 0.2^{o}C$ to $26.2 \pm 0.3^{o}C$, were watered twice daily by an overhead sprinkler.

Ten weeks later, the shoot material of each plant was removed at ground level, dried at $80^{o}C$ and weighed. A t-test was used to compare the high density with low density plants. Separate F-tests (randomised complete block with sub-samples) were used for the low density and high density plants to compare the susceptible and resistant biotype. Allowance was made with the high density plants using covariance adjustment for plants growing at the edge of each tray which received less competition than plants in the middle of the tray.

7.3 RESULTS AND DISCUSSION

Trials investigating competitive differences between herbicide-resistant and susceptible biotypes by Conard and Radosevich (1979), and Bourdot *et al* (1990), used the interplanting replacement series technique discussed by Harper (1977). Although growing two biotypes together at a range of different ratios may give more information

about competitive interactions between the biotypes than using only a 1:1 ratio, the primary objective was to determine whether one biotype was more competitive than the other. Comparison of dry matter production by the two biotypes when grown under the same conditions of competitive stress was considered a satisfactory indication of relative competitive abilities. To ensure the resistant and susceptible nodding thistle biotypes were subjected to the same level of stress, equal numbers of resistant and susceptible plants were grown together at equal spacings. To ensure plants were being exposed to competitive stress, a second series of plants were grown under identical conditions but at a lower density. If competition did not occur at the higher density, plant size would be the same at both densities. Bourdot *et al* (1990) did not show that competition had occurred when investigating the relative fitness of giant buttercup biotypes. Thus they could only conclude that either fitness differences were not detected or competition had not occurred.

As average plant size at the higher planting density was less than 20% of that at the lower planting density (Fig 7.1; Plates 7.1 and 7.2), significant levels of competition did occur at the high density. However, despite this severe competition, there was no difference between the resistant and susceptible nodding thistle populations in average plant size. The difference in size of resistant and susceptible plants at the low density was also not significant ($p = 0.05$). However there was insufficient competitive stress within the 10 week period to cause any plant death for either biotype.

Competition between plants probably occurred for nutrients rather than water or light. The twice daily irrigation kept the potting mixture moist at all times. Although plants were only 6 cm apart, the average length of the longest leaf was 3.9 cm so little shading of neighbouring plants occurred. No fertilizer was added to the potting mixture which would have contained minimal nutrients initially, and the overhead irrigation would have leached away mobile nutrients. Therefore the results showed that growth rates were the same for phenoxy-resistant and susceptible nodding thistle plants when exposed to nutrient stress.

The relative growth rates of these biotypes may have differed if water or light had been the limiting factor. The susceptibility of these two biotypes to control by pasture management techniques may also differ with respect to their seed germination or seed production. However, although this trial was relatively simple in design compared with some past fitness experiments, it would probably have detected the reduced fitness of the triazine-resistant biotypes studied by Conard and Radosevich (1979), and the zinc-resistant biotypes discussed by Hickey and McNeilly (1976). Inefficient photosynthesis in triazine-resistant biotypes would have resulted in less root production to compete for the limited nutrients available. An increased requirement

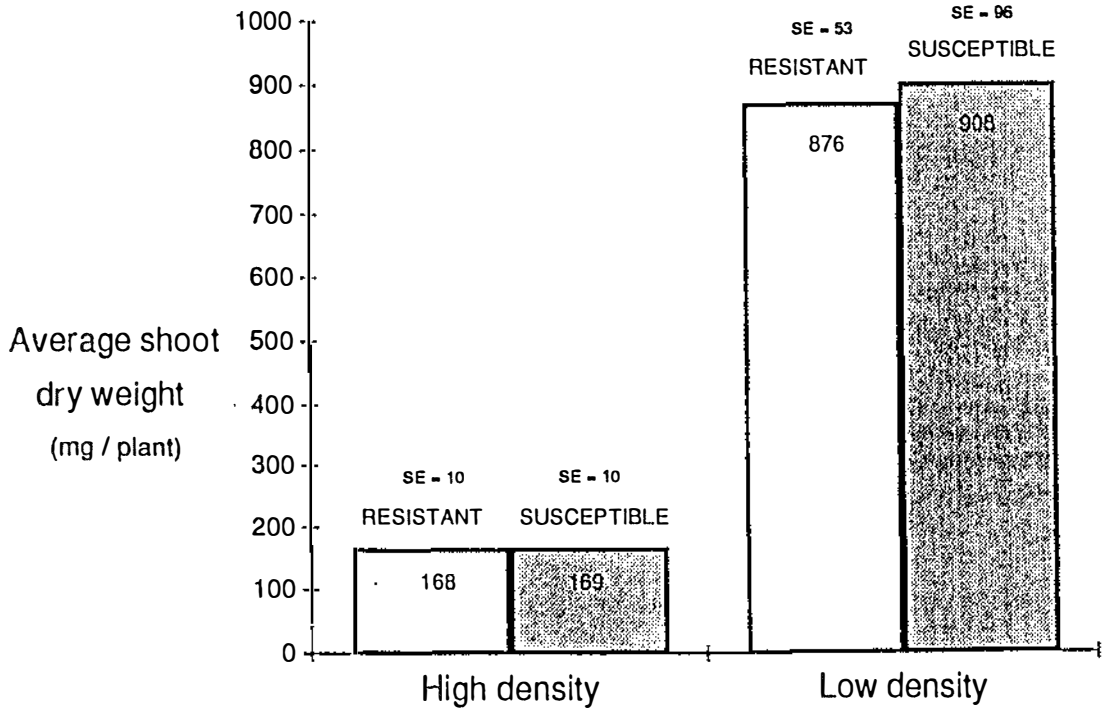


Fig 7.1 Average shoot dry weight of phenoxy-resistant (Argyll) and susceptible (Matapiro) nodding thistle plants grown at two densities for 10 weeks.

for zinc by zinc-resistant biotypes would not have been satisfied by the low nutrient supply.

The only measure of competition was shoot production following 10 weeks of growth. The root systems were too intermingled to allow separation and accurate measurement of these, though presumably significant differences in root growth between the two biotypes would have been reflected in shoot growth by 10 weeks of age. The destructive harvesting of the shoot systems prevented measurements of seed production from the competing plants. Thus the trial measured the short-term impact of competition on thistle infestations rather than the long-term potential for proportionally less seed to be produced by resistant than susceptible plants. However, if plant mortality or seed production subsequent to the harvest date had been correlated to plant size at 10 weeks of age, significant differences would not have been expected.

With most cases of pesticide resistance, significant competition exists between pests prior to pesticide application, and this competition is greatly reduced after application. For example, triazine herbicides are often used to keep ground bare in nurseries,

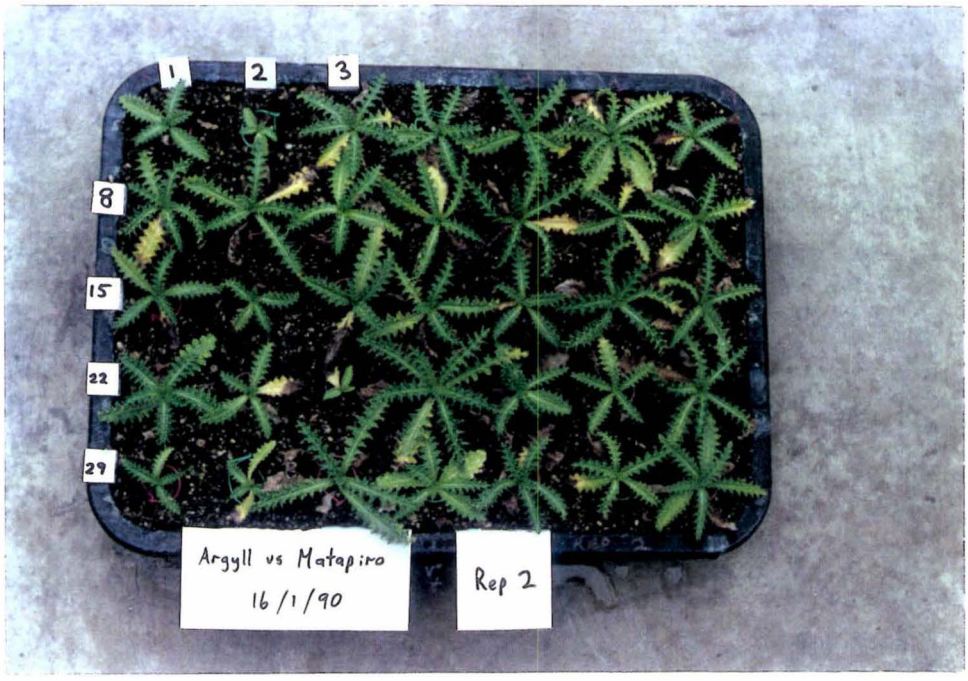


Plate 7.1: Argyll (*eg* numbers 8 and 22) and Matapiro (*eg* numbers 1, 15 and 29) plants after 2 months of growing together in a tray at a density of 35 plants per tray.

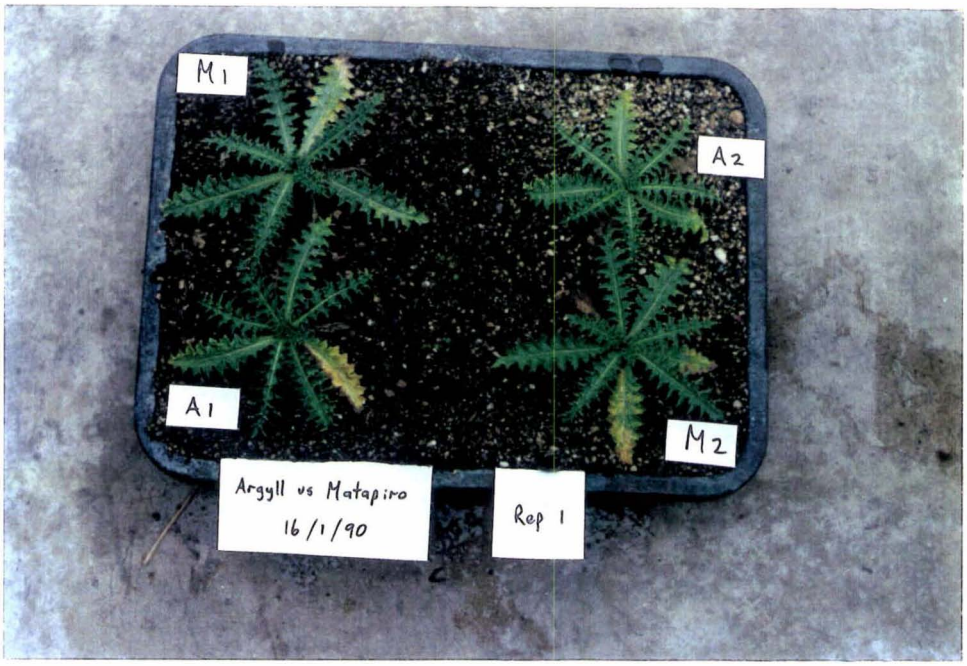


Plate 7.2: Argyll (A1 and A2) and Matapiro (M1 and M2) plants after 2 months of growing together in a tray at a density of 4 plants per tray.

orchards, waste-land and between widely spaced rows of maize. Other weeds competing with triazine-resistant plants are removed by herbicide application, allowing unrestricted growth of survivors. Although some competition may occur between nodding thistle plants at very high densities, the main form of competition for nodding thistle in New Zealand pastures is probably from pasture species, especially grasses. As grasses are unaffected by phenoxy herbicides, phenoxy-resistant nodding thistle plants will be subjected to similar levels of competition whether herbicides have been applied or not.

Nodding thistle appeared as dense and aggressive at the sites visited in 1988 at which resistance was later confirmed as at sites where plants were still susceptible to herbicides. As these resistant plants had germinated, established and flowered in reasonably competitive pastures, any disadvantage in fitness within these plants was presumably more subtle than that suffered by the triazine-resistant redroot and groundsel plants discussed earlier.

The nodding thistle fitness trial did not conclusively show resistant and susceptible biotypes to be of equal fitness for all factors listed by Gressel and Segel (1982). However no observations made during the germination of seed and establishment of populations of susceptible and resistant nodding thistle biotypes under many different conditions over the 5 year duration of this project suggested that fitness differences do exist.

7.4 CONCLUSIONS

No differences were found in the competitive ability of resistant and susceptible nodding thistle biotypes under nutrient stress. If other differences in the overall fitness of the biotypes do exist, they are probably less marked than those found by Conard and Radosevich (1979) with triazine-resistance and by Hickey and McNeilly (1976) with zinc-resistance.

CHAPTER 8: INITIAL PHYSIOLOGICAL INVESTIGATIONS

8.1 INTRODUCTION

Switching to alternative herbicides appeared not to be a feasible option for selectively controlling resistant nodding thistle populations in pastures (Chapter 5). Thus a better understanding of the resistance mechanisms was needed to help overcome the problem. Several experiments were conducted to investigate how resistant nodding thistle plants survive exposure to phenoxy herbicides.

Another objective of these initial physiological investigations was to determine how to differentiate resistant plants from susceptible plants. Morphologically there appeared to be no way of determining whether a plant was resistant until it was sprayed. As a quick identification of resistant plants would be useful for both research purposes and confirmation of resistance problems for farmers, potential differences between resistant and susceptible plants suitable for such identification were investigated.

8.2 LEAF TRICHOMES

8.2.1 Introduction

Despite growing thousands of nodding thistle plants during this project, no consistent morphological difference between herbicide-resistant and susceptible plants was observed. Comparisons of plant morphology using light and electron microscopy also failed to detect significant differences. However on many occasions plants were found to differ in leaf trichome density (Plates 8.1 and 8.2), and generally higher densities were found on plants from populations which had no herbicide resistance. Although this phenomenon did not occur consistently, it was investigated on two occasions when it was particularly noticeable, to decide whether the trait could be used to identify resistant populations. Leaf trichomes can also influence absorption of foliar-applied herbicides by increasing droplet retention and improving passage of herbicide through the cuticle via the cells at the base of trichomes (Kirkwood 1987). However a high trichome density can also decrease herbicide efficacy by preventing herbicide droplets from reaching the leaf surface. Thus if significant differences between ecotypes in trichome density existed, the mechanism of resistance might be linked.

Plate 8.1: A nodding thistle leaf with few trichomes.

Plate 8.2: A nodding thistle leaf with a high trichome density.

8.2.2 1987 Field Trial

8.2.2.1 **Introduction**

The opportunity was taken to look at possible relationships between trichome density and herbicide resistance when plants from the resistant Argyll and susceptible Ohiti nodding thistle populations were transplanted into a pasture near Massey University in early 1987 for the trial described in Chapter 4. The untreated control plants were used later in the trial investigating cross-resistance to clopyralid (Chapter 5). Prior to commencement of the clopyralid trial, high densities of trichomes were noticed in some Ohiti plants while nearby Argyll plants had leaves with almost no trichomes on the upper leaf surface. The trichomes were tapered multicellular unbranched structures 1-3 mm in length and 7-10 µm in diameter at the base. All untreated Argyll and Ohiti plants were consequently scored for trichome density on their upper leaf surfaces to quantify these observations.

8.2.2.2 **Methods and Materials**

Details regarding the establishment of the Argyll and Ohiti populations were covered in Section 4.2. Plants were scored subjectively for trichome density on 1 October 1987, 6-9 months after being transplanted to the site. Only those plants which had not received MCPA in May were assessed, totalling 77 Argyll plants and 63 Ohiti plants. Scores ranging from 0 for plants with very few trichomes on the upper leaf surface to 5 when trichome density was high were assigned to each plant. The origin of each plant was unknown by the scorer to prevent biased results. Plants scoring 5 had trichome densities of approximately 350/cm^2 averaged over all leaves (trichome densities were usually higher on the youngest leaves), whereas plants scoring 0 were almost completely glabrous on the upper leaf surface though there were usually still some trichomes on the lower leaf surface. Differences between populations in allocation of scores were compared using chi-square analysis.

8.2.2.3 **Results and Discussion**

A significantly higher proportion ($p = 0.01$) of Ohiti plants had a high trichome density (score of 5) than did the Argyll plants (Fig 8.1). However nearly 30% of the Argyll plants were also assigned a score of 5. If those Argyll plants were resistant to phenoxys, this result would indicate that trichome density is an unreliable indicator of herbicide susceptibility, and would not explain why plants are resistant. However

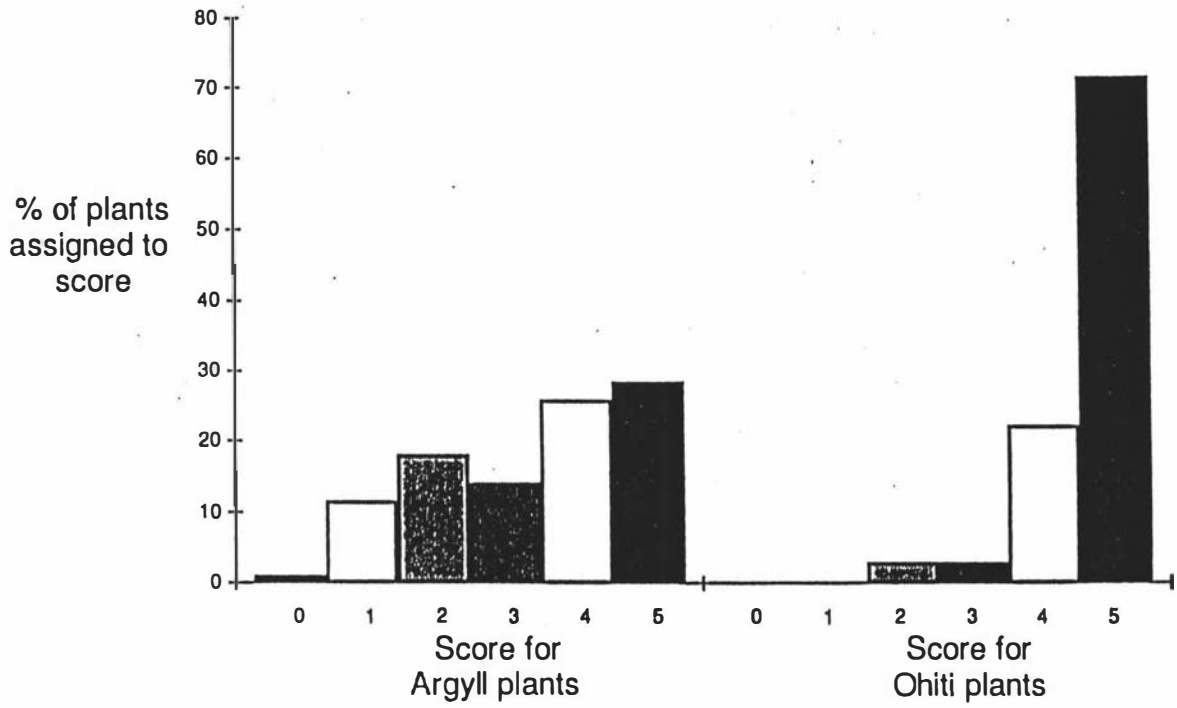


Fig 8.1: The percentage of field-grown Argyll and Ohiti plants assigned to each of the trichome density scores on 1 October 1987, where 0 = few trichomes on upper leaf surfaces and 5 = high trichome density.

another possible explanation for this result was that 30% of the Argyll population was susceptible and that some of the Ohiti plants were resistant. Results shown in Fig 4.1 offer some support for this hypothesis. For example, at the MCPA concentration of 8 mg ai / 5 ml which killed 27% of the 4-month-old Argyll plants, 8% of the 4-month-old Ohiti plants survived.

Thus the significant difference in trichome density between the two populations appeared worthy of further investigation.

8.2.3 1988 Glasshouse Trial

8.2.3.1 **Introduction**

As discussed in Section 6.3, nodding thistle plants were established under glasshouse conditions in 1988 using seed taken from a number of sites, and these plants were subsequently sprayed with a single dose of MCPA to determine which came from herbicide-resistant populations. Variability in trichome density was noticed among these plants, so they were scored for trichome density prior to spraying to investigate

further the correlation between trichome density and herbicide resistance.

8.2.3.2 **Methods and Materials**

Details regarding the establishment, growth and treatment of the nodding thistle plants are described in Section 6.3.1. Trichome density was assessed for each plant from 14 of the populations using the system described in Section 8.2.2.2 immediately prior to herbicide application on 18 August 1988 when plants were 14 weeks old. Differences in allocation of scores were compared using chi-square analysis.

8.2.3.3 **Results and Discussion**

Although the subjective nature of the scoring system made comparison between this and the earlier field trial difficult, the highest trichome density in the glasshouse trial appeared to be considerably less than in the field trial. Consequently no plants received a score of 5. However there were still significant differences ($p = 0.01$) between the populations in allocation within the other five trichome density categories (Table 8.1).

The three populations which sustained greater than 85% mortality following application of MCPA had high trichome densities, and the two populations which suffered no mortality had very low trichome densities. However many of the other resistant populations did have moderately high trichome densities, thus creating a poor correlation ($r = +0.41$) between population mortality and trichome density (Fig 8.2).

The possibility existed that a better correlation existed between trichome density and resistance to MCPA for individual plants rather than populations consisting of both hirsute and glabrous plants. The mortality data for all plants given the same trichome density score were bulked disregarding the origin of each plant, and chi-square analysis was used to determine whether plants with low trichome density scores were more resistant to MCPA. The proportion of plants killed by MCPA did not differ significantly ($p = 0.05$) between the five groups of plants with different trichome densities (Fig 8.3). Although a lower proportion of the plants with low trichome densities were killed by MCPA than plants with higher trichome densities, 25% of the plants scoring 0 died.

Table 8.1 The percentage of glasshouse-grown plants from 14 different original sites assigned to various trichome density scores on 18 August 1988, where 0 = few trichomes on upper leaf surfaces and 5 = high trichome density. The mean trichome score and percentage of plants killed by 3.0 mg ai MCPA / 5 ml (taken from Fig 6.5) for each population are also included.

Population	% kill with 3.0 mg ai MCPA / 5 ml	% plants assigned trichome density score of:			Mean trichome density score
		0 - 1	2	3-4	
Mason Ridge	93	13	33	53	2.6
Rangitoto	90	33	20	47	2.2
Poukawa	87	30	37	33	2.0
Mananui	77	37	50	13	1.8
Tauhara	43	3	50	47	2.5
Raukawa	33	20	37	43	2.3
Kia Ora	27	100	0	0	1.0
Rotoma	27	47	23	30	2.0
Te Onepu	20	30	47	23	2.0
Arohena	20	47	40	13	1.7
Glenalvon	13	30	70	0	1.7
Argyll	10	23	23	53	2.4
Waotu	0	63	23	13	1.4
Maungatautari	0	70	25	5	0.9

8.2.4 Conclusions

Although nodding thistle populations grown under the same environmental conditions did differ significantly in both trichome density and resistance to MCPA, there was no close relationship between these two variables. Therefore trichome density was not considered useful for identifying resistant plants, and was probably not involved with the mechanism of resistance to MCPA.

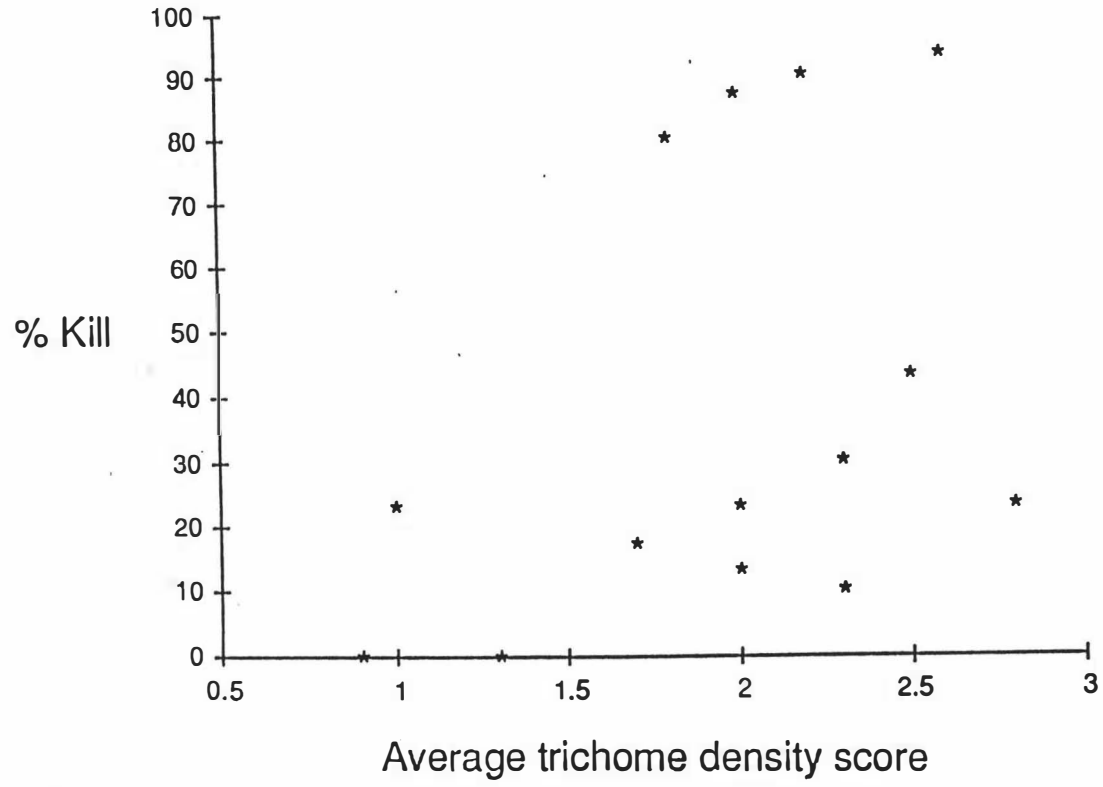


Fig 8.2: The relationship between the average foliar trichome density of 14 nodding thistle populations and the percentage of plants killed within each population when treated with 3.0 mg ai MCPA / 5 ml immediately following assessment of trichome density. THe correlation coefficient (r) for this relationship was +0.41. Scoring of trichome density ranged from 0 (almost no trichomes) to 5 (high trichome density).

8.3 CUTICLE DAMAGE AND SOIL APPLICATION

8.3.1 Introduction

One possible mechanism by which nodding thistle plants resistant to MCPA may survive higher application rates of this herbicide than normal is reduced foliar penetration. For example, resistant plants may have thicker cuticles or different wax composition compared with susceptible plants. To investigate this possibility, a glasshouse experiment was conducted in which the relative susceptibility of Argyll and Ohiti plants to MCPA was determined using both plants with intact cuticles and cuticles which were mechanically damaged immediately prior to spraying. The difference in susceptibility of Argyll and Ohiti plants should have become smaller if resistance was caused by poor cuticular penetration.

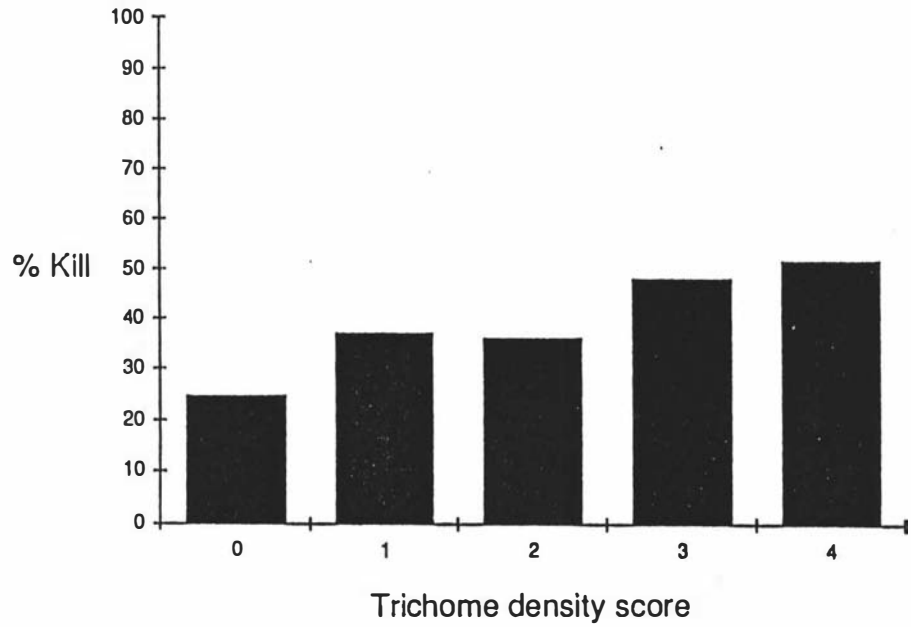


Fig 8.3: The percentage of nodding thistle plants assigned to each of the trichome density score categories which were subsequently killed by 3.0 mg ai MCPA / 5ml. There was no significant difference (p = 0.05) in mortality between the categories.

Although normally applied to the foliage of plants, MCPA and 2,4-D can enter plants via the root system (Klingman and Ashton 1982). If herbicide resistance resulted from poor foliar penetration, applying the herbicide to the soil should have overcome resistance as the herbicide would have then entered the plant via the roots, eliminating the need to penetrate the cuticle. An experiment was also conducted to test this hypothesis.

8.3.2 Methods and Materials

An experiment investigating the effect of cuticle damage and root uptake on herbicide resistance was run in conjunction with the experiment described in Section 3.4 which compared the susceptibility of Argyll and Ohiti nodding thistle populations to MCPA under glasshouse conditions. Argyll and Ohiti nodding thistle plants were established for these experiments at the same time and under the same conditions as discussed in Section 3.4.2, with germination beginning in late November 1986 and treatments with MCPA being applied on 23 February 1987. The cuticle of some plants was damaged immediately prior to foliar application of MCPA by a device consisting of five wire brushes attached to each other and weighing 1.10 kg being dropped on to each plant

from a constant height of 5.0 cm (Plates 8.3 and 8.4). MCPA was then applied as usual in 5 ml of solution using the sprayer described in Section 2.2.4. Other plants were treated by injecting 5 ml of MCPA solution into their rooting zones using a syringe with no needle. The concentrations of MCPA used and the number of plants from each population receiving each treatment are shown in Table 8.2. The corresponding information for the experiment discussed in Section 3.4 is also shown. The number of plants allocated to each treatment was influenced by the number available for the experiment and the predicted usefulness of information likely to be obtained from each treatment for calculating LD50 values based on past glasshouse experiments. Plant survival was assessed on 25 May 1987. Probit analysis of data disregarded anomalous results obtained at low herbicide concentrations for the reasons discussed in Section 3.4.3.

Table 8.2 The number of Argyll and Ohiti nodding thistle plants treated at various MCPA concentrations on 23 February 1987.

Concentration of MCPA applied (mg ai / 5 ml)	Foliar application to intact cuticles		Foliar application to damaged cuticles		Application to plant rooting zone	
	Argyll	Ohiti	Argyll	Ohiti	Argyll	Ohiti
0	10	10	20	20	10	10
0.023	0	10	0	10	0	10
0.047	0	10	0	10	0	10
0.094	0	10	10	10	10	10
0.19	10	10	10	10	10	10
0.37	10	20	10	20	10	10
0.75	10	20	10	10	10	10
1.5	10	20	10	10	10	10
3	10	10	10	10	10	10
6	10	10	10	10	10	10
12	10	10	10	10	10	10
24	10	10	10	10	10	10
48	10	10	0	10	10	10

Plate 8.3: A nodding thistle plant placed into position through a hole in the top of a wooden box ready to be scarified by the collection of wire brushes shown in the foreground.

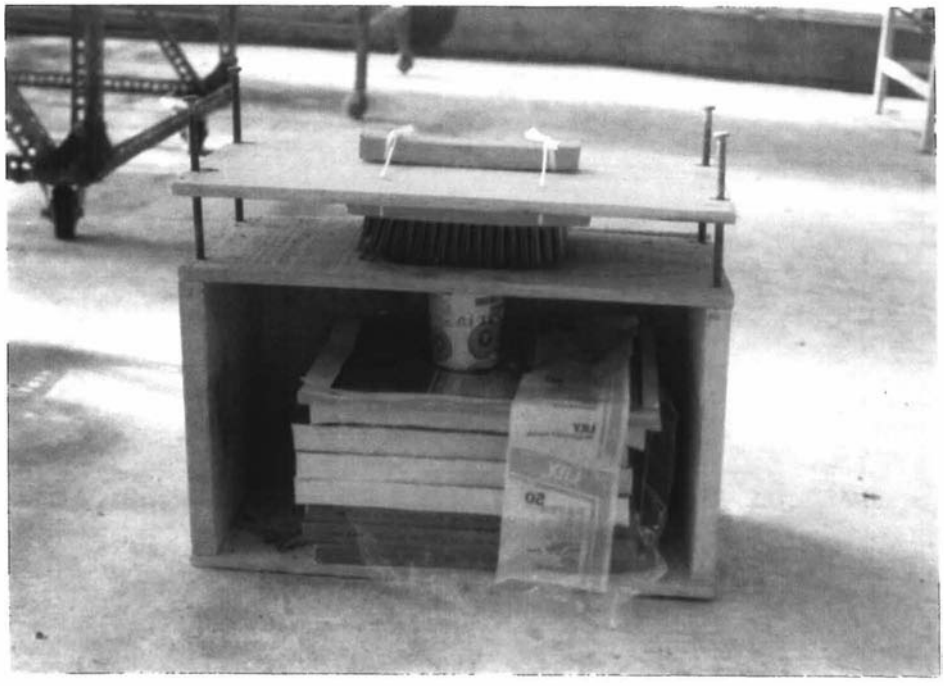


Plate 8.4: Immediately after the scarifier had been dropped 5 cm on to the nodding thistle plant.

8.3.3 Results and Discussion

8.3.3.1 **Effect of Cuticle Damage**

The unfitted dose response curve for Ohiti nodding thistle plants which sustained cuticle damage prior to MCPA application varied little from that for Ohiti plants treated with intact cuticles (Fig 8.4). In contrast, the unfitted dose response curve for Argyll plants indicated that damaging the cuticles may have reduced resistance to MCPA slightly. Although the LD50 calculated for Argyll plants damaged prior to spraying was only half that for plants sprayed with intact cuticles (Table 8.3), this difference between the two LD50 values was not significant ($p = 0.05$).

Despite severe damage to the cuticles, Argyll plants were still nine times more resistant to MCPA than Ohiti plants. This result therefore suggested that reduced penetration of cuticles was not the mechanism of resistance.

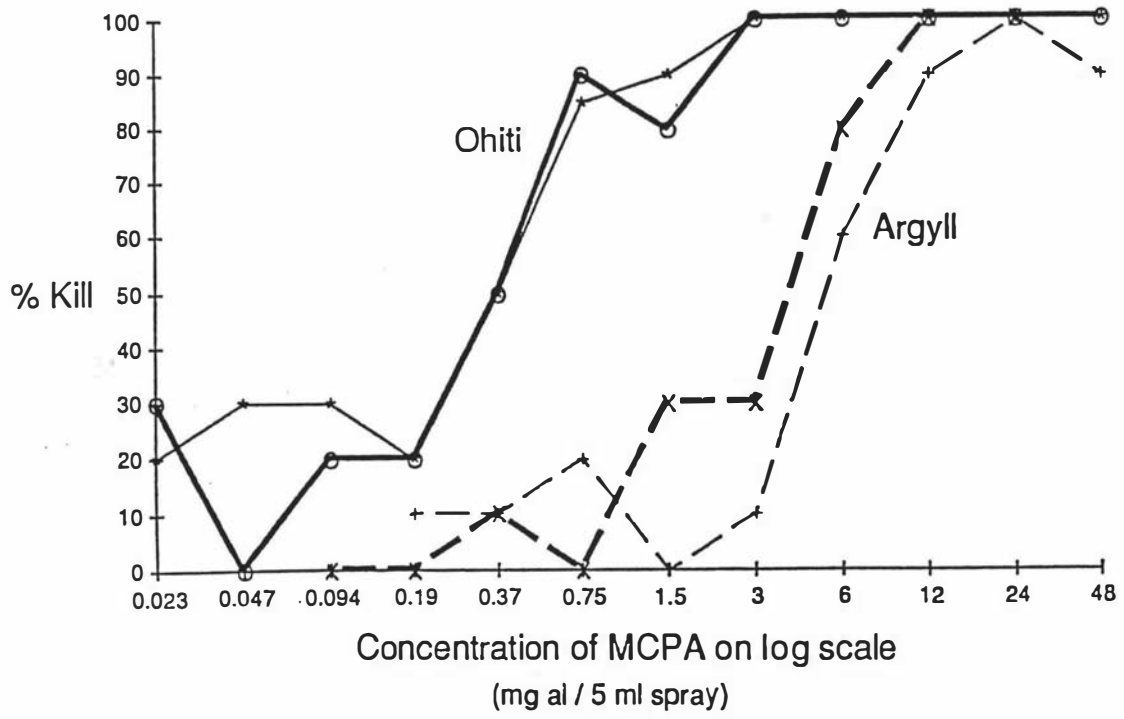


Fig 8.4: The unfitted dose response curves for glasshouse-grown Ohiti (solid lines) and Argyll (broken lines) nodding thistle populations sprayed with MCPA on to intact (light lines) and damaged (heavy lines) cuticles in February 1987.

Table 8.3: The effect of damaging plant cuticles on the susceptibility to MCPA of phenoxy-resistant (Argyll) and phenoxy-susceptible (Ohiti) nodding thistle plants. All values have been obtained from probit analysis of data presented in Fig 8.4.

	Plants sprayed with intact cuticles	Plants sprayed with damaged cuticles
Argyll plants:		
LD50	6.3	3.3
95% confidence limits	3.4 - 11.3	2.1 - 4.8
Ohiti plants:		
LD50	0.38	0.38
95% confidence limits	0.21 - 0.58	0.26 - 0.53
Argyll LD50 / Ohiti LD50	16.6	8.7

8.3.3.2 **Effect of Root Uptake**

If poor cuticular penetration was the sole mechanism of resistance in Argyll plants, root absorption should have resulted in identical dose response curves for the Argyll and Ohiti populations. However there was a statistically significant ($p = 0.05$) 3.1-fold difference between the two curves (Fig 8.5) which again suggested poor cuticular penetration was not involved. However, root uptake did reduce the magnitude of the susceptibility difference between Argyll and Ohiti populations from 17-fold to 3-fold. One possible explanation for this could be that several mechanisms act together to protect Argyll plants from MCPA, and one of these mechanisms is reduced cuticular penetration.

A more probable explanation was that herbicide movement from the potting mixture to the site of action within the plant by-passed regions of the plant where the herbicide would normally be immobilized or deactivated. MCPA would probably kill nodding thistle through activity in the crown or the root system (Ashton and Crafts 1981), so

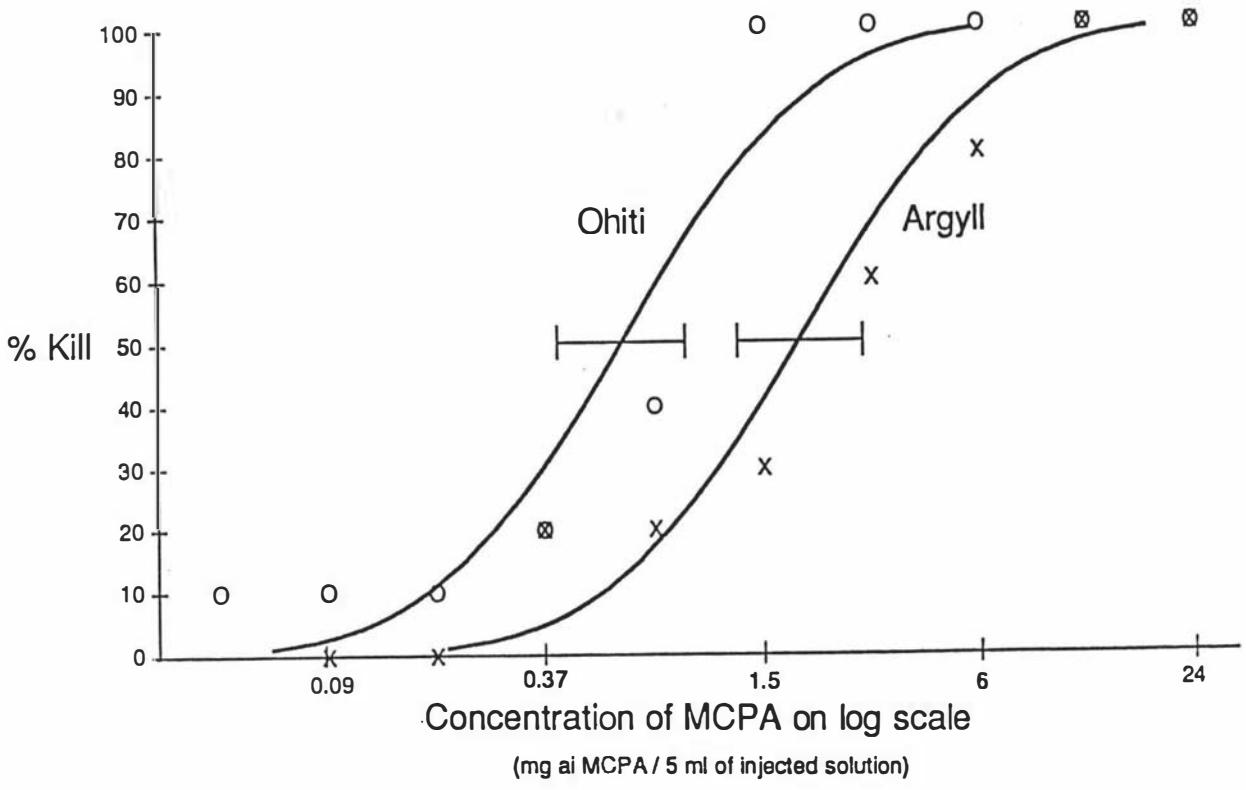


Fig 8.5: The fitted dose response curves for glasshouse-grown Argyll and Ohiti nodding thistle populations to which MCPA was applied by injection into the potting mixture of individual plants in February 1987. The raw data points for the Argyll (X) and Ohiti (O) populations are also shown. Horizontal bars show the 95% confidence intervals for the LD50 of each population.

little movement of the herbicide would be necessary to reach these sites following root uptake. If enzymes existed in the foliage to deactivate the herbicide, exposure of the herbicide to these enzymes would be minimal.

8.3.4 Conclusion

Results obtained in a trial reported earlier which tested many types of herbicides suggested poor penetration of the cuticle was not the mechanism of herbicide resistance in Argyll nodding thistle plants (Section 5.5.2). Damaging plant cuticles prior to foliar application of MCPA and applying herbicide via the root system both gave results which also indicated that herbicide absorption by the leaves of Argyll and Ohiti plants does not differ.

CHAPTER 9: PHYSIOLOGICAL STUDIES USING RADIOLABELLED 2,4-D

9.1 INTRODUCTION

Evidence obtained from herbicide screening work (Chapter 5) and experiments involving damaged cuticles and root uptake of herbicide (Chapter 8) suggested that herbicide resistance in nodding thistle did not involve reduced cuticular penetration. To confirm this finding and to investigate the mechanism of herbicide resistance in nodding thistle further, experiments were conducted using ^{14}C-labelled 2,4-D.

Radiolabelled herbicides have been used frequently in the past to study the absorption and translocation of herbicides in plants (Thompson *et al* 1986). Herbicide molecules are produced which contain atoms of ^{14}C. The β-emission from these atoms allows calculation of herbicide concentrations in plant tissue by extraction of the herbicide from the tissue into solutions containing substances called scintillators. These scintillators convert the kinetic energy of the β-particles into light photons which can then be detected and measured. This is known as liquid scintillation spectrometry (Coupland 1986).

A known quantity of ^{14}C-labelled herbicide can be placed on leaf surfaces, and then the ^{14}C concentrations present at various locations within the plant can be measured after an appropriate period to determine the extent of herbicide penetration and translocation (Thompson *et al* 1986).

If the herbicide is not easily extracted from the tissue, sub-samples of the tissue can be burnt in a continuous stream of oxygen and the resulting $^{14}CO_2$ is trapped in a solution containing scintillators and can be measured (Coupland 1986).

Radiolabelled herbicides can also be used to study how plants deactivate these compounds (Eastin and Basler 1977). Extraction and sample preparation procedures vary greatly depending on the chemical being studied, though chromatographic techniques such as thin-layer chromatography are often used to separate the various metabolites of a herbicide once they are extracted (Weete 1977). Following separation, the proportion of ^{14}C present in each fraction can be measured. Numerous studies have been conducted on the metabolism of 2,4-D by plants, and some of these have been reviewed by Loos (1975) and Pillmoor and Gaunt (1981).

Several biotypes of plant species tolerant of phenoxy herbicides have been treated with ^{14}C-labelled herbicides to investigate the mechanism of tolerance. A clone of field

bindweed (*Convolvulus arvensis* L.) tolerant of 2,4-D was found by Whitworth and Muzik (1967) to differ very little from a susceptible clone in uptake and translocation of radiolabelled herbicide.

Davis and Linscott (1986) used ^{14}C-2,4-D (labelled in the side-chain) to determine why the "T-68" cultivar of birdsfoot trefoil (*Lotus corniculatus* L.) tolerated 2,4-D better than the "Viking" cultivar. There was more herbicide binding to cellular constituents in T-68, resulting in less translocation of the herbicide through the plant. Five times more $^{14}CO_2$ was produced by the tolerant cultivar, suggesting there was also greater breakdown of the side-chain of 2,4-D molecules in these plants.

Several lines of soya bean (*Glycine max* (L.)Merrill) root callus tissue capable of growing in higher concentrations of 2,4-D than normal were studied by Davidonis *et al* (1982). Some differences in the rate of uptake of ^{14}C-2,4-D were noted between resistant and susceptible callus lines. Resistant tissue also converted more of the ^{14}C-2,4-D molecules into hydroxylated glycosides than susceptible tissue, *ie* hydroxyl groups were attached to the phenyl ring to deactivate the herbicide, and the molecule was then conjugated to glucose. However the differences in both rate of uptake and rate of deactivation were not consistent enough to wholly explain the observed resistance.

All experiments in the present project with nodding thistle also used ^{14}C-2,4-D. Radiolabelled 2,4-D was more readily available than MCPA. There was also more information available from other studies on the behaviour of ^{14}C-2,4-D in plants, particularly with respect to preparation of samples and analysis by chromatography. Although most experiments with resistant nodding thistle to date had used MCPA, 2,4-D and MCPA are very similar chemicals (Section 1.3) and several trials had shown cross-resistance to exist for 2,4-D (Sections 1.2.4 and 5.3). Resistance in nodding thistle was probably initially caused by selection pressure exerted by 2,4-D application (Section 6.4).

9.2 FIRST PENETRATION AND TRANSLOCATION EXPERIMENT

9.2.1 Introduction

The objective of initial radiolabelled herbicide studies with herbicide-resistant nodding thistle was to become familiar with the techniques necessary for this work. Although some problems did arise, the results obtained were considered sufficiently reliable to be reported.

9.2.2 Methods and Materials

9.2.2.1 **Preparation of Herbicide**

All experiments reported in this chapter used ring-labelled 2,4-D, *ie* all six carbon atoms in the phenyl ring of the 2,4-D molecule were ^{14}C, but the carbon atoms in the carboxyl side chain were unlabelled. The herbicide was obtained unformulated as an acid, which was a powder weighing 1.21 mg and containing 9.22 MBq of activity. The herbicide was dissolved in 5.0 ml of acetone, of which 4.0 ml was stored at -18^{o}C for future experiments. The acetone from the remaining 1.0 ml was evaporated off, and a 25% ammonia solution was added to the residue to formulate the 2,4-D as an ammonium salt. Excess ammonia was evaporated off using a rotary evaporator (Buchi Rotavapor R) and the solution was made up to 1.0 ml with distilled water, resulting in a concentration of 0.019% w/v of 2,4-D ammonium salt. The activity of this solution, determined by the Beckman LS3801 liquid scintillation counter used for most measurements of radioactivity in this project, was 1.45 kBq/µl. The scintillation fluid used was 4.0 g/l of 2,5-diphenyloxazole (PPO) in a toluene / Triton X-100 (2:1 v/v) mixture.

To ensure that plants treated with ^{14}C-2,4-D were subjected to sufficient quantities of herbicide to induce "normal" damage responses, unlabelled 2,4-D was applied in addition to the labelled material. The unlabelled 2,4-D was prepared as described above, *ie* 2,4-D acid was dissolved in 25% ammonia solution, excess ammonia was evaporated off, and distilled water added to produce a solution containing 0.77% w/v of 2,4-D ammonium salt. As all commercial formulations of 2,4-D contain surfactant, oxysorbic surfactant (Tween 20) was added to produce a 0.1% v/v solution.

9.2.2.2 **Application to Plants**

Plants from the Maungatautari (resistant) and Mason Ridge (susceptible) populations were established in plastic pots in May 1988 and grown as described in Section 6.3.1 with plants used in an earlier experiment. Four plants from each population were placed with their pots in individual aluminium foil trays prior to treatment and watered to a constant weight daily by pouring water into these trays.

The unlabelled herbicide was applied individually to nodding thistle rosettes on 7 December 1988 at 5 ml / plant using the sprayer described in Section 2.2. When

treated, the plants were 29 weeks old and the average shoot and root masses were 1.82 and 3.01 g DW respectively. Immediately after spraying each plant, 20 µl (29.0 kBq) of ^{14}C-2,4-D solution was applied as 1 µl droplets from a microsyringe, with 14 droplets being placed on the upper surface of a tagged mature leaf and six droplets on a tagged young leaf approximately half the length of mature leaves. Each plant received approximately 0.48 mg ai of 2,4-D, of which 0.8% was radiolabelled. It was estimated from work described in Chapter 6 that the concentration of MCPA used to differentiate between resistant and susceptible populations was equivalent to approximately 0.6 mg ai / plant.

9.2.2.3 **Recovery of Radioactivity**

To determine the proportion of herbicide that remained on leaf surfaces, treated leaves were removed individually and rinsed in 50 ml of water then 25 ml of chloroform, both for 60 sec. Aliquots were then taken from these solutions, added to scintillation fluid and radioactivity levels were measured. The effectiveness of these rinses in removing radioactivity adhering to external leaf surfaces was determined by rinsing leaves from one resistant and one susceptible plant immediately after application. The other six plants were left in a glasshouse for 7 days following application before the treated leaves were removed and rinsed.

After rinsing, each treated leaf was macerated in 95% ethanol with a high-speed laboratory blender and then the ethanol/leaf slurry was shaken gently for 24 hours. The remainder of each treated plant was separated into three lots of tissues: all remaining foliage, the crown of the rosette and the root system (which was carefully extracted from the potting medium by rinsing in water). These three groups of tissue were also individually macerated in 95% ethanol and shaken for 24 hours. The ethanol was separated from the tissue by filtration and the residue was rinsed twice with 95% ethanol. Tissue from two of the plants was then mixed with 80% ethanol and shaken for a further 24 hours. As this second ethanol extraction did not yield any further radioactivity from the tissue, it was not repeated with the other plants.

The solutions were concentrated by evaporating the ethanol, and distilled water was added to bring each solution to 25.0 ml. Aliquots were taken and radioactivity levels were measured. The pigments present in the solutions reduced the intensity of light emission following addition to the scintillation fluid ("quenching"). Data was adjusted to allow for this quenching by adding known quantities of radioactivity to solutions with varying levels of pigment, measuring the extent of quenching and programming the scintillation counter to automatically allow for this.

The filtrate was dried at 80°C and sub-samples were sent to the Forest Research Institute in Rotorua where radioactivity levels were assessed by combustion.

9.2.3 Results and Discussion

9.2.3.1 **Foliar Penetration**

All of the herbicide applied to one of the two plants harvested directly after application was successfully recovered using our rinsing technique (Table 9.1). Although 70% of the 20.0 µl of herbicide was supposedly applied to the mature leaf and the remainder on the younger leaf, the results suggest that slightly more than intended was applied to the second leaf. When removing the treated young leaf from the resistant plant, the leaf was dropped and this apparently caused 26% of the freshly applied herbicide to be

Table 9.1: The quantity of radioactivity (kBq) recovered from two plants which had their leaves rinsed with water then chloroform immediately following application of ^{14}C-2,4-D.

	Resistant Plant	Susceptible Plant
Mature leaf:		
- kBq of ^{14}C applied	20.3	20.3
- kBq washed off by water	18.8	20.0
- kBq washed off by chloroform	0.03	0.04
- % recovery	92.9	98.6
Young leaf:		
- kBq of ^{14}C applied	8.70	8.70
- kBq washed off by water	6.42	9.17
- kBq washed off by chloroform	0.03	0.02
- % recovery	74.1	105.6
Total % recovery	87.3	100.7

dislodged from the leaf surface. Losses of this magnitude were less likely on the other six plants as they were moved very little following application, and the treated leaves were not removed until 7 days later.

Most of the applied ^{14}C-2,4-D was recovered from the surface of the treated leaves when rinsed 7 days after application (Table 9.2). The average recovery rate was 83%, suggesting that only 17% of applied 2,4-D penetrated the cuticle. Radioactivity remaining on the outside of treated leaves gave an indirect measure of herbicide penetration. Another estimate of foliar absorption was obtained by summing the radioactivity detected within the various tissues of each plant and comparing these totals with the radioactivity found on leaf surfaces of the plant (Table 9.3). Both methods of estimating herbicide absorption showed no significant differences between herbicide-resistant and susceptible plants in uptake of 2,4-D.

Table 9.2: The percentage of radioactivity applied to leaves of herbicide-resistant and susceptible nodding thistle plants as ^{14}C-2,4-D ammonium salt that was recovered by rinsing the treated leaves 7 days after application. Differences between means are not significant at p = 0.05.

	% of applied ^{14}C recovered		
	Young leaf	Mature leaf	Total
Resistant plants:			
Rep 1	80.2	75.6	76.9
Rep 2	73.0	99.4	91.5
Rep 3	84.7	80.5	81.7
Mean	**79.3**	**85.2**	**83.4**
Susceptible plants:			
Rep 1	54.8	88.0	78.0
Rep 2	79.0	89.7	86.5
Rep 3	78.5	84.1	82.4
Mean	**70.8**	**87.3**	**82.3**

Table 9.3: Estimates of herbicide penetration derived from comparing quantities of ^{14}C found within plants with those located on leaf surfaces. Differences between mean values for resistant and susceptible plants are not significant at $p = 0.05$.

	^{14}C recovered (kBq)		Estimate of % penetration
	Leaf surfaces	Within plant	
Resistant plants:			
Rep 1	22.3	4.05	15.4
Rep 2	26.5	2.57	8.8
Rep 3	23.7	2.84	10.7
Mean	**24.2**	**3.15**	**11.6**
Susceptible plants:			
Rep 1	22.6	2.83	11.1
Rep 2	25.1	2.34	8.5
Rep 3	23.9	2.67	10.0
Mean	**23.9**	**2.61**	**9.9**

Plants from which lower quantities of radioactivity were measured in leaf washings had higher quantities detected within the plants than in other plants (Table 9.3), suggesting that variability in recoveries from washing leaves did result from differences in penetration rate rather than experimental error.

The proportion of 2,4-D that penetrates foliage varies considerably and is influenced by many different factors (Ashton and Crafts 1981, Fletcher and Kirkwood 1982; see also Section 1.3.3). Some of the reported trials in which penetration rates of ^{14}C-2,4-D have reached 80% involved application of the chemical as the parent acid dissolved in ethanol (Bhan *et al* 1970, Coble *et al* 1970), which probably would allow greater penetration of lipophilic cuticles than 2,4-D formulated as an ammonium salt and dissolved in water. Solvents such as ethanol were not used as carriers for 2,4-D in our experiments to avoid masking possible differences in permeability of the cuticles of resistant and susceptible nodding thistle plants to herbicides.

There was some indication that herbicide penetration was greater in young leaves than mature leaves (Table 9.2), but variability in results meant this difference was not significant statistically ($p = 0.05$). Hull (1970) stated that absorption of most organic and inorganic solutes is generally greater in relatively young leaves.

Treated leaf surfaces were rinsed with chloroform following the water rinse to determine whether any radioactivity had lodged in the waxes, thus preventing both penetration and removal by rinsing with water. Thompson *et al* (1986) reported that almost 50% of ^{14}C-fluroxypyr applied to leaves of field pansy (*Viola arvensis* Murr.) had penetrated into cuticular waxes 4 hours after application and was recovered by washing the treated area with chloroform. The radioactivity located in chloroform washes of the nodding thistle leaves 7 days after treatment was generally less than 1% of that found in water washes, and so this second rinsing was not used in later experiments. The low recovery of radioactivity from the chloroform rinses indicated that the ammonium salt of 2,4-D does not readily become immobilized in the cuticular waxes of nodding thistle.

Radioactivity not located either within plants or on leaf surfaces amounted to 6% and 9% of that originally applied to resistant and susceptible plants respectively. These differences in unaccounted losses were not significantly different ($p = 0.05$) and so were probably not related to the mechanism of resistance.

9.2.3.2 **Translocation**

Seven days after applying ^{14}C-2,4-D to the nodding thistle plants, approximately half of the radioactivity located within the plants was still present within the treated leaves (Table 9.4). Differences between resistant and susceptible ecotypes in the proportion of radioactivity remaining within treated tissues were not significant ($p = 0.05$) due to variability in the results.

The main tissue where radioactivity accumulated after leaving treated leaves was the crown (Table 9.4). The crown represents the stem of rosette plants through which assimilates must pass when travelling from one leaf to other leaves or to the roots, and it also contains the actively growing apical meristem. Movement of 2,4-D within plants is generally from photosynthesizing leaves to areas of high utilization of photosynthate (Ashton and Crafts 1981). Presumably radioactivity was located in the crown mainly because of accumulation at the shoot apical meristem, though some may have been passing through this tissue when harvested. However immobilization of

Table 9.4: The distribution of ^{14}C within nodding thistle plants to which ^{14}C-2,4-D was applied to a young leaf and a mature leaf 7 days earlier. Figures are expressed as a percentage of the total amount of radioactivity located within the plant both as ethanol-soluble and insoluble fractions.

	% of total ^{14}C located within plant				
	Treated young leaf	Treated mature leaf	Untreated foliage	Crown	Roots
Resistant plants:					
Rep 1	16.9	24.1	11.5	36.7	10.8
Rep 2	40.8	31.3	4.0	17.2	6.7
Rep 3	23.3	35.7	4.6	32.6	3.8
Mean	**27.0**	**30.4**	**6.7**	**28.8**	**7.1**
Susceptible plants:					
Rep 1	12.8	17.9	27.9	25.9	15.5
Rep 2	21.9	22.4	7.8	28.8	19.0
Rep 3	14.2	33.8	24.0	12.6	15.5
Mean	**16.3**	**24.7**	**19.9**	**22.4**	**16.7**
LSD at p = 0.05*:	NS	NS	NS	NS	6.5

* NS = means not significantly different at p = 0.05.

2,4-D while passing though the stem cannot be discounted. The concentration of 2,4-D used in this experiment may have been high enough to damage phloem tissue within the stem, reducing translocation through this tissue (Fletcher and Kirkwood 1982).

Radioactivity which had passed through the crown to other plant parts was found in approximately equal quantities in untreated leaves and the root system. The radiolabelled 2,4-D had probably moved with photoassimilates to actively growing young leaves and root tips, and also perhaps storage regions of the tap root. Over

twice as much radioactivity was found in these tissues in the susceptible nodding thistle ecotypes as in resistant ones (Table 9.4), though variability between plants meant this difference was significant only for the root tissue ($p = 0.05$). These results suggested 2,4-D may not have moved as freely in resistant plants as in susceptible plants, similar to the poor translocation of 2,4-D measured in a resistant birdsfoot trefoil cultivar by Davis and Linscott (1986).

9.2.3.3 **Insoluble Residues**

Radioactivity present within the plant which did not get extracted by ethanol was measured by combusting the material. This procedure showed most of the radioactivity was removed by the ethanol extractions (Table 9.5), but there were consistent differences in the apparent extraction of radioactivity from some types of tissue. The most marked difference was the total absence of radioactivity detected in combusted root material from the three resistant plants, yet root material from the three

Table 9.5: The percentage of the radioactivity located within plant organs which was not soluble in ethanol. The pooled standard deviation was 2.8.

	Treated young leaf	Treated mature leaf	Untreated foliage	Crown	Roots
Resistant plants:					
Rep 1	2.6	8.8	0.7	2.7	0
Rep 2	2.3	6.0	0	0.1	0
Rep 3	1.7	6.8	11.4	1.7	0
Mean	**2.2**	**7.2**	**4.0**	**1.5**	**0**
Susceptible plants:					
Rep 1	4.4	5.3	13.1	2.4	15.2
Rep 2	4.6	5.4	6.9	1.4	8.5
Rep 3	4.1	5.8	14.3	1.6	8.6
Mean	**4.4**	**5.5**	**11.4**	**1.8**	**10.8**

susceptible plants had relatively high levels of radioactivity not removed by ethanol extraction.

Two possible reasons for radioactivity being found in combusted samples were that the ethanol extraction procedure was not thorough enough to remove all material, or that the ^{14}C-2,4-D had become bound to cell constituents and could not be removed using ethanol. Thoroughness of the extraction technique should not have caused consistent differences between ecotypes for the same type of tissue. However, if this observation was related to the mechanism of resistance, binding of molecules would have appeared more likely in the resistant plants. Davis and Linscott (1986) found that reduced translocation of chain-labelled 2,4-D in resistant birdsfoot trefoil plants was caused by increased herbicide binding to cellular constituents. Such binding is common with chain-labelled 2,4-D as the carboxyl group is readily cleaved from the phenyl ring and then incorporated into structural carbohydrates (Pillmoor and Gaunt 1981). However all radiolabelled carbon atoms in our 2,4-D molecules were contained within the phenyl ring which is much less susceptible to being broken down.

One possible explanation for the increased levels of insoluble residues in susceptible plants in our results is that binding was required before the herbicide could influence plant growth. Phenoxy herbicides and auxin are similar in structure and activity, and some evidence suggests that auxin binds to membrane-bound receptor sites prior to influencing plant growth (Libbenga *et al* 1986).

9.2.4 Conclusions

This first experiment with ^{14}C-2,4-D allowed techniques to be developed and refined for future work. Results suggested that the mechanism of herbicide resistance did not involve differential rates of cuticular penetration. Significant differences were detected in the translocation of radioactivity into the root system, and in the ethanol solubility of this material once in the root system. However, in view of the variability in the results, these experiments were repeated to confirm the findings.

9.3 SECOND PENETRATION AND TRANSLOCATION EXPERIMENT

9.3.1 Introduction

One objective for the second radiolabelled 2,4-D experiment was to confirm the tentative conclusions drawn in the first experiment by repeating the procedure and

increasing replication to six plants per treatment. The other main objective was to increase the quantity of ^{14}C-2,4-D penetrating into the plants as higher levels of radioactivity within tissues would assist with future metabolism studies. Low concentrations of radiolabelled herbicide make detection of minor metabolites difficult when using techniques such as thin-layer chromatography.

9.3.2 Methods and Materials

9.3.2.1 **Preparation of Herbicide**

Solutions of labelled and unlabelled 2,4-D were prepared as described in Section 9.2.2.1, though the final concentrations differed slightly between experiments. Radioactivity levels were measured at 2.08 kBq/µl in the second experiment, 43% higher than the concentration of 1.45 kBq/µl used in the first experiment. The concentration of the unlabelled 2,4-D ammonium salt solution was 0.54% w/v, 70% of that used in the first experiment.

9.3.2.2 **Application to Plants**

Nodding thistle populations at Argyll and Matapiro were shown to be resistant and susceptible respectively to MCPA in an earlier trial (Section 6.2). Seed collected for that trial was used to establish resistant and susceptible plants from these populations for the present ^{14}C-2,4-D trial, with germination beginning on 27 September 1989. Seedlings were transplanted into individual 230 ml plastic pots containing coarse sand (particle diameter exceeding 1.7 mm). This medium was used to overcome root extraction problems previously experienced when using pumice or peat (*eg* Section 9.2), and also the apparent soil aeration problems experienced in past work (*eg* Section 2.5) with finer sand used without pumice or peat. Plants were grown in a glasshouse, irrigated regularly by an overhead watering system, and nutrients were applied periodically using a commercially available complete nutrient mixture (Peter's Peat Lite).

When plants were 2 months old, six Argyll and six Matapiro plants similar in size were transferred to a growth cabinet in which temperature fluctuated between 19^{o}C and 21^{o}C. Each pot was placed in an aluminium tray and water was added daily to a weight determined earlier following overhead irrigation. Light was supplied to plants at 190 Wm^{-2} for 16 hours each day. Average shoot and root masses were 1.11 and 0.81 g DW respectively.

Herbicide was applied to all 12 plants on 29 November 1989, 3 days after they were transferred to the cabinet. An atomiser was used to apply 50 µl of the ^{14}C-2,4-D solution to all foliage of each plant. The atomiser consisted of a stream of pressurised air (1.2 l/min) blowing across the end of a microsyringe and was constructed as described by Bucholtz and Hess (1988). The atomiser allowed more rapid transfer of radioactive herbicide to the foliage, and also resulted in formation of droplets more similar in size to those from conventional spraying equipment than in the first experiment. A sheet of chromatography paper was positioned under the foliage of each plant during spraying to absorb all herbicide not intercepted by the leaves. Plants were left for 60 minutes after application to allow some herbicide penetration into leaves and also drying of the spray. The plants were then resprayed with 200 µl of unlabelled 2,4-D, using the same atomiser as with the labelled herbicide. Thus each plant received approximately 0.88 mg ai of 2,4-D, of which 1.5% was radiolabelled.

Following the second herbicide application, sprayed plants were immediately placed into a large clear perspex box within the growth cabinet for 24 hours. The relative humidity within this box was approximately 100% to encourage absorption of the herbicide by plants. The temperature was 23^{o}C, and lights remained on for 12 hours after application. After removal from the perspex box, plants remained in the growth cabinet for a further 7 days before being harvested.

9.3.2.3 **Recovery of Radioactivity**

The sheets of paper placed beneath the foliage of each plant were cut up once herbicide applications were finished and placed into 100 ml of methanol overnight to extract any radioactivity within the paper. Aliquots from these solutions were then assayed for activity.

After 7 days, all foliage from each plant was removed just below the crown and rinsed in 150 ml of distilled water for 2 min. The foliage was allowed to drain for a further 1 min before being blended into a slurry with 95% ethanol. The root systems were carefully extracted from the pots and were also blended into a slurry with 95% ethanol. The slurries were gently shaken for 48 hours, then the extract was filtered, the residue was rinsed twice with ethanol, dried at 80^{o}C and sub-samples were assayed for activity by combustion at the Forest Research Institute in Rotorua. The ethanol extracts were concentrated using a rotary evaporator, and aliquots were assayed for activity. The remaining solution was frozen at -18^{o}C for future analysis of metabolites.

The potting mixture for each plant was placed into flasks, sediment from the pots and trays was washed into the flasks with water, and then water was removed from the flasks in an oven at 80°C. The sediment was transferred into beakers with 200 ml of methanol, and shaken before aliquots were assayed for activity.

9.3.3 Results and Discussion

9.3.3.1 **Foliar Penetration**

The percentage of applied ^{14}C-2,4-D found on the paper backing sheets ranged from 2.1% to 13.0%. The average interception of radioactivity by plant foliage was calculated to be 93.6%, equating to 97.1 kBq / plant. This was 3.3 times more than was applied per plant in the first experiment, though the quantity per treated leaf was probably similar or higher in the earlier experiment when only two leaves per plant were treated.

As with the first experiment, two estimates of foliar penetration by the ^{14}C-2,4-D have been calculated (Table 9.6). One technique assumes all ^{14}C not present in the leaf washings or on the paper penetrated into the plant. This could overestimate penetration if some herbicide was not intercepted by either the foliage or the backing paper. The other estimate compares the total ^{14}C recovered within the plant and rooting zone with that located in the leaf washings. This technique could underestimate penetration if some radioactivity was lost when plant material was processed for assaying. If all radioactivity had been recovered, the two techniques would have given the same result. However, unrecovered ^{14}C amounted to between 4.4% and 18.9% of the total amount applied (Table 9.6). Differences between resistant and susceptible plants in unrecovered radioactivity were not significant ($p = 0.05$).

As in the first experiment, neither estimate showed any significant difference between resistant and susceptible nodding thistle biotypes in the penetration of 2,4-D into the foliage. These results offer conclusive evidence to reinforce findings from several earlier experiments suggesting that the mechanism of resistance to phenoxy herbicides in nodding thistle does not involve differences in foliar penetration. Differences in foliar uptake of phenoxy herbicides have also not been detected in several other species with resistant biotypes, *eg* field bindweed (Whitworth and Muzik 1967) and birdsfoot trefoil (Davis and Linscott 1986).

Results in Table 9.6 show that herbicide penetration in the second experiment was 2-3 times higher than in the first experiment, reaching 30-40% in the later experiment

Table 9.6: Estimates of the percentage of ^{14}C-2,4-D applied to Argyll (resistant) and Matapiro (susceptible) nodding thistle plants which penetrated into the foliage. Estimate 1 assumes all ^{14}C applied to the leaf not found in the washings was absorbed. Estimate 2 compares the ^{14}C found inside the plant with that found in the leaf washings. The percentage of applied ^{14}C not recovered in the experiment is included as this caused the differences between the two estimates.

	% penetration of ^{14}C-2,4-D		% of applied ^{14}C not recovered
	Estimate 1	Estimate 2	
Resistant plants:			
Rep 1	45.1	31.3	18.9
Rep 2	41.8	33.0	12.3
Rep 3	36.5	32.2	5.9
Rep 4	26.0	21.4	5.4
Rep 5	31.5	27.6	5.2
Rep 6	40.4	33.2	10.1
Mean	**36.9**	**29.8**	**9.6**
Susceptible plants:			
Rep 1	42.9	29.6	16.5
Rep 2	38.9	29.3	13.2
Rep 3	40.4	35.8	6.5
Rep 4	33.3	30.2	4.4
Rep 5	41.5	30.8	13.7
Rep 6	34.2	28.8	7.5
Mean	**38.5**	**30.7**	**10.3**
LSD at p = 0.05:	7.4	4.8	6.7
Overall mean for Expt 1	17.1	10.7	7.3

compared with 10-20% earlier. The main reason for this increased penetration was probably the conditions of high humidity, warm temperatures and exposure to light for many hours following herbicide application, factors all recognised by Ashton and Crafts (1981) as being conducive to good foliar uptake of phenoxy herbicides. In the first experiment, plants remained in a dimly lit (20 Wm^{-2}) laboratory for many hours after herbicide application.

Applying the radiolabelled herbicide prior to the unlabelled material may have also assisted penetration of the ^{14}C-2,4-D. The gradient between internal and external concentrations of 2,4-D would have been high initially when no 2,4-D was inside the plant. In the first experiment, unlabelled 2,4-D would have already entered the plant when ^{14}C-2,4-D was applied, decreasing the concentration gradient. Assuming that diffusion of labelled 2,4-D occurred as freely as unlabelled 2,4-D through the cuticle, only 0.8% of 2,4-D penetrating the cuticle would have been labelled. In the second experiment, all 2,4-D entering the plant would have been labelled for the first hour while internal 2,4-D concentrations were low. Drying droplets were then rewetted with the application of unlabelled herbicide to allow diffusion to continue, and the much higher concentration of this herbicide (compared with ^{14}C-2,4-D) would have increased the concentration gradient again.

The reduced droplet size produced by the atomiser in the second experiment may have also assisted penetration. McKinley *et al* (1972) found that approximately three times and six times as much 2,4-D was needed to give the same result with 200 µm and 400 µm droplets respectively as with 100 µm droplets. The average droplet size in the second experiment was 150 µm (diameter), compared with 3000 µm for the first experiment.

The three-fold increase in ^{14}C-2,4-D applied to plants combined with the three-fold increase in penetration rate resulted in the average quantity of activity recovered within plants rising from 2.9 kBq in the first experiment to 26.2 kBq in the present experiment. This proved useful in later metabolism studies.

9.3.3.2 **Translocation**

Numerous researchers have reported the leakage of phenoxy herbicides from roots following the transport of the compounds to the roots from a foliar application (Ashton and Crafts 1981). The extent of this leakage from nodding thistle plants was estimated in the second experiment by measuring the quantity of ^{14}C present in the potting mixture of plants one week after herbicide application. The potting mixture was

covered by chromatography paper during herbicide application to ensure no ^{14}C was inadvertantly sprayed on to the mixture.

There was significantly more ^{14}C located in the potting mixture of susceptible plants than for resistant plants (Table 9.7). Although only 0.7% of the radioactivity located following penetration of resistant plants was released from the roots, six times as much radioactivity was released from the roots of susceptible plants. This amounted to 19% of all radioactivity translocated underground by the susceptible plants being released into the potting mixture, compared with 4% for resistant plants.

Table 9.7: The percentage of ^{14}C recovered from the potting mixture, presumably due to exudation from roots, expressed relative to total ^{14}C recovered from the plant following absorption, and relative to total ^{14}C translocated down into the root system.

	% of absorbed ^{14}C exuded from roots		% of ^{14}C translocated into roots that was exuded	
	susceptible plants	resistant plants	susceptible plants	resistant plants
Rep 1	2.4	0.45	15.5	2.3
Rep 2	4.0	0.58	18.1	2.2
Rep 3	3.2	0.45	18.7	2.5
Rep 4	6.2	0.89	23.0	3.6
Rep 5	5.1	0.57	18.7	4.3
Rep 6	3.8	1.12	22.5	9.6
Mean*	**4.1**	**0.68**	**19.4**	**4.1**

* Mean vaues for herbicide-resistant plants were significantly lower ($p = 0.01$) than mean values for susceptible plants in both comparisons.

If the increased release of radioactivity had been from the roots of the resistant plants, active exudation of herbicide may have been involved, suggesting a possible mechanism of resistance. However as the release was from the susceptible plants, this phenomenon was probably a symptom of herbicide damage, indicating that the root system of sensitive plants was no longer intact enough to properly contain root contents. Another possible explanation is that exudation of 2,4-D is normal from roots, and that the 2,4-D molecules within resistant plants had either been immobilized or transformed into new substances which were not easily exuded.

As all foliage of each plant received ^{14}C-2,4-D in the second experiment, less information on translocation within the plant could be gleaned compared with the first experiment where only a few leaves were treated. However translocation into the root system was measured, and this could be calculated more accurately than in the earlier experiment as the extent of exudation or release from roots was also measured. Although significantly less ^{14}C was translocated into the roots of resistant plants in the first experiment compared with susceptible plants, no difference in translocation was measured in the second experiment (Table 9.8).

Although release of radioactivity from plant roots was not measured in the first experiment, such measurements were unlikely to have explained the difference in results between the two experiments. The difference in ^{14}C translocated into the roots of susceptible and resistant plants would have been even greater than was actually measured if susceptible plants had released more radioactivity than resistant plants into the potting medium in the first experiment.

As translocation of radioactivity to the root system did not differ significantly between susceptible and resistant plants in the second experiment, it appears unlikely that resistance results from differential rates of translocation. However the difference in results between experiments suggests the actual mechanism of resistance may influence subsequent translocation of 2,4-D or its metabolites under some conditions.

9.3.3.3 **Insoluble Residues**

The percentage of radioactivity found within treated plants which had not been removed by ethanol extraction was significantly higher ($p = 0.05$) in susceptible plants than in resistant plants for both the foliage and the roots (Table 9.9). Likewise, radioactivity was more successfully extracted by ethanol from the root tissue than the foliage for both resistant and susceptible plants. These results confirmed the significant difference in insoluble residues measured for root tissue in the first

Table 9.8: The percentage of recovered ^{14}C absorbed by plants that was translocated into the root system of herbicide-resistant and susceptible plants. Figures which do not account for ^{14}C loss into the potting mixture are included to allow comparison with Experiment 1.

	% of absorbed ^{14}C recovered in roots		% of absorbed ^{14}C translocated into roots	
	susceptible plants	resistant plants	susceptible plants	resistant plants
Experiment 2:				
Rep 1	12.9	19.1	15.3	19.5
Rep 2	18.1	25.9	22.1	26.5
Rep 3	14.0	17.4	17.2	17.8
Rep 4	20.8	24.0	27.0	24.9
Rep 5	22.2	12.6	27.3	13.2
Rep 6	13.1	10.6	16.9	11.7
Mean*	**16.8**	**18.3**	**21.0**	**18.9**
Experiment 1:				
Mean	16.7	7.1	-	-

* Means for susceptible and resistant plants do not differ significantly at p = 0.05.

experiment (Table 9.5), though the magnitude of this difference was less marked in the second experiment.

As was discussed in Section 9.2.3.3, one possible explanation for the increased percentage of insoluble radioactive material within susceptible plants was that 2,4-D molecules bind to membranes in order to influence plant growth. The resistance mechanism may reduce the extent of such binding and thus render the plant less susceptible to the herbicide. If this was the explanation, the binding would have needed to be quite strong to withstand the ethanol extraction procedure. Results in

Table 9.9: The percentage of ^{14}C located within foliage or roots of treated resistant and susceptible nodding thistle plants which was not removed by extraction with 95% ethanol.

	% ^{14}C insoluble to ethanol in foliage		% ^{14}C insoluble to ethanol in roots	
	susceptible plants	resistant plants	susceptible plants	resistant plants
Rep 1	21.3	13.8	12.0	7.4
Rep 2	21.6	13.5	10.7	9.4
Rep 3	18.4	19.5	15.7	7.1
Rep 4	24.2	16.5	16.7	9.3
Rep 5	20.3	15.5	13.9	8.4
Rep 6	19.6	14.3	14.3	8.1
Mean*	**20.9 c**	**15.5 b**	**13.9 b**	**8.3 a**

* Mean values with different letters are significantly different at p = 0.05.

Table 9.9 indicate that if differences in binding were occurring, there was 1.3 times as much binding in susceptible leaves as resistant leaves, and 1.7 times as much in susceptible compared with resistant roots. These differences may not be large enough to fully explain the 7-fold differences in resistance commonly measured in earlier experiments.

The quantities of radioactivity not removed by ethanol in this experiment were higher than in the first experiment (Table 9.5). If the ethanol extraction technique had been less thorough for the second experiment, differences in results between experiments could be explained. However similar extraction techniques in both experiments suggested that there might have been genuine differences in quantities of non-soluble radioactive material between experiments. As translocation of radioactivity to the roots also appeared to differ between experiments (Section 9.3.3.2), differences in

growing conditions or herbicide application procedures between the two experiments may have affected the results obtained.

9.3.4 Conclusions

The second experiment with ^{14}C-2,4-D confirmed that entry of phenoxy herbicides into resistant and susceptible nodding thistle plants did not differ. However the reduced translocation of radioactivity into the roots of resistant plants in the first experiment was not confirmed in the second trial. Compared with resistant plants, significantly more radioactivity was released from the roots of susceptible plants, and less of the radioactivity located within plant tissues could be extracted with ethanol. As these findings did not clearly indicate the mechanism of resistance, investigations were now required into the possible degradation of 2,4-D molecules within the plants.

9.4 METABOLISM EXPERIMENTS

9.4.1 Introduction

As discussed in Section 1.3.6, 2,4-D can be metabolised in plants by conjugation with amino acids, cleavage of the side chain or hydroxylation of the phenyl ring. Following hydroxylation, conjugation often occurs with glucose molecules to form glycosides (Ashton and Crafts 1981). Metabolites usually remain radiolabelled, especially when the original molecule was ring-labelled. Thus in experiments reported above, it was not known whether the radioactivity being measured indicated the presence of active 2,4-D molecules or inactive metabolites.

To discover whether 2,4-D has been metabolised within plants, differences between the chemical properties of 2,4-D and the resultant metabolites have been used in past experiments. For example, 2,4-D metabolism has been investigated in soyabean callus tissue by Feung *et al* (1971, 1972, 1973b), Davidonis *et al* (1982), and Zama and Mumma (1983). Other 2,4-D metabolism work includes that conducted in bean plants (Hamilton *et al* 1971), oats (Feung *et al* 1974), sweet corn (*Zea mays* L.), tobacco (*Nicotiana tabacum* L.), carrot (*Daucus carota* L.) and sunflower (*Helianthus annuus* L.) (Feung *et al* 1975), rice (Feung *et al* 1976) and wheat (Bristol *et al* 1977).

The ring hydroxylated glycosides of 2,4-D are water soluble and thus can be separated from 2,4-D and amino acid conjugates of 2,4-D as these are soluble in ether (Feung *et al* 1971). Cleavage of the side-chain probably results in the formation of

2,4-dichorophenol which then forms a glycoside and thus would also become water-soluble (Luckwill and Lloyd-Jones 1960). The amino acid conjugates can then be separated from 2,4-D and identified using paper and thin-layer chromatography (Feung *et al* 1973a). Likewise the glucose can be cleaved from the glycosides using β-glucosidase and the resulting molecules (aglycones) can be separated and identified by chromatography (Feung *et al* 1973b). The proportion of 2,4-D present in each of the various forms identified can be quantified by using ^{14}C-2,4-D and comparing radioactivity levels in each fraction.

Metabolism of 2,4-D could be the cause of resistance in nodding thistle, so techniques described by the researchers mentioned above were used to compare the fate of the ^{14}C-2,4-D applied to the resistant and susceptible plants discussed in Section 9.3.

9.4.2 Methods and Materials

9.4.2.1 **Ether Partitioning**

The ethanol extracts from the roots of four susceptible and four resistant nodding thistle plants obtained earlier (see Section 9.3.2) were thawed out following storage at -18^{o}C. Ethanol was removed from the solutions using a rotary evaporator at 30^{o}C and distilled water was added to bring solutions to a volume of 25.0 ml. The solutions were acidified to a pH of 2.0 with HCl, then each solution was partitioned three times with 25 ml of diethyl ether. Several 1 ml aliquots were then taken from the water and ether fractions and these were assayed for radioactivity. All procedures described in Section 9.4.2 are summarised in Fig 9.1.

9.4.2.2 **Separation of the Ether Soluble Components**

The ether fractions from the roots of a susceptible and a resistant plant were evaporated separately to dryness in a rotary evaporator at 30^{o}C and then the residues were each dissolved in 1 ml of acetone. Half of each acetone solution was spotted on to Whatman No 1 chromatography paper (30 cm x 10 cm) and the other half was spotted on to a glass plate uniformly coated in a thin layer of silica gel G (Merck). The solvents used for chromatographic separation were butanol / ethanol / 3N ammonium hydroxide (4 : 1 : 5) for the paper and diethyl ether / petroleum ether / formic acid (70 : 30 : 2) for the thin-layer plate, as used by Feung *et al* (1973a). Some ^{14}C-2,4-D ammonium salt was also spotted for comparison.

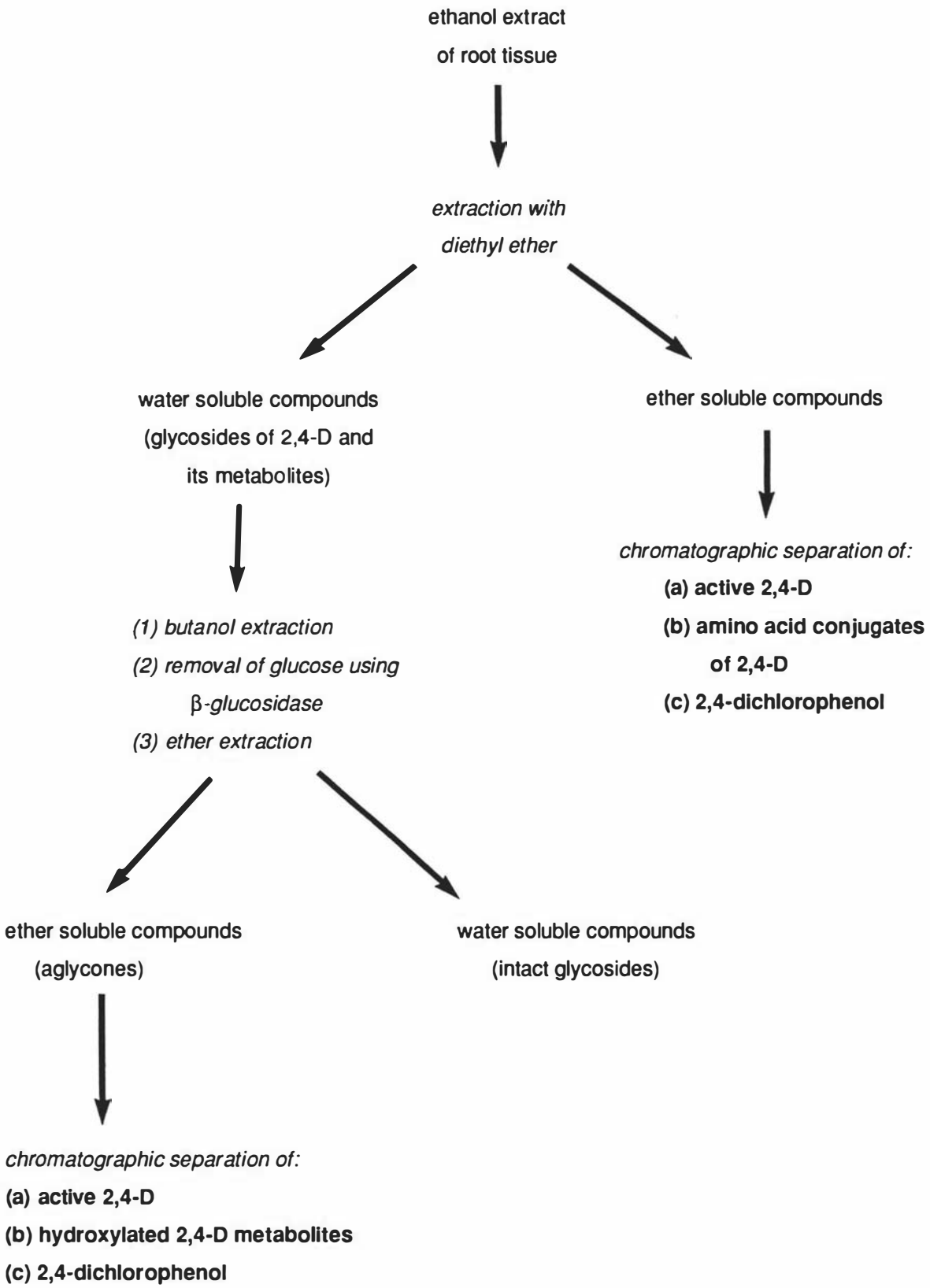


Fig 9.1: A summary of the procedures used in this project to study the possible metabolism of radiolabelled 2,4-D within resistant and susceptible nodding thistle plants.

Following chromatography, the chromatograms were held against x-ray plates within sealed black plastic bags for 5 days at -18^{o}C, then the x-ray plates were developed to find which portions of the chromatogram contained radioactivity. The radioactivity within the various portions of thin-layer plates was quantified by scraping off the silica gel from these portions, adding the gel to 1 ml of ethanol in scintillation vials, adding scintillant and assaying for radioactivity. Radioactivity in portions of paper chromatograms was quantified by cutting out radioactive portions, shaking these in 20 ml of ethanol overnight then assaying 1 ml aliquots.

A large quantity of brown pigmented material within the acetone solution hindered movement of substances during chromatography. When the ether fractions of the next four plants (two resistant and two susceptible) were prepared for chromatography, a technique described by Feung *et al* (1976) was used to reduce this problem. The ether fractions were evaporated to dryness, and the residue was dissolved in 10 ml of 90% ethanol, placed in centrifuge tubes and stored at -18^{o}C overnight. The chilled solutions were centrifuged for 5 min at 0^{o}C and the precipitate, which contained no radioactivity, was discarded. The solutions were then stored again overnight at -18^{o}C, centrifuged the following day to remove more precipitate, then evaporated to dryness and taken up in 1 ml of acetone, ready for chromatography as described above.

9.4.2.3 **Separation of the Water Soluble Components**

The water fraction obtained from the ether partitioning was extracted twice with butanol, then the butanol fraction was evaporated to dryness and the residue dissolved in 10 ml of distilled water. The pH of this solution was adjusted to 4.5 with 1% $NaHCO_3$ then 30 mg of β-glucosidase (5.3 units/mg) was added and the solution shaken gently for 3 days at room temperature to allow glucose to be removed from the glycosides. The solutions were then made up to 30 ml with distilled water, acidified to pH 2.0 with HCl and partitioned three times with 30 ml of diethyl ether. Several 1 ml aliquots were then taken from both the water and ether fractions and these were assayed for radioactivity to determine whether glucose had been successfully removed from the metabolites.

The ether fraction was cleaned by centrifugation and the aglycones separated by paper and thin-layer chromatography as described in Section 9.4.2.2. This procedure was repeated for the root extracts of three resistant and three susceptible plants.

9.4.3 Results and Discussion

9.4.3.1 **Ether Partitioning**

A significantly higher ($p = 0.01$) proportion of the radioactivity extracted from the roots of susceptible nodding thistle plants was soluble in ether compared to resistant plants (Table 9.10). Susceptible plants contained an average of 37% ether soluble ^{14}C and 73% water soluble ^{14}C in the root system, compared with 15% and 85% respectively for resistant plants. The ether soluble fraction would have contained unmetabolized ^{14}C-2,4-D plus amino acid conjugates, whereas the water soluble materials would have been glycosides of 2,4-D and its metabolites. The high proportion of radioactivity in the water soluble fraction suggested both resistant and susceptible plants had metabolized much of the applied 2,4-D during the 7 days following spraying.

However the percentage of water soluble metabolites produced 7 days after application of 2,4-D has been found in past experiments to vary from 13% to 44% in susceptible dicotyledonous species and from 56 to 72% in resistant monocotyledons (Bristol *et al* 1977). Thus our figures of 73 to 85% appear rather high, though figures quoted by Bristol *et al* were all obtained from plant callus tissue cultures incubated on 2,4-D treated media, and so may not relate well to our whole plant experiment for compounds which had been translocated away from the site of application. Amino

Table 9.10: The percentage of radioactive material extracted by ethanol from the roots of phenoxy-resistant and susceptible nodding thistle plants which was soluble in ether. Mean values were significantly different at $p = 0.01$.

Replicate	Susceptible Plant	Resistant Plant
1	38.9	18.3
2	37.7	18.1
3	39.5	7.5
4	33.7	16.4
Mean	**37.4**	**15.1**

acid conjugates of 2,4-D formed in treated leaves apparently do not get translocated out of these leaves (Ashton and Crafts 1981), and our measurements were from root tissue. Despite this, our high figures could have also resulted from 2,4-D metabolism during processing and storage of samples by microbial or chemical reactions. Such metabolism should have been minimal because techniques used were similar to those descibed by other workers (see earlier discussion), and samples were stored at -18^{o}C prior to processing to prevent microbial action.

Despite discrepancies with other work, the significantly greater proportion of water soluble materials in the resistant plants indicated that rates of metabolism differed between resistant and susceptible biotypes and thus could be related to the mechanism of resistance.

9.4.3.2 **Separation of the Ether Soluble Components**

The thin layer chromatograms that were produced suggested much of the ether soluble material present within susceptible plants was 2,4-D because it had an Rf value similar to that measured for 2,4-D (0.8) (Table 9.11). In contrast, most of the ether soluble components in resistant plants appeared to be metabolites of 2,4-D as these materials had Rf values that were not 0.8. By combining mean results from Tables 9.10 and 9.11, the quantity of active 2,4-D present in the roots of susceptible plants was estimated to be 13.8 times greater than in resistant plants (Table 9.12). This difference would be large enough to completely explain the mechanism of resistance.

The ether soluble materials with Rf values different from 2,4-D were probably amino acid conjugates of 2,4-D (Feung *et al* 1973b). The quality of chromatograms did not allow good identification of the compounds present. However significantly more material from the resistant plants was relatively immobile (Rf < 0.1) on the thin layer plates than from susceptible plants (Table 9.11). Investigations by Feung *et al* (1973a) showed that conjugates of 2,4-D with glycine, lysine, histidine, arginine or hydroxyproline would all be immobile with the solvent system used for our thin layer plates.

The quality of the paper chromatograms produced was poor due to overloading of the paper with sediment even after the clean-up process using centrifugation. The radioactivity detected on these chromatograms suggested there was no separation of 2,4-D from metabolites. However Feung *et al* (1973a) showed that most of the amino acid conjugates listed above have Rf values similar to the Rf value of 2,4-D (0.75) following chromatographic separation on paper using the butanol / ethanol /

Table 9.11: The percentage of radioactive ether soluble compounds isolated from the roots of herbicide resistant and susceptible nodding thistle plants which had Rf values similar to 2,4-D (0.8), or with Rf values below 0.1, following chromatographic separation on thin layer silica gel G plates using a diethyl ether / petroleum ether / formic acid (70 : 30 : :2) solvent.

Replicate	% compounds with Rf < 0.1		% compounds with Rf = 0.8	
	susceptible plants	resistant plants	susceptible plants	resistant plants
1	21.8	36.0	57.1	22.6
2	11.9	72.9	73.8	6.3
3	6.8	45.3	79.9	4.4
Mean*	**13.5** a	**51.4** b	**70.3** b	**11.1** a

* Mean values with different letters are significantly different at p = 0.05.

ammonium hydroxide solvent. The arginine conjugate had the closest value at 0.76, and the other four had values between 0.61 and 0.68. The poor quality of the paper chromatograms meant the presence of compounds at any of these Rf values could not be discounted as the resulting radioactive bar merged with that for 2,4-D.

However none of these amino acid conjugates have been identified in species investigated by other workers. The amino acids most commonly found conjugated to 2,4-D are glutamic acid and aspartic acid (Ashton and Crafts 1981). The conjugates of glutamic acid and aspartic acid should have been present at Rf values of 0.21 and 0.26 respectively on the thin-layer plates, and at 0.43 and 0.36 respectively on the paper chromatograms (Feung *et al* 1973a). As explanation of the radioactivity found at Rf < 0.01 is difficult using published results, the possibility exists that radioactivity may have been immobile on the thin layer plates simply due to poor preparation techniques. However the differences between resistant and susceptible plants then become difficult to explain.

Table 9.12: Estimation of the differences in absolute quantities of active radiolabelled 2,4-D in the roots of resistant and susceptible nodding thistle plants 7 days after foliar application.

	Susceptible plants	Resistant plants
Total radioactivity absorbed by plants (kBq)	26.5	26.0
Percentage of absorbed radioactivity found in roots	16.8%	18.3%
Total radioactivity found in roots (kBq)	4.45	4.76
Percentage of root radioactivity extracted by ethanol	86.1%	91.7%
Total radioactivity extracted from roots (kBq)	3.83	4.36
Percentage of extracted radioactivity soluble in ether	37.4%	15.1%
Total radioactivity soluble in ether (kBq)	1.43	0.66
Percentage of ether-soluble material with Rf = 0.8	70.3%	11.1%
Estimate of total active radiolabelled 2,4-D (kBq)	1.01	0.073
Difference between resistant and susceptible plant roots in quantity of active 2,4-D	13.8 times	

If resistance in the nodding thistle plants is to be explained by production of amino acid conjugates, this would require that these conjugates were herbicidally inactive. However Feung *et al* (1974) showed that all amino acid conjugates of 2,4-D stimulated elongation of oat coleoptiles in a similar manner to 2,4-D, leading them to conclude that amino acid conjugation does not deactivate 2,4-D. These results have since been disputed as these conjugates are readily hydrolysed back to 2,4-D, which could have occurred during the oat coleoptile assay (Pillmoor and Gaunt 1981). This ready conversion back to 2,4-D does suggest that amino acid conjugation is a temporary form of deactivation. Davidonis *et al* (1980) showed in soyabean callus tissue that much of the applied 2,4-D was converted to amino acid conjugates within 24 hours of

application, then conjugates were hydrolysed back to 2,4-D over subsequent days and also converted to water soluble metabolites by hydroxylation. Although this process eventually leads to more permanent deactivation of 2,4-D by hydroxylation, it also provided a constant supply of active 2,4-D within the plant (Pillmoor and Gaunt 1981).

Unexplained losses of radioactivity during sample preparation may have affected the validity of results with the chromatography of ether soluble compounds. Losses were not measured for the first replicate, but with Replicates 2 and 3, the loss of radioactivity while preparing the ether solutions for chromatography was 30% and 17% respectively for the susceptible plants, and 55% and 44% respectively for resistant plants. Further investigation of these losses showed they occurred whenever samples were evaporated to dryness, which occurred once for the first replicate, and twice for the other two replicates with the addition of the centrifugation clean-up step. The radioactivity appeared to be lost into the rotary evaporator as glassware was checked after residues were redissolved to ensure radioactivity was not left behind. These losses were particularly unsatisfactory because they were greater for the resistant plants, suggesting differences between ecotypes in the compounds present were not being measured by the chromatography. An attempt to overcome these losses by not evaporating to dryness during the sample preparation procedure failed as the sample then could not be successfully applied to chromatographic plates.

9.4.3.3 **Separation of the Water Soluble Components**

Following chromatographic separation of the first two samples of water soluble compounds, it was discovered that over 90% of the radioactivity had been lost during sample preparation. Losses of radioactivity were subsequently monitored closely throughout the preparation of the other four samples chromatographed. The monitoring confirmed that most of the radioactivity was being lost during sample preparation for both resistant and susceptible plants (Table 9.13). Further investigations indicated that large losses were occurring whenever solutions were evaporated to dryness, with the radioactivity apparently disappearing into the rotary evaporator. Only some of this radioactivity could be recovered subsequently from vessels within the evaporator. A chemist at the DowElanco laboratory in New Plymouth contacted about this problem stated they avoid evaporating solutions of phenoxy chemicals to dryness as they have experienced similar losses of material (J. Cowles, pers comm). Time constraints prevented development of more satisfactory sample preparation techniques to permit further analysis of water soluble compounds.

Table 9.13: Losses of radioactivity recorded during the processing of samples containing water soluble compounds from two replicates of resistant and susceptible nodding thistle plants.

	Resistant plants		Susceptible plants		Mean
	Rep 2	Rep 3	Rep 2	Rep 3	
Radioactivity in water soluble fraction (Bq)	5029	4396	2896	2504	3706
% lost during butanol step	23.3	38.9	33.7	41.7	34.4
% remaining water soluble following β-glucosidase	20.5	16.7	14.1	23.7	18.7
% lost producing 90% ethanol solution	46.9	81.2	55.0	23.8	51.7
% lost producing acetone solution	69.8	41.3	66.7	31.3	52.3
Radioactivity in acetone solution (Bq)	453	215	227	453	337
Total % loss of radioactivity	91.0	95.1	92.2	81.9	90.0

The large losses of radioactivity meant valid comparisons of compounds obtained from susceptible and resistant plants was not possible. The low quantities of radioactivity remaining after sample preparation also resulted in poor quality chromatograms. However some chromatograms did indicate that at least three different water soluble compounds containing radioactivity were present in the roots of the nodding thistle plants. The Rf value of a major compound was the same as for the 2,4-D applied to chromatograms as a reference point, suggesting that some 2,4-D was conjugated to sugar without first being hydroxylated. Formation of such glucose esters of 2,4-D commonly occurs in plants (Ashton and Crafts 1981).

9.4.4 Conclusions

Susceptible nodding thistle plants appeared to contain significantly higher

concentrations of unmetabolised 2,4-D molecules 7 days after application than resistant plants. The 2,4-D molecules were probably converted more rapidly in the resistant plants into both water soluble and ether soluble metabolites. Details of these breakdown pathways could not be ascertained due to difficulties with sample preparation.

9.5 DISCUSSION

Pillmoor and Gaunt (1981) have discussed the difficulty of assessing the exact mechanism of selectivity with phenoxy herbicides. Both amino acid conjugates and sugar esters of 2,4-D can easily convert back to active 2,4-D molecules within the plant. Resistant plants may differ in sensitivity from susceptible plants because the equilibrium between active and inactive forms of 2,4-D differs, with resistance resulting from concentrations of active herbicide molecules being maintained below toxic levels in sensitive tissues.

Further research could be usefully conducted on 2,4-D metabolism in nodding thistle by monitoring the changes in metabolite levels throughout the days following application, rather than just at 7 days after spraying. Likewise, studies of metabolism in tissues other than just the roots would assist with interpretation of results.

Many similarities exist between the resistance to phenoxy herbicides in nodding thistle and giant buttercup. For example, the magnitude of resistance is similar between the two species, and the selection pressure which resulted in the resistance was probably also similar (Bourdot *et al* 1990). Since the experiments with radiolabelled 2,4-D were completed with nodding thistle, similar experiments have been conducted with ^{14}C-MCPA on the phenoxy-resistant giant buttercup (McNaughton 1991). This study also showed no difference between susceptible and resistant biotypes in foliar penetration of herbicide. McNaughton concluded that resistance in giant buttercup resulted from a combination of reduced translocation to the stolons and decarboxylation of MCPA.

The MCPA molecules used in the giant buttercup work contained ^{14}C only in the carboxyl side-chain, unlike the ring-labelled molecules used in our work. Large quantities of radioactivity were lost from the giant buttercup tissue, and in some cases there was more lost from resistant than susceptible plants. Although no ^{14}C could be detected in the air near treated tissue, it was assumed the side chain was being cleaved from the MCPA molecule and radioactivity was lost through subsequent production of $^{14}CO_2$. This form of phenoxy degradation has been detected in a number of plant species (Pillmoor and Gaunt 1981).

If decarboxylation had occurred in our experiments, radioactivity would not have been lost. The ^{14}C atoms would have remained in the resulting phenol metabolite, and this molecule in turn would have been conjugated with glucose to become water soluble (Pillmoor and Gaunt 1981). Our results were consistent with this scenario because a higher quantity of water soluble materials were detected in resistant plants (Section 9.4.3.1). However further work would be required to prove it, either by repeating the experiments using chain-labelled 2,4-D, or improving the identification procedure for the water soluble compounds.

Whether increased rates of degradation involved decarboxylation, hydroxylation, amino acid conjugation or some other metabolic pathway, presumably there were greater quantities of the enzyme necessary to control this metabolism within the resistant plants. Very little is known of the enzymes responsible for the metabolism of the phenoxyacetic acids, though experiments have suggested that inducible enzymes may be involved in some cases (Pillmoor and Gaunt 1981). This increase in enzyme quantity over time as a result of exposure to herbicide would be consistent with observations in our trials. There was usually no difference between resistant and susceptible plants in severity of symptoms for the first few days after treatment, then the resistant plants recovered and the susceptible plants continued to die. An inducible enzyme system that has been implicated with detoxification of foreign molecules in plants is the microsomal cytochrome P450 monooxygenase system, which has been shown to catalyze hydroxylation of 2,4-D (Jones and Casely 1989).

Hydroxylation of the phenyl ring followed by glucose conjugation has been shown recently to be a major metabolism pathway for tribenuron-methyl in wheat (Ryan and Dulka 1990). If cross-resistance to this herbicide did exist in nodding thistle, as suggested by results reported in Chapter 5, this would be explained by the presence of an enzyme system which catalyses hydroxylation.

An understanding of resistance mechanisms for herbicides in plants can occasionally assist with designing control strategies for such plants. For example, if resistance was caused by poor penetration of cuticles, the addition of a surfactant to the herbicide could help overcome this resistance. One possible strategy for phenoxy-resistant nodding thistle could be to prevent the functioning of the enzyme system responsible for deactivating the herbicides. Research has shown that the activity of cytochrome P450 can be blocked by several chemicals (*eg* aminobenzotriazole), thus inhibiting the metabolism of some herbicides (Jones and Casely 1989). If this enzyme system was responsible for herbicide resistance in nodding thistle, the addition of a chemical such as aminobenzotriazole to the herbicide applied to weeds could overcome the

resistance.

One chemical readily available in New Zealand which inhibits cytochrome P450 activity is paclobutrazol (Burden *et al* 1987), a plant growth regulator used in this country to increase crop yields in stonefruit species (O'Connor 1989). Paclobutrazol inhibits a cytochrome P450 mediated oxidation of kaurene to kaurenoic acid, which therefore prevents production of the plant growth hormone gibberellic acid (Burden *et al* 1987).

Field trials were conducted in 1990 by DowElanco staff to determine whether the addition of paclobutrazol to 2,4-D would improve the control of phenoxy-resistant nodding thistle. Despite several application rates being used, no improvement in control of the nodding thistle plants was obtained (B. Harris, pers comm). However application rates ranged from 0.05 - 0.40 kg ai/ha, yet effects on plants are often not measurable until rates exceed 0.5 kg ai/ha (Hebblethwaite 1987). Therefore this trial did not conclusively show that paclobutrazol is ineffective at overcoming the resistance mechanism. Gressel (1990) has discussed how there are probably many different types of monooxygenases involved with inhibiting metabolism of herbicides. Thus, even if paclobutrazol is ineffective at overcoming the resistance mechanism, monooxygenases may still be involved and inhibition may be possible using other chemicals. Gressel listed several other chemicals which have been used to inhibit monooxygenases in plants, and any of these may be effective at eliminating resistance in nodding thistle.

Although it would be worthwhile testing these chemicals, there may be practical problems with using them with 2,4-D to improve control of nodding thistle in New Zealand. If a chemical were to prevent deactivation of 2,4-D by nodding thistle, it may also prevent such deactivation in white clover or even grass species, thus increasing damage to desirable pasture species. The addition of another chemical to 2,4-D might also increase the cost of the herbicide treatment substantially, making control of nodding thistle uneconomic.

9.6 CONCLUSION

The mechanism by which resistant nodding thistle plants survived applications of phenoxy herbicides did not involve reduced foliar penetration nor increased exudation from the roots. There appeared to be a small but significant increase in binding of 2,4-D within susceptible plants. However the main mechanism of resistance apparently involved deactivation of herbicide molecules before damage to the plant

could occur. This may have involved hydroxylation of the phenyl ring or decarboxylation of the side-chain, followed by formation of sugar conjugates, or increased rates of amino acid conjugation or sugar ester formation. Although the identity of the resulting compounds was not established, significant differences were detected between resistant and susceptible plants in radiolabelled metabolites. It may be possible to block this deactivation of 2,4-D by adding a chemical which inhibits the enzymes involved with this metabolism.

CHAPTER 10: CONCLUDING DISCUSSION

10.1 INTRODUCTION

As stated in Section 1.1, the objective of this project was to gain an understanding of why MCPA and 2,4-D consistently give poor control of nodding thistle in parts of Hawkes Bay. It was considered that an understanding of this phenomenon might lead to improved recommendations for the control of this weed.

The development of herbicide resistance within some nodding thistle populations was soon found to be the cause of control problems (Chapters 3 and 4). My discovery of resistance to MCPA and 2,4-D within nodding thistle populations was one of the first documented cases in the world of resistance developing to phenoxy herbicides. The relationship between previous spraying history and the incidence of phenoxy herbicide resistance shown in Chapter 6 gave better proof of resistance having developed as a result of selection pressure from the herbicides than previously documented cases. Other experiments conducted as part of this study showed that cross-resistance occurred to MCPA, MCPB and 2,4-D, but not other "hormone herbicides" such as mecoprop, clopyralid or picloram (Chapter 5). Phenoxy-resistant nodding thistle populations were located at numerous other sites throughout Hawkes Bay and Waikato (Chapter 6). No fitness differential was found between resistant and susceptible biotypes for vegetative growth under nutrient stress (Chapter 7). Physiological investigations showed resistance was due to differences in metabolism between biotypes rather than to differences in herbicide absorption or translocation (Chapters 8 and 9).

Each experiment provided more information about resistance, and implications of the findings were discussed in the appropriate chapters dealing with each experiment. However it would be useful now to draw these findings together in a final discussion of herbicide resistant nodding thistle populations and options available for their control.

10.2 REASONS FOR RESISTANCE DEVELOPING

10.2.1 Introduction

Much of the discussion of herbicide resistance in recent years has made use of a model developed by Gressel and Segel (1982) and which was revised only recently (Gressel

and Segel 1990). This model identifies factors which determine the rate at which resistance will develop within a plant population. A simplified version of the model is given by the following equation (Gressel 1991):

$$N_n = N_o \left[1 + \frac{f\ (\ \alpha_r\ /\ \alpha_s\ )\ -\ 1}{y} \right]^n$$

where:
N_n = the proportion (frequency) of resistant individuals (N) after n years of treatment.
N_o = the initial frequency of resistant individuals in the population.
f = the overall competitive fitness of resistant individuals relative to the wild type when the herbicide is absent. Values of 0.1 to 0.5 have been calculated for most atrazine-resistant weeds (Gressel 1991).
y = the average residence time (years) in the soil seed bank. This factor was modified for rotational cropping situations by Gressel and Segel (1990).
α_r = the proportion of resistant plants remaining to set seed at the end of a season following application of a herbicide.
α_s = the proportion of susceptible plants remaining to set seed at the end of a season following application of a herbicide.
α_r/α_s = the selection pressure of the herbicide.

This model has successfully explained most herbicide resistance phenomena to date (Gressel 1991), although it has also been used to argue that resistance is unlikely to develop with phenoxy herbicides (Gressel and Segel 1982). The factors determined by the Gressel and Segel models to be important in controlling the development of resistance are discussed below in relation to findings from the nodding thistle experiments.

10.2.2 Selection Pressure of Herbicides

The selection pressure of a herbicide influences the rate at which herbicide resistance develops within a plant population by determining the ratio of resistant versus susceptible plants setting seed each year. Most of the earlier documented cases of herbicide resistance involved triazine herbicides such as simazine (Gressel *et al* 1982). The persistence of these compounds ensured that susceptible individuals were controlled as they established for many months after application, so seed production from susceptible plants was very low compared with that from resistant plants. Herbicides such as 2,4-D with low persistence were considered less likely to exert sufficient selection pressure to create resistant populations because susceptible plants would germinate and set seed following herbicide application (Gressel and Segel 1982).

However less persistent herbicides could create sufficient selection pressure if they were applied several times annually, and this has occurred with paraquat (Powles and

Holtum 1990; Matsunaka and Itoh 1991). Gressel (1991) predicted that for weeds which have a single major productive flush of germination, resistance could evolve if the timing of low persistence herbicide applications coincided with full germination.

The appearance of resistant populations of nodding thistle suggests that 2,4-D has exerted sufficient selection pressure despite its short persistence. One contributing factor must be the length of the nodding thistle life cycle, usually considered to be biennial. Although nodding thistle can grow as an annual under some growing conditions, it still takes many months to produce seeds (Popay and Medd 1990) compared with the relatively short-lived annuals such as groundsel (*Senecio vulgaris* L.), fathen (*Chenopodium album* L.) and redroot (*Amaranthus powellii* S.Wats.) (Bandeen *et al* 1982) which have developed resistance to triazines. Although 2,4-D or MCPA are usually applied only once every year to control nodding thistle infestations, most plants establish successfully only in autumn and the application is usually timed to kill all of these plants (Popay and Medd 1990). Some of the farms with resistant nodding thistle populations have been sprayed twice a year (Chapter 6) which would further increase the selection pressure.

Although most other New Zealand pasture weeds also have many months of vegetative growth prior to seed production, nodding thistle has probably been subjected to greater selection pressure than many other species because of its special status as a Noxious Plant (Section 6.5).

Apart from the timing of herbicide applications, another factor which influences the selection pressure on weed populations is the proportion of susceptible individuals not killed by the herbicide at the time of application (Gressel and Segel 1982). If a number of susceptible nodding thistle plants within a paddock were missed during spraying, they would subsequently produce seeds and therefore lower the selection pressure of the herbicide. The proportion of plants missed during spraying potentially could be quite high in the hilly terrain on which nodding thistle often grows in New Zealand. However the development of resistance in many populations within New Zealand hill country indicates that few susceptible plants do get missed, and this is probably due to the efficiency of aerial application used on most of these properties. Trials with aerial application of herbicides in the Argyll area have shown the reliability of this practice (McLean and Dixon 1972).

Age of plants and environmental conditions at time of application can also influence the proportion of susceptible plants surviving herbicide applications. As discussed in Sections 1.2.4 and 1.4.1, nodding thistle becomes more tolerant of phenoxy herbicides as it becomes older or when growth rates are low. Control of nodding thistle by

MCPA or 2,4-D can be very poor when plants have begun flowering, and also in mid-winter when growth rates are slow (Popay *et al* 1989). Even when application is timed correctly so that the majority of thistles in a paddock are young and actively growing, most populations will have a few plants present which are older following germination earlier in the season than the other plants (Popay and Kelly 1986). These older plants will be less susceptible to phenoxy herbicides (Popay *et al* 1989) and thus may not die. Tolerance due to age or environmental factors will allow plants with a "susceptible" genotype to survive herbicide applications and successfully set seed.

Presumably selection pressures exerted by phenoxy herbicides on nodding thistle populations will differ significantly between neighbouring farms depending on the ability of individual farmers to optimize timing of spraying, and on the ability of spraying contractors to successfully apply the herbicide. However, although selection pressure for nodding thistle populations may be reasonably high on some properties, it generally appears to be quite low because most farmers have large numbers of new plants germinating every year despite past spraying practices. This has necessitated the annual spraying of paddocks which has apparently led to resistance establishing. Thus, although selection pressure may be higher than originally anticipated on some properties due to efficient and timely herbicide application, factors other than high selection pressure must also be important for development of resistance in nodding thistle.

10.2.3 Initial Frequency of Resistance Gene

The initial frequency of the gene(s) responsible for resistance within a population was another variable identified by Gressel and Segel (1982) as important in determining how long it takes for resistance to become evident. They stated that the initial frequency depends on the number of genes involved, the dominance and the ploidy.

The genetics of resistance within nodding thistle is an aspect which has not been addressed in the present study. To fully understand the resistance of nodding thistle to phenoxy herbicides, information on the inheritance of the trait would be useful. At present it is not known whether only one gene is involved or whether resistance in nodding thistle is controlled polygenically.

Anecdotal evidence presented in Chapter 6 suggested that noticeable levels of resistance were appearing in some populations after only 10 years of spraying. However using Gressel and Segel's (1982) model, this does not allow the initial gene frequency to be estimated unless good information on the other factors influencing rate

of resistance build-up can be obtained. There was some evidence that resistance may have built up more rapidly in populations which have established in an area since the late 1960s (Chapter 6). As discussed in Section 6.4.4, the seeds from which these populations established may have come from neighbouring populations which had been subjected to phenoxy herbicides. Thus the initial frequency of the resistance gene(s) may have been higher in these new populations than in populations which were well established before the advent of phenoxy herbicides.

10.2.4 Seed Bank

A major factor identified by Gressel and Segel (1982) influencing the rate of increase in resistance is the buffering effect of susceptible seeds germinating after remaining dormant in the soil for many years. This is doubtlessly important in traditional cropping situations where the soil is cultivated frequently, allowing dormant seeds to be brought to the surface. In assessing the success of their model, Gressel and Segel (1990) noted that resistance was most likely to occur in situations where the soil was not cultivated, such as orchards, road-sides and in no-till agriculture. Susceptible seeds would remain buried in such habitats, and seeds left on the surface would be short-lived.

Minimal soil disturbance is another factor which would favour the build-up of resistance within pasture species. The soil is very seldom cultivated in pastoral enterprises, especially in the rolling hill country where nodding thistle was studied in this project. Thus most nodding thistle seed would remain near the soil surface. Some seeds may become buried by falling down cracks, being trodden under by livestock when the soil is wet, being covered by earthworm casts or being deposited underground following ingestion by earthworms (Popay and Medd 1990). Any seeds that do get buried must be brought back to the soil surface to germinate. Earthworms have been shown to bring weed seeds to the surface in their casts without affecting the seed viability (McRill 1974). Experiments have shown that those earthworm species typically found in Hawkes Bay and Waikato pastures can ingest nodding thistle seeds and deposit them in casts without the seeds losing viability (J. Springett, unpub data). This appears to be the only likely mechanism by which buried seeds could be brought back to the soil surface. Unfortunately no quantitative data could be found for the number of nodding thistle seeds brought to the surface by earthworms, but presumably it is much lower than would occur with cultivation. Although nodding thistle seed can last many years in the soil when buried below 4 cm, seed within 2 cm of the soil surface survives only 2-3 years at the most (Popay *et al* 1987). Thus it would appear that buried seeds of susceptible individuals are unlikely to reduce the rate of resistance

build-up significantly in pastures.

10.2.5 Fitness of Resistant Individuals

As discussed in Chapter 7, a common characteristic of populations resistant to pesticides has often been a reduced fitness of the resistant individuals compared with susceptible individuals. Gressel and Segel (1982) considered reduced fitness to be important in decreasing the rate at which resistance to herbicides would develop within weed populations. However plants resistant to a herbicide will still produce much more seed than susceptible plants following treatment by the herbicide. Reduced fitness will adversely influence the development of resistance within a population only when the herbicide is absent, at which time susceptible plants will produce more seed than their resistant counterparts. This influence of fitness on the development of resistance in years when the herbicide is absent was discussed by Gressel and Segel (1990) when they revised their model. They discussed evidence indicating that reduced fitness of herbicide resistant individuals decreased seed production relative to susceptible individuals during competition, and also increased their susceptibility to cultivation, diseases and herbicides from other chemical groups ("negative cross-resistance").

Results presented in Chapter 7 suggest that resistant nodding thistle plants do not differ significantly in fitness from susceptible plants. However much further research is required to determine definitively that fitness does not differ between nodding thistle biotypes. Gressel and Segel (1982) stated that reduced fitness might manifest itself as a reduction in the: (a) proportion of seed germinating at a given time; (b) rate of germination; (c) success in establishment following self-thinning; (d) growth rate; (e) plasticity; and (f) seed size, and yield per flower and per plant. They have since discussed some of these variables further (Gressel and Segel 1990), and emphasised that fitness should be measured at various densities and ratios of sensitive to resistant individuals, not just a 1:1 ratio as used for our study.

The competition study at a 1:1 ratio of Argyll (resistant) and Matapiro (susceptible) plants under severe nutrient stress gave no indication of reduced fitness, and observations of relative seed size, germination, susceptibility to diseases, susceptibility to non-phenoxy herbicides, growth rates under various environmental conditions and seed production have not indicated differences in fitness. However, apart from formalising these observations with careful measurements, further factors which need investigating include relative susceptibility to drought conditions, shading by pasture plants at establishment, competition with pasture species, seed longevity and dormancy

mechanisms.

Plants resistant to residual herbicides would generally face little competition from neighbouring vegetation for months after application of the herbicides because most germinating plant species continue to be killed while residues of the herbicide remain active. In contrast, herbicide resistant nodding thistle plants must be competitive to survive in New Zealand pastures even if phenoxy herbicides have been applied because pasture grasses continue to grow strongly. Thus it would appear unlikely that herbicide resistance could have ever developed in nodding thistle if significant fitness disadvantages existed within resistant individuals. However, without detailed studies, caution is required in reaching such a conclusion. For example, nodding thistle often germinates in drought-damaged pastures, so competition with resident vegetation is initially minimal. If fitness differentials only affect plants at this stage, resistant plants may not be adversely affected.

10.2.6 Conclusions

Although MCPA and 2,4-D are not residual herbicides, several factors have apparently combined to allow these herbicides to apply sufficient selection pressure to nodding thistle populations to induce resistance. Nodding thistle effectively germinates only once a year because pasture competition prevents germination at any time other than autumn (Popay and Kelly 1986). The biennial life cycle of nodding thistle means that correctly timed annual applications of phenoxy herbicide are sufficient to remove almost all nodding thistle plants which are not resistant before they can set seed (Popay *et al* 1989). Although nodding thistle often grows on hilly terrain, aerial application of herbicides can result in very high kill rates (McLean and Dixon 1972). The desire of farmers to eradicate this species from their properties, and the legal compulsion to control it in most areas, have resulted in farmers applying herbicides to nodding thistle populations every year for many years. Nodding thistle seed only persists in the soil if it is buried (Popay *et al* 1987), so the minimal soil disturbance which occurs in New Zealand hill country pastures prevents much reinfestation of pastures from susceptible seeds buried in earlier years. There does not appear to be major fitness differences between resistant and susceptible individuals to slow the rate at which resistance can develop.

10.3 PREVENTING FURTHER DEVELOPMENT OF RESISTANCE TO PHENOXY HERBICIDES

10.3.1 Nodding Thistle

As will be discussed in Section 10.4, the options available to control phenoxy resistant nodding thistle selectively and economically in pastures are rather limited once resistance has developed. The best strategy would be to avoid resistance developing initially. The exact details of factors discussed in Section 10.2 responsible for resistance developing in New Zealand populations of nodding thistle probably do not need understanding before strategies can be designed to curb further cases of resistance occurring.

General strategies for preventing development of resistance have been discussed in the past in a number of publications (*eg* Gressel and Segel 1982; Slife 1986; Gressel 1987; Gressel 1991). However many of these strategies are not particularly appropriate to the New Zealand hill country pastoral situation. Crop rotation to allow different herbicide types to be used is not particularly practical in hill country suited only to grassland production. Some fodder crops such as marrow-stem kale (*Brassica oleracea* L. var. *medullosa* Thell.) are sown in these areas, but usually only on a very small proportion of the farm and only for less than one year before replanting in pasture. Rotation of herbicides or using mixtures of herbicides is also not very practical since cross-resistance exists between the three main herbicides that could be used, *ie* MCPA, 2,4-D and MCPB. Other herbicides are not selective enough to be used in clover-based pastures, and this will be discussed further in Section 10.4.

Most strategies to prevent herbicide resistance developing usually involve decreasing the selection pressure by a herbicide on a weed population. The formula used to define selection pressure in Section 10.2.1 suggests selection pressure can be decreased either by increasing the number of susceptible plants setting seed, or decreasing seed production by resistant plants. If the fitness of resistant plants is low, seed production from these plants might be more susceptible to strategies such as grazing pressure, competition from pastures or adverse environmental conditions such as drought. However, as discussed above, any fitness differential between susceptible and resistant plants is probably too small to be useful. Another strategy to prevent seed production from resistant plants when resistance was just developing would be to spot-spray every nodding thistle plant which survived applications of phenoxy herbicides with a herbicide such as dicamba or clopyralid. However, given the extensive hilly terrain on which these populations grow and the relatively low return per hectare from grassland farming, this strategy would probably also not be feasible economically or practically.

The most appropriate strategy to prevent resistance occurring in these populations is probably to allow greater numbers of susceptible plants to set seed. As discussed in Chapter 6, herbicide resistance generally occurs on farms where herbicides are applied frequently in an attempt to eradicate the species from the property. Those farmers who only spray occasionally when thistle numbers are excessively high, and thus are seeking merely to control rather than eradicate populations, do not have resistance problems. This less rigorous attitude to nodding thistle control has been forced on many farmers recently, not by the threat of resistance occurring, but by a difficult economic climate.

Research has shown that densities of a similar thistle species, Scotch thistle (*Cirsium vulgare*), must reach 1.7 plants/m^2 before it is economic to apply MCPA in New Zealand pastures (Hartley 1983). At densities below this, the damage caused to the clover component of the pasture by MCPA reduces animal production more than the reduced utilization of the pasture caused by the presence of thistles. The similarity in size and growth between nodding thistle and Scotch thistle suggests a similar economic threshold exists for the control of nodding thistle. For properties where nodding thistle is well established, it may be prudent to spray only when these high densities are reached. By keeping the pasture dense through good grazing management and growing the most competitive pasture species available, nodding thistle should be kept below the threshold density in most years. If phenoxy herbicides are used only when pasture management techniques fail to keep thistle densities low, the selection pressure over past years will have been low enough to ensure good control is obtained.

Nodding thistle is a species which is still spreading within New Zealand (Popay and Medd 1990). A strategy of minimal spraying would be unacceptable on properties where nodding thistle has only begun establishing. Annual applications of phenoxy herbicides should be encouraged if infestations are small enough to attempt eradication. However it would appear important that plants which survive phenoxy applications are destroyed either by hand-grubbing or spot-application with a herbicide such as dicamba or clopyralid. This would be practical if only a small part of the property is infested. Not only should this decrease the number of years required to eradicate the population by ensuring no seed is set, it should also stop herbicide resistance from developing. With herbicide resistance present on many properties in Hawkes Bay and Waikato, it is possible that the resistance gene(s) would be present at a high initial frequency in populations establishing on a property from seed brought in from another area.

10.3.2 Other Pasture Species

Although this project has concentrated only on nodding thistle, it appears possible that resistance to phenoxy herbicides could develop in other pasture weed species in New Zealand as these herbicides are used to control many species (O'Connor 1989). It has developed in giant buttercup (Bourdot *et al* 1989) and may have developed in slender winged thistle (Section 6.3). If other pasture weed species were investigated, it is probable that further cases of phenoxy resistance would be discovered. Suspicions have existed for some years among New Zealand weed scientists that resistance has developed in some localities in species such as winged thistle (*Carduus tenuiflorus*), Californian thistle (*Cirsium arvense*), variegated thistle (*Silybum marianum*), ragwort (*Senecio jacobaea*) and blackberry (*Rubus fruticosus*) (Popay *et al* 1991).

Pasture species in which resistance is most likely to develop are those which farmers consider serious enough to warrant annual spraying. Such species will probably be highly visual and capable of forming dense infestations, motivating farmers to attempt eradication of the species from their properties. The Noxious Plants Act in New Zealand may also influence which species will get sprayed often enough to allow resistance to develop.

As with nodding thistle, strategies to prevent resistance from building up should probably involve decreasing applications to well established populations, and increasing control efforts with new establishing populations. Once a species is well established on a property, pasture management techniques could be used to minimize problems caused by the weed. Phenoxy herbicides should only be used in those years when the weed density becomes particularly high. Leathwick and Bourdot (1991) have suggested applying MCPA to giant buttercup only after flowering has occurred so that seeds of susceptible plants will survive the spraying and thus minimize the build-up of resistant biotypes. When new populations of a species are establishing on a property, annual applications of phenoxy herbicides should be followed by spot-treatment of any surviving plants with an alternative herbicide or a grubbing hoe.

Resistance could also develop in desirable pasture species which are exposed to annual applications of phenoxy herbicides to nearby weed species. White clover was found to recover very rapidly from high application rates of phenoxy herbicides at some of our nodding thistle trial sites in Hawkes Bay. When white clover plants were collected from all of our Hawkes Bay sites (Fig 6.1) and treated with phenoxy herbicides in a glasshouse, significant variability in tolerance was noted (Popay, pers comm). If a gene conferring resistance to phenoxy herbicides could be incorporated into

commercial white clover cultivars, advantages could be obtained for pastoral farming from this otherwise troublesome phenomenon. Phenoxy herbicides could be used for pasture weed control without the subsequent drop in production from the clover component of pastures that presently occurs (Thompson and Saunders 1984). However this might also lead to greater use of phenoxy herbicides than at present, and thus more cases of herbicide resistance in pasture weeds could develop.

10.3.3 Other Land Uses

Although resistance has developed to phenoxy herbicides in New Zealand pastures, it appears unlikely that resistance to these herbicides will develop in non-pasture situations. The clover component of New Zealand pastures and low returns from pastoral enterprises generally restricts herbicide use in these situations to MCPB, MCPA and 2,4-D because clovers can recover successfully following their application and these herbicides are relatively inexpensive.

In other New Zealand situations where phenoxy herbicides are used, many alternative herbicides are available. In cereal production, alternatives to phenoxy herbicides include chlorsulfuron, mecoprop, dichlorprop, clopyralid, dicamba, ioxynil, bromoxynil and trifluralin. Weed control in turf involves use of alternatives such as ioxynil, bromoxynil, mecoprop, dichlorprop, clopyralid, dicamba, picloram, bentazone and bromofenoxim. Use of alternative herbicides in these situations is common to control those weed species not susceptible to phenoxy herbicides, so any development of resistance within susceptible species would be stopped.

In New Zealand, 2,4-D is also used for control of some scrub species and weeds of waste areas, *eg* wineberry (*Aristotelia serrata* (Forst.) Oliver), silver poplar (*Populus alba* L. cv Nivea), greater bindweed (*Calystegia silvatica* (Kit.) Griseb.), and goat's rue (*Galega officinalis* L.) (O'Connor 1989). However alternative herbicides also exist for many of these species, including amitrole, glyphosate, dicamba, metsulfuron and picloram. Some of these alternative herbicides are routinely mixed with 2,4-D to improve control. Thus resistance would be unlikely to develop for most of these species.

10.4 CONTROL OF PHENOXY-RESISTANT NODDING THISTLE

10.4.1 Introduction

The preceding section discussed ways of preventing herbicide resistance from developing. However results presented in Chapter 6 indicated that many populations of nodding thistle resistant to phenoxy herbicides are already present. Potential strategies for controlling these populations are discussed below.

10.4.2 Alternative Herbicides

The potential to use alternative herbicides to control these populations has already been explored in some depth in Chapter 5 (especially Section 5.5.1). Cross-resistance exists to MCPA, 2,4-D and MCPB. Those herbicides which still effectively control phenoxy-resistant nodding thistle plants are very damaging to pasture species. Clopyralid, picloram, dicamba and mecoprop severely damage clovers, while glyphosate and paraquat/diquat also damage grasses. The current recommendation to farmers with resistance problems is to add 30 g ai/ha of clopyralid to normal rates of 2,4-D or MCPA (O'Connor 1989). However this low rate still causes substantial damage to clovers (Section 5.5.1). Wick-boom application of glyphosate or picloram to bolting thistles is an option, but this only controls thistles after they have severely reduced pasture utilization for many months as vegetative rosettes.

The range of herbicides that could be used would increase markedly if pastures were grown without a legume component. In many agricultural systems in other parts of the world, nitrogen fertilizers are used rather than legumes to supply the nitrogen requirements of pasture grasses (Keeney and Gregg 1982). However the low economic return from pastoral farming in New Zealand makes it unlikely that farmers would be prepared to replace nitrogen fixation by clovers with annual applications of nitrogen fertilizer simply to allow better control of thistles.

Nodding thistle can be controlled by bentazone (Popay 1986) and this herbicide is selective in clover-based pastures (O'Connor 1989). It was not tested for cross-resistance in this project because it costs approximately nine times more per hectare than the 2,4-D currently used for thistle control. Thus bentazone may be effective at controlling phenoxy-resistant nodding thistle but application of this herbicide on sheep farms is unlikely to be economic.

10.4.3 Improving Effectiveness of Phenoxy Herbicides

If resistance had been caused by poor penetration of the foliage, the resistance mechanism could have been overcome using surfactants to increase foliar uptake. However results obtained throughout the project have shown that penetration of the foliage is not a limiting factor.

There is some evidence that phenoxy resistant nodding thistle plants may be more susceptible to 2,4-D when they are young seedlings, allowing control of field populations of "resistant" nodding thistle seedlings by farmers using only slightly increased application rates (Section 6.4.4). This practice would require very careful timing of applications but has apparently been used successfully. Difficulties would arise if seedlings germinated over several months in autumn, as this would probably necessitate several herbicide applications to ensure all seedlings were young enough to die when sprayed.

If phenoxy herbicides were applied to resistant plants while they were young, susceptibility of seedlings could be increased further by adding a surfactant. Bourdot *et al* (1989) found that addition of surfactants to phenoxy-resistant giant buttercup did not affect the relative difference in susceptibility between resistant and susceptible populations, but it did decrease the LD50 for both populations by 25%. Thus the addition of a surfactant could cause some improvement in control of nodding thistle seedlings, but consideration would also be needed with respect to the cost of adding a surfactant and whether clovers would suffer increased damage. Bourdot *et al* (1989) found addition of surfactant to be less expensive than increasing herbicide application rates but did not determine how much extra clover damage resulted from adding surfactants.

The use of livestock to graze nodding thistle following application of sub-toxic doses of MCPA was discussed in Section 1.2.3.1. Plants become more palatable to livestock, resulting in severe defoliation which can lead to plant death. This "spray-grazing" strategy could be useful to increase pressure on resistant seedlings which may not have received quite enough herbicide to cause outright death.

Results presented in Section 8.3.3 showed that the resistance mechanism could be overcome to some extent by applying MCPA to roots of nodding thistle plants rather than to the foliage. As herbicide degradation appears to be responsible for resistance (Chapter 9), this result suggests the enzymes responsible for degradation may exist mainly in the foliage. If phenoxy herbicides were applied to pastures as granules, it is possible that the subsequent root uptake of herbicides as they were released from

granules by rainfall might control phenoxy resistant nodding thistle plants without damaging clovers. However, even if clovers did not also become more susceptible to the herbicide when applied this way, costs would probably be prohibitive. Phenoxy herbicides are not available in New Zealand as granules, but generally granulated formulations are expensive to produce and transport (Green *et al* 1987). Increased application rates of active ingredient would probably also be necessary to allow for the rapid microbial degradation of herbicide that is likely before uptake by plant roots (Loos 1975).

It may be possible to block the enzyme system responsible for deactivating the herbicide within resistant plants by adding a synergist to the herbicide (Gressel 1990), and this has already been discussed in some detail (Section 9.5). This is probably the most promising strategy for improving the control of resistant populations by phenoxy herbicides. However it is similar to other strategies discussed in this section in that, even if such a synergist could be discovered, it is quite possible that the cost of such a chemical or the increase in damage to clovers that could be caused may make such a treatment impractical.

10.4.4 Non-Chemical Techniques

The various strategies that could be used to control phenoxy-resistant herbicides using chemicals have been outlined above, and generally they involve damage to the pasture or require further research. Another strategy could be to abandon the use of chemicals and to control the resistant nodding thistle populations using the various non-chemical techniques discussed in Section 1.2.3. This option would be more attractive if resistant plants were significantly less fit than usual as suggested by Gressel and Segel (1982). Unfortunately there has been no indication that reduced fitness does exist in resistant plants (Section 10.2.5). Most techniques which do not use chemicals have drawbacks (Section 1.2.3).

10.5 FURTHER RESEARCH

As this is one of the first documented cases of resistance developing to phenoxy herbicides, further research into a number of aspects would be useful to help understand the phenomenon of herbicide resistance. The genetics of the resistance mechanism needs investigating as this will have a major influence on the rate at which resistance develops. If a single gene is responsible for resistance and this can be identified, it may be possible to transfer this gene into crop species which are normally

susceptible to phenoxy herbicides.

The proportion of susceptible individuals which escape control by phenoxy herbicides and set seed each year under various spraying regimes should be investigated to determine the typical selection pressure for resistance in New Zealand pastures. This would give some indication as to how selection pressure could be decreased sufficiently to prevent resistance developing.

The extent of buffering of resistance development through seed burial and reemergence by earthworms would be interesting. If there is little movement of seeds from herbicide resistant plants into the soil, occasional deep cultivation of affected pastures might significantly alter the proportion of susceptible to resistant plants germinating at the surface. If substantial movement of seeds by earthworms was discovered at some sites, encouragement of earthworm activity at other sites might be a feasible option for slowing the build up of resistance.

The relative fitness of resistant and susceptible plants also requires further investigation. If no differences exist, this would be of interest to herbicide resistance researchers. If differences can be discovered, these might be useful for devising control strategies.

The most promising area of research for controlling resistant nodding thistle plants would appear to be in confirming whether monooxygenases or similar such systems are responsible for metabolism of 2,4-D and MCPA, and then screening potential synergists for their ability to prevent this metabolism.

BIBLIOGRAPHY

Abel, A.L. (1954). The rotation of weed killers. Proceedings British Weed Control Conference 1: 249-255.

Aberg, E. and Stecko, V. (1975). Internal factors affecting toxicity. In: Audus, L.J. (ed): Herbicides - physiology, biochemistry, ecology. Vol 2, pp 175-201. Academic Press, London.

Agbakoba, C.S.O. and Goodin, J.R. (1970). Absorption and translocation of ^{14}C-labelled 2,4-D and picloram in field bindweed. Weed Science 18: 168-170.

Allan, H.H. (1940). A handbook of the naturalized flora of New Zealand. DSIR Bulletin No 83, Government Printer, Wellington. 344 pp.

Ashton, F.M. and Crafts, A.S. (1981). Mode of action of herbicides. John Wiley and Sons, New York. 525 pp.

Anderson, W.P. (1977). Weed science: principles. West Publishing Co, New York. 598 pp.

Atkinson, G.C. and Meeklah, F.A. (1980). Weed control in lucerne. Aglink FPP 403, Ministry of Agriculture and Fisheries, Wellington. 2 pp.

Bandeen, J.D., Stephenson, G.R. and Cowett, E.R. (1982). Discovery and distribution of herbicide-resistant weeds in North America. In: LeBaron, H.M. and Gressel, J. (eds): Herbicide resistance in plants, pp 9-30. John Wiley and Sons, New York.

Bartels, P.G. (1985). Effect of herbicides on chloroplast and cellular development. In: Duke, S.O. (ed): Weed physiology. Vol II. Herbicide physiology, pp 63-90. CRC Press, Florida.

Bell, A.R., Nalewaja, J.D. and Schooler, A.B. (1972). Response of kochia selections to 2,4-D, dicamba and picloram. Weed Science 20: 458-462.

Bendall, G.M. (1973). The control of slender thistle, *Carduus pycnocephalus* L. and *C. tenuiflorus* Curt. (Compositae), in pasture by grazing management. Australian Journal of Agricultural Research 24: 831-837.

Beyer, E.M., Brown, H.M. and Duffy, M.J. (1987). Sulphonylurea herbicide soil relations. Proceedings 1985 British Crop Protection Conference - Weeds: 531-540.

Bhan, V.M., Stoller, E.W. and Slife, F.W. (1970). Toxicity, absorption and metabolism of 2,4-D in yellow nutsedge. Weed Science 18: 733-737.

Bourdot, G.W., Harrington, K.C. and Popay, A.I. (1989). The appearance of phenoxy-herbicide resistance in New Zealand pasture weeds. Brighton Crop Protection Conference - Weeds - 1989: 309-316.

Bourdot, G.W. and Hurrell, G.A. (1988). Differential tolerance of MCPA among giant buttercup (*Ranunculus acris*) biotypes in Takaka, Golden Bay. Proceedings NZ Weed and Pest Control Conference 41: 231-234.

Bourdot, G.W., Hurrell, G.A. and Saville, D.J. (1989). Biological and economic effects of surfactant addition to MCPA for giant buttercup control. Proceedings of NZ Weed and Pest Control Conference 42: 43-47.

Bourdot, G.W., Hurrell, G.A. and Saville, D.J. (1990). Variation in MCPA-resistance in *Ranunculus acris* L. subsp. *acris* and its correlation with historical exposure to MCPA. Weed Research 30: 449-457.

Bouse, L.F. and Bovey, R.W. (1967). A laboratory sprayer for potted plants. Weeds 15: 89-91.

Bovey, R.W. (1980). Physiological effects of phenoxy herbicides in higher plants. In: Bovey, R.W. and Young, A.L. (eds): The science of 2,4,5-T and associated phenoxy herbicides, pp 217-238. John Wiley and Sons, New York.

Bradshaw, A.D. (1982). Evolution of heavy metal resistance - an analogy for herbicide resistance. In: LeBaron, H.M. and Gressel, J. (eds): Herbicide resistance in plants, pp 293-307. John Wiley and Sons, New York.

Bristol, D.W., Ghanuni, A.M. and Oleson, A.E. (1977). Metabolism of 2,4-D by wheat cell suspension cultures. Journal of Agricultural and Food Chemistry 25: 1308-1314.

Bucholtz, D.L. and Hess, F.D. (1988). An atomizer for application of very low volumes of herbicide solutions. Weed Science 36: 406-409.

Burden, R.S., Carter, G.A., Clark, T., Cooke, D.T., Croker, S.J., Deas, A.H.B., Hedden, P., James, C.S. and Lenton, J.R. (1987). Comparative activity of the enantiomers of triadimenol and paclobutrazol as inhibitors of fungal growth and plant sterol and gibberellin biosynthesis. Pesticide Science 21: 253-267.

Cartright, R. and Kok, L.T. (1985). Growth responses of musk and plumeless thistles (*Carduus nutans* and *C. acanthoides*) to damage by *Trichosirocalus horridus* (Coleoptera: Curculionidae). Weed Science 33: 57-62.

Cherry, J.H. (1976). Actions on nucleic acid and protein metabolism. In: Audus, L.J. (ed): Herbicides: physiology, biochemistry, ecology. Vol 1, pp 525-546. 2nd Ed. Academic Press, London.

Christopher, J.T., Burnet, M., Powles, S.B., Holtum, J.A.M. and Liljegren, D.R. (1992). Sulfonylurea resistance in annual ryegrass (*Lolium rigidum*) in Australia. Proceedings 1st International Weed Control Congress 2: 126-128.

Clapham, A.R., Tutin, T.G. and Warburg, E.F. (1962). Flora of the British Isles. Cambridge University Press, London. 1269 pp.

Close, R.C. and Noonan, M.J. (1984). Plant protection in wheat - a survey of grower practices. Proceedings NZ Weed and Pest Control Conference 37: 300-303.

Coble, H.D., Slife, F.W. and Butler, H.S. (1970). Absorption, metabolism and translocation of 2,4-D by honeyvine milkweed. Weed Science 18: 653-656.

Conard, S.G. and Radosevich, S.R. (1979). Ecological fitness of *Senecio vulgaris* and *Amaranthus retroflexus* biotypes susceptible or resistant to atrazine. Journal of Applied Ecology 16: 171-177.

Congdon, R.J. (1978). The Noxious Plants scheme. Conference Proceedings of the Noxious Weeds' Institute (1978): 7-12.

Coupland, D. (1986). Sample preparation for liquid scintillation counting. Aspects of Applied Biology 11: Biochemical and physiological techniques in herbicide research, pp 55-66.

Davidonis, G.H., Hamilton, R.H. and Mumma, R.O. (1980). Metabolism of 2,4-D in soybean root callus. Plant Physiology 66: 537-540.

Davidonis, G.H., Hamilton, R.H. and Mumma, R.O. (1982). Metabolism of 2,4-diclorophenoxyacetic acid in 2,4-dichlorophenoxyacetic acid-resistant soybean callus tissue. Plant Physiology 70: 104-107.

Davis, C. and Linscott, L. (1986). Tolerance of birdsfoot trefoil (*Lotus corniculatus*) to 2,4-D. Weed Science 34: 373-376.

Desrochers, A.M., Bain, J.F. and Warwick, S.I. (1988). The biology of Canadian weeds. 89. *Carduus nutans* and *Carduus acanthoides*. Canadian Journal of Plant Science 68: 1053-1068.

Delahunty, E. (1960). Control of nodding thistle. Proceedings NZ Weed Control Conference 13: 108-111.

Delahunty, E. (1961). Nodding thistle control in pastures with chemicals. NZ Journal of Agriculture 103: 23-25.

Doing, H., Biddiscombe, E.F. and Knedlhans, S. (1969). Ecology and distribution of the *Carduus nutans* group (nodding thistles) in Australia. Vegetatio 17: 313-351.

Donaldson, T.W., Bayer, D.E. and Leonard, O.A. (1973). Absorption of 2,4-D and monuron by barley roots. Plant Physiology 52: 638-645.

Eastin, E.F. and Basler, E. (1977). Absorption, translocation and degradation of herbicides by plants. In Truelove, B. (ed): Research methods in weed science, pp 89-96. Southern Weed Science Society, Alabama.

Edmonds, D.K. and Popay, A.I. (1983). Effect of pasture competition on the survival and flowering of nodding thistle. Proceedings NZ Weed and Pest Control Conference 36: 89-92.

Ellis, M. and Kay, Q.O.N. (1975). Genetic variation in herbicide resistance in scentless mayweed (*Tripleurospermum inodorum* (L.) Schultz Bip.). I. Differences between populations in response to MCPA. Weed Research 15: 307-315.

Featherstone, C.I. (1957). The progress of chemical weed control in Hawkes Bay. Proceedings NZ Weed Control Conference 10: 7-12.

Fenemore, P.G. (1977). Fungus gnats - little known pests of glasshouse plants. Commercial Horticulture 9(2): 14-15.

Ferguson, D.T., Schehl, S.E., Hageman, L.H., Lepone, G.E. and Carraro, G.A. (1985). DPX-L5300 - a new cereal herbicide. Proceedings 1985 British Crop Protection Conference - Weeds: 43-48.

Feung, C.S., Hamilton, R.H. and Mumma, R.O. (1973a). Metabolism of 2,4-D. IV. Mass spectra and chromatographic properties of amino acid conjugates. Journal of Agricultural and Food Chemistry 21: 632-637.

Feung, C.S., Hamilton, R.H. and Mumma, R.O. (1973b). Matabolism of 2,4-D. V. Identification of metabolites in soybean callus tissue cultures. Journal of Agricultural and Food Chemistry 21: 637-640.

Feung, C.S., Hamilton, R.H. and Mumma, R.O. (1975). Metabolism of 2,4-D. VII. Comparison of metabolites from five species of plant callus tissue cultures. Journal of Agricultural and Food Chemistry 23: 373-376.

Feung, C.S., Hamilton, R.H. and Mumma, R.O. (1976). Metabolism of 2,4-D. 10. Identification of metabolites in rice root callus tissue cultures. Journal of Agricultural and Food Chemistry 24: 1013-1015.

Feung, C.S., Hamilton, R.H. and Witham, F.H. (1971). Metabolism of 2,4-D by soybean cotyledon callus tissue cutlures. Journal of Agricultural and Food Chemistry 19: 475-479.

Feung, C.S., Hamilton, R.H., Witham, F.H. and Mumma, R.O. (1972). The relative amounts and identification of some 2,4-D metabolites isolated from soybean cotyledon callus cultures. Plant Physiology 50: 80-86.

Feung, C.S., Mumma, R.O. and Hamilton, R.H. (1974). Metabolism of 2,4-D. VI. Biological properties of amino acid conjugates. Journal of Agricultural and Food Chemistry 22: 307-309.

Field, R.J. and Peel, A.J. (1971). The metabolism and radial movement of growth regulators and herbicides in willow stems. New Phytologist 70: 743-749.

Finney, D.J. (1971). Probit analysis. 3rd Ed. Cambridge University Press, London. 333 pp.

Finney, D.J. (1978). Statistical method in biological assay. 3rd Ed. Charles Griffen and Co, London. 508 pp.

Fletcher, W.W. and Kirkwood, R.C. (1982). Herbicides and plant growth regulators. Granada, London. 408 pp.

Fryer, J.D. and Makepeace, R.J. (1977). Weed control handbook. Vol 1. Principles. 6th Edition. Blackwell Scientific Publications, Oxford. 510 pp.

Fryer, J.D. and Makepeace, R.J. (1978). Weed control handbook. Vol 2. Recommendations. 8th Edition. Blackwell Scientific Publications, Oxford. 532 pp.

Green, M.B., Hartley, G.S. and West, T.F. (1987). Chemicals for crop improvement and pest management, 3rd Edition. Pergamon Press, Oxford. 370 pp.

Gressel, J. (1987). Strategies for prevention of herbicide resistance in weeds. In Brent, K.J. and Atkin, R.K. (eds): Rational pesticide use, pp 183-196. Cambridge University Press, Cambridge.

Gressel, J. (1988). Multiple resistances to wheat selective herbicides: new challenges to molecular biology. Oxford Surveys of Plant Molecular and Cell Biology 5: 195-203.

Gressel, J. (1990). Synergizing herbicides. Reviews of Weed Science 5: 49-82.

Gressel, J. (1991). Why get resistance? It can be prevented or delayed. In: Casely, J.C., Cussans, G.W. and Atkin, R.W. (ed): Herbicide resistance in weeds and crops, pp 1-25. Butterworth-Heinemann, Oxford.

Gressel, J., Ammon, H.U., Fogelfors, H., Gasquez, J., Kay, Q.O.N. and Kees, H. (1982). Discovery and distribution of herbicide resistant weeds outside America. In: LeBaron, H.M. and Gressel, J. (eds): Herbicide resistance in plants, pp 31-56. John Wiley and Sons, New York.

Gressel, J. and Segel, L.A. (1982). Interrelating factors controlling the rate of appearance of resistance: the outlook for the future. In: LeBaron, H.M. and Gressel, J. (eds): Herbicide resistance in plants, pp 325-348. John Wiley and Sons, New York.

Gressel, J. and Segel, L.A. (1990). Herbicide rotations and mixtures: effective strategies to delay resistance. In: Green, M.B., LeBaron, H.M. and Moberg, W.K. (eds): Managing resistance to agrochemicals, pp 430-458. American Chemical Society Symposium Series No 421, Washington.

Grossbard, E. and Atkinson, D. (1985). The herbicide glyphosate. Butterworths, London. 490 pp.

Guthrie-Smith, H (1953). Tutira - the story of a New Zealand sheep station. 3rd Edition. William Blackwood and Sons Ltd, Edinburgh. 444 pp.

Hamilton, R.H., Hurter, J., Hall, J.K. and Ercegovich, C.D. (1971). Metabolism of 2,4-D and 2,4,5-T by bean plants. Journal of Agricultural and Food Chemistry 19: 480-483.

Hanson, J.B. and Slife, F.W. (1969). How does 2,4-D kill a plant? Illinois Research 3: 3-4. Cited by Ashton and Crafts (1981).

Harper, J.L. (1977). Population biology of plants. Academic Press, London. 892 pp.

Harrington, K.C. (1983). The influence of growth stage and application site on movement and effect of glyphosate in **Cirsium arvense** (L)Scop. M Agr Sc thesis, Massey University, Palmerston North, NZ. 149 pp.

Harrington, K.C. (1987). A technique for comparing the susceptibilty of two weed populations to a herbicide. Proceedings NZ Weed and Pest Control Conference 40: 230-232.

Harrington, K.C. (1989). Distribution and cross-tolerance of MCPA-tolerant nodding thistle. Proceedings NZ Weed and Pest Control Conference 42: 39-42.

Harrington, K.C. (1990). Spraying history and fitness of nodding thistle, *Carduus nutans*, populations resistant to MCPA and 2,4-D. Proceedings Australian Weeds Conference 9: 201-204.

Harrington, K.C. and Popay, A.I. (1987). Differences in susceptibility of nodding thistle populations to phenoxy herbicides. Proceedings Australian Weeds Conference 8: 126-129.

Harrington, K.C., Popay, A.I., Robertson, A.G. and McPherson, H.G. (1988). Resistance of nodding thistle to MCPA in Hawkes Bay. Proceedings NZ Weed and Pest Control Conference 41: 219-222.

Hartley, M.J. (1983). Effect of Scotch thistles on sheep growth rates. Proceedings NZ Weed and Pest Control Conference 36: 86-88.

Hartley, M.J. and James, T.K. (1979). Cost benefit of selective control of Californian thistle in pasture. Proceedings NZ Weed and Pest Control Conference 32: 245-249.

Heap, I.M. (1991). Resistance to herbicides in annual ryegrass (*Lolium rigidum*) in Australia. In: Casely, J.C., Cussans, G.W. and Atkin, R.W. (ed): Herbicide resistance in weeds and crops, pp 57-66. Butterworth-Heinemann, Oxford.

Hay, J.R. (1976). Herbicide transport in plants. In: Audus, L.J. (ed): Herbicides: physiology, biochemistry, ecology. Vol 1, pp 365-396. 2nd Ed. Academic Press, London.

Hebblethwaite, P.D. (1987). The chemical control of growth, development and yield in *Lolium perenne* grown for seed. Journal of Applied Seed Production 5: 54-59.

Hess, F.D. (1985). Herbicide absorption and translocation and their relationship to plants tolerances and susceptibility. In: Duke, S.O. (ed): Weed physiology. Vol II. Herbicide physiology, pp 191-214. CRC Press, Florida.

Hickey, D.A. and McNeilly, T. (1976). Competition between metal-tolerant and normal plant populations: a field experiment in normal soil. Evolution 29: 458-464.

Hill, R. (1986). Biological control of gorse: implications for the natural environment and for primary production. DSIR Entomology Division Report 6: 1-130.

Hodgson, J.M. (1970). The response of Canada thistle ecotypes to 2,4-D, amitrole and intensive cultivation. Weed Science 18: 253-255.

Holst, P.J. (1980). The use of goats in grazing systems and their place in weed control. Australian Society of Animal Production Proceedings 13: 188-191.

Holzner, W. (1982). Concepts, categories and characteristics of weeds. In: Holzner, W. and Numata, N. (eds): Biology and ecology of weeds, pp 3-20. Dr W Junk Publishers, The Hague.

Hull, H.M. (1970). Leaf structure as related to absorption of pesticides and other compounds. Residue Reviews 31: 1-155.

Hurrell, G.A., Bourdot, G.W. and Butler, J.H.B. (1983). Timing of herbicide applications for nodding thistle control in Canterbury. Proceedings NZ Weed and Pest Control Conference 36: 93-95.

Hyde-Wyatt, B.H. and Morris, D.I. (1980). The noxious and secondary weeds of Tasmania, pp 26-27. Department of Agriculture, Tasmania, Australia.

Ivens, G.W. (1980). Weed science and technology. In: Perspectives in world agriculture, pp 181-206. CAB, London.

Jansen, L.L. (1965). Effect of structural variations in ionic surfactants on phytotoxicity and physical-chemical properties of aqueous sprays of several herbicides. Weeds 13: 117-123.

Jentes, C.E. (1985). Control of musk thistle with picloram by ropewick and broadcast methods. Down to Earth 41(2): 16-20.

Jessep, C.T. (1975). Introduction of a weevil for biological control of nodding thistle. Proceedings NZ Weed and Pest Control Conference 28: 205-206.

Jessep, C.T. (1981). Nodding thistle receptacle weevil life cycle. DSIR Information Series No 105/37.

Jessep, C.T. (1989a). *Carduus nutans* L., nodding thistle (Asteraceae). In: Cameron, P.J., Hill, R.L., Bain, J. and Thomas, W.P. (eds): A review of biological control of insect pests and weeds in New Zealand 1874 to 1987. Technical Communication, CAB International Institute of Biological Control 10: 339-342. CAB International, Wallingford, UK.

Jessep, C.T. (1989b). Introduction of the crown weevil (*Trichosirocalus horridus*) as an additional biocontrol agent against nodding thistle. Proceedings NZ Weed and Pest Control Conference 42: 52-54.

Jones, O.T.G. (1991). Cytochrome P450 and herbicide resistance. In: Casely, J.C., Cussans, G.W. and Atkin, R.W. (ed): Herbicide resistance in weeds and crops, pp 213-226. Butterworth-Heinemann, Oxford.

Jones, O.T.G. and Casely, J.C. (1989). Role of cytochrome P450 in herbicide metabolism. Brighton Crop Protection Conference - Weeds - 1989: 1175-1184.

Keeney, D.R. and Gregg, P.E.H. (1982). Nitrogen fertilizers and the nitrogen cycle. In: Lynch, P.B. (ed): Nitrogen fertilizers in New Zealand agriculture, pp 19-28. Ray Richards Publisher, Auckland.

Kelly, D., Cameron, H. and Alex, J. (1988). Wind dispersal of nodding thistle seeds and pappi. Proceedings NZ Weed and Pest Control Conference 41: 207-209.

Kelly, D., McCallum, K., Schmidt, C.J. and Scanlan, P.M. (1990). Seed predation in nodding and slender winged thistles by nodding thistle receptacle weevil. Proceedings NZ Weed and Pest Control Conference 43: 212-215.

Kelly, D. and Popay, A.I. (1985). Pasture production lost to unsprayed thistles at two sites. Proceedings NZ Weed and Pest Control Conference 38: 115-118.

Kendall, M. and Stuart, A. (1979). The advanced theory of statistics. Vol 2. Inference and relationship. 4th Ed. Charles Griffen and Co, London. 748 pp.

King, L.J. (1966). Weeds of the world: biology and control. Leonard Hill, London. 526 pp.

Kirkwood, R.C. (1987). Uptake and movement of herbicides from plant surfaces and effects of formulation and environment upon them. In Cottrell, H.J. (ed): Pesticides on plant surfaces, pp 1-25. John Wiley and Sons, Chichester.

Klingman, G.C. and Ashton, F.M. (1982). Weed science: principles and practices. 2nd Ed. John Wiley and Sons, New York. 449 pp.

Leathwick, D.M. and Bourdot, G.W. (1991). Application time influences the development of herbicide resistance in giant buttercup (*Ranunculus acris*). Proceedings NZ Weed and Pest Control Conference 44: 275-279.

LeBaron, H.M. (1982). Introduction. In: LeBaron, H.M. and Gressel, J. (eds): Herbicide resistance in plants, pp 1-8. John Wiley and Sons, New York.

LeBaron, H.M. (1991). Distribution and seriousness of herbicide-resistant weed infestations worldwide. In: Caseley, J.C., Cussans, G.W. and Atkin, R.K. (eds): Herbicide resistance in weeds and crops, pp 27-43. Butterworth Heinemann, Oxford.

LeBaron, H.M. and Gressel, J. (1982). Herbicide resistance in plants. John Wiley and Sons, New York. 401 pp.

Little, T.M. and Hills, F.J. (1978). Agricultural experimentation: design and analysis. John Wiley and Sons, New York. 350 pp.

Loos, M.A. (1975). Phenoxyalkanoic acids. In: Kearney, P.C. and Kaufman, D.D. (eds): Herbicides: chemistry, degradation and mode of action. Vol 1, pp 1-128. 2nd Ed. Marcel Dekker, New York.

Luckwill, L.C. and Lloyd-Jones, C.P. (1960). Metabolism of plant growth regulators. II. Decarboxylation of 2,4-D in leaves of apple and strawberry. Annals of Applied Biology 48: 626-636.

Lutman, P.J.W. and Lovegrove, A.W. (1985). Variations in the tolerance of *Galium aparine* (cleavers) and *Stellaria media* (chickweed) to mecoprop. 1985 British Crop Protection Conference - Weeds: 411-418.

Maclean, S.M. (1957). Effect of time of spraying on pasture production. Proceedings NZ Weed Control Conference 10: 34-43.

MAFQual (1987). Seed certification 1987-1988: field and laboratory standards. Scientific Liason Unit, MAFTech, Lincoln. 70 pp.

Martin, P., Fullerton, D.K. and James, T.K. (1990). Weed trials using a rotary weed wiper. Proceedings NZ Weed and Pest Control Conference 43: 262-265.

Martin, P. and Rahman, A. (1988a). Emergence of nodding thistle seedlings in pasture at two sites in the Waikato. Proceedings NZ Weed and Pest Control Conference 41: 210-213.

Martin, P. and Rahman, A (1988b). Influence of sheep grazing on the survival and fate of nodding thistle rosettes. Proceedings NZ Weed and Pest Control Conference 41: 214-218.

Martin, P., Thompson, A. and Rahman, A. (1988). Spot treatment of ragwort and nodding thistle with DPX-L5300. Proccedings NZ Weed and Pest Control Conference 41: 223-225.

Matsunaka, S. and Itoh, K. (1991). Paraquat resistance in Japan. In: Casely, J.C., Cussans, G.W. and Atkin, R.W. (ed): Herbicide resistance in weeds and crops, pp 77-86. Butterworth-Heinemann, Oxford.

Matthews, L.J. (1971). Weed control - nodding thistle. NZ Journal of Agriculture 122(2): 72-73.

Matthews, L.J. (1975). Weed control by chemical methods. A.R. Shearer, Government Printer, Wellington. 710 pp.

McCallum, K. and Kelly, D. (1990). Pre- and post-dispersal predation of nodding thistle seeds by birds and rodents. Proceedings NZ Weed and Pest Control Conference 43: 216-219.

McKinley, K.S., Brandt, S.A., Mosse, P. and Ashford, R. (1972). Droplet size and phytotoxicity of herbicides. Weed Science 22: 31-34.

McLean, J.R.F. and Dixon, A.T. (1972). Selective aerial treatment of barley grass. Proceedings of NZ Weed and Pest Control Conference 25: 51-55.

McNaughton, A.S. (1991). Physiological and genetic aspects of herbicide resistance in giant buttercup (Ranunculus acris L. ssp acris). Masterate thesis, Lincoln University, New Zealand.

McRill, M. (1974). The ingestion of weed seed by earthworms. Proceedings British Weed Control Conference 12: 519-524.

McWhorter, C.G. (1985). The physiological effects of adjuvants on plants. In: Duke, S.O. (ed): Weed physiology. Vol II. Herbicide physiology, pp 141-158. CRC Press, Florida.

Medd, R.W. and Lovett, J.V. (1978a). Biological studies of *Carduus nutans* (L.) ssp. *nutans*. I. Germination and light requirement of seedlings. Weed Research 18: 363-367.

Medd, R.W. and Lovett, J.V. (1978b). Biological studies of *Carduus nutans* (L.) *nutans*. II. Vernalization and physiological development. Weed Research 18: 369-372.

Meeklah, W.F.A. (1958). Comparison of the substituted phenoxyacetics and the phenoxybutyrics on a white clover-perennial ryegrass pasture. Proceedings NZ Weed Control Conference 11: 61-67.

Moore, W.B., Doyle, C.J. and Rahman, A. (1989). Economics of controlling *Carduus nutans* on grazed pasture in New Zealand. Crop Protection 8: 16-24.

Muntan, L. and Bencivelli, A. (1987). Weed control in winter cereals with DPX-L5300 in Mediterranean countries. 1987 British Crop Protection Conference - Weeds: 445-451.

Muzik, T.J. (1976). Influence of environmental factors on toxicity to plants. In: Audus, L.J. (ed): Herbicides - physiology, biochemistry, ecology, Vol 2, pp 203-247. Academic Press, London.

Neill, S.A. (1952). Noxious weeds. Proceedings NZ Weed Control Conference 5: 75-80.

Norman, A.G., Minarik, C.E. and Weintraub, R.L. (1950). Herbicides. Annual Review of Plant Physiology 1: 141-168.

Nufarm (1989). Spray-grazing with Nufarm MCPA 500 or Amicide 500. Promotional pamphlet, Nufarm Ltd, Laverton North, Victoria, Australia. 2 pp.

O'Connor, B.P. (1984). New Zealand agrichemical manual. 1st Ed. Agpress/Novasearch, Wellington. 228 pp.

O'Connor, B.P. (1989). New Zealand agrichemical and plant protection manual. 3rd Ed. WHAM/Novasearch, Wellington. 287 pp.

Parsons, W.T. (1973). Noxious weeds of Victoria. Inkata Press, Melbourne. 300 pp.

Phung, H.T. and Popay, A.I. (1981). Effect of pasture cover on the germination of certain weed seeds. Proceedings NZ Weed and Pest Control Conference 34: 111-113.

Pierce, J.R. (1990). Controlling saffron thistle with goats in Western Australia. Cashmere Newsletter 11(2): 30-32.

Pillmoor, J.B. and Gaunt, J.K. (1981). The behaviour and mode of action of the phenoxyacetic acids in plants. In Hutson, D.H. and Roberts, T.R. (eds): Progress in pesticide biochemistry, Vol 1, pp 147-218. John Wiley, Chichester.

Popay, A.I. (1978). Recent research into the control of nodding thistle. Proceedings Noxious Weed Inspectors Conference 1978: 12-17.

Popay, A.I. (1986). Nodding thistle - crop and pasture weed - biology, significance, control. Aglink FPP 359 (1st revision), MAF, Wellington. 2pp.

Popay, A.I., Bourdot, G.W., Harrington, K.C. and Rahman, A. (1991). Herbicide resistance in weeds in New Zealand. In Casely, J.C., Cussans, G.W. and Atkin, R.K. (eds): Herbicide resistance in weeds and crops, pp 470-471. Butterworth-Heinemann, Oxford.

Popay, A.I., Butler, J.H. and Meeklah, F.A. (1989). Chemical control of nodding thistle (*Carduus nutans* L.) in New Zealand pastures. Weed Research 29: 21-28.

Popay, A.I. and Kelly, D. (1986). Seasonality of emergence, and survival, of nodding thistle. Proceedings NZ Weed and Pest Control Conference 39: 187-191.

Popay, A.I., Lyttle, L.A., Edmonds, D.K. and Phung, H.T. (1984). Incidence of nodding thistle receptacle weevil on nodding and slender winged thistles. Proceedings NZ Weed and Pest Control Conference 37: 28-32.

Popay, A.I. and Medd, R.W. (1990). The biology of Australian weeds. 21 *Carduus nutans* L. ssp. *nutans*. Plant Protection Quarterly 5(1): 3-13.

Popay, A.I., Mitchell, R.B. and Thompson, A. (1979). Age, size and fate of nodding thistles. Proceedings NZ Weed and Pest Control Conference 32: 21-26.

Popay, A.I. and Thompson, A. (1979). Some aspects of the biology of *Carduus nutans* in New Zealand pastures. Proceedings Asian-Pacific Weed Science Society Conference 7: 343-346.

Popay, A.I., Thompson, A. and Bell, D.D. (1987). Germination and emergence of nodding thistle, *Carduus nutans* L. Proceedings Australian Weeds Conference 8: 175-178.

Porter, J.F. (1979). Development and use of a herbicide spot-gun applicator. Proceedings NZ Weed and Pest Control Conference 32: 211-214.

Powles, S.B. and Holtum, J.A.M. (1990). Herbicide resistant weeds in Australia. Proceedings Australian Weeds Conference 9: 185-193.

Radosevich, S.R. and Holt, J.S. (1982). Physiological responses and fitness of susceptible and resistant weed biotypes to triazine herbicides. In: LeBaron, H.M. and Gressel, J. (eds): Herbicide resistance in plants, pp 163-183. John Wiley and Sons, New York.

Reid, M. (1982). Nodding thistle survey. Waikato Bee Notes 15: 10.

Richardson, R.G. (1977). A review of foliar absorption and translocation of 2,4-D and 2,4,5-T. Weed Research 17: 259-272.

Robertson, M.M. and Kirkwood, R.C. (1970). The mode of action of foliage-applied translocated herbicides with particular reference to the phenoxy-acid compounds. II. The mechanisms and factors influencing translocation, metabolism and biochemical inhibition. Weed Research 10: 94-120.

Rolston, M.P., Lambert, M.G., Clarke, D.A. and Devantier, B.P. (1981). Control of rushes and thistles in pastures by goat and sheep grazing. Proceedings NZ Weed and Pest Control Conference 34: 117-121.

Ross, M.A. and Lembi, C.A. (1985). Applied weed science. Burgess Publishing Co, Minneapolis. 340 pp.

Rutherford, G.R., Bell, P.D. and Forgie, C.D. (1981). 3,6-dichloropicolinic acid/MCPB for control of nodding thistle in pasture. Proceedings NZ Weed and Pest Control COnference 34: 122-125.

Ryan, D.L. and Dulka, J.J. (1990). Metabolism of DPX-L5300 in wheat. Proceedings of American Chemical Society Division of Agrochemicals National Meeting 200: 61.

Sanders, P. (1990). Influence of grazing and phenoxy herbicides on nodding thistle. Proceedings NZ Weed and Pest Control Conference 43: 220-224.

Sargent, J.A. and Blackman, G.E. (1972). Studies on foliar penetration. Journal of Experimental Botany 23: 830-841.

Scott-Russell, R. (1977). Plant root systems: their functions and interaction with the soil. McGraw-Hill, London. 298 pp.

Sexsmith, J.J. (1964). Morphological and herbicide susceptibility differences among strains of hoary cress. Weeds 12: 19-22.

Sharma, M.P. and Vanden Born, W.H. (1970). Foliar penetration of picloram and 2,4-D in aspen and balsam poplar. Weed Science 18: 57-63.

Shaw, W.C. and Swanson, C.R. (1952). Techniques and equipment used in evluating chemicals for their herbicidal properties. Weeds 1: 352-365.

Slife, F.W. (1986). Resistance in weeds. In Committee on Strategies for the Management of Pesticide Resistant Pest Populations (eds): Pesticide resistance: strategies and tactics for management, pp 327-334. National Academy Press, Washington.

Smith, I.D. (1971). Nodding thistle threatens South Otago. NZ Journal of Agriculture 123(5): 61.

Steel, R.G.D. and Torrie, J.H. (1981). Principles and practices of statistics: a biometrical approach. McGraw-Hill, Auckland. 633 pp.

Stevens, P.J.G., Gaskin, R.E. and Zabkiewicz, J.A. (1988). Silwet L-77: a new development in spray adjuvants. Proceedings NZ Weed and Pest Control Conference 41: 141-145.

Stevenson, P. (1989). Goats take to thistles. The NZ Farmer 111(29): 22.

Stryckers, J. (1958). Onderzoekingen naar de toepassings-mogelijkheden van synthetische groeistoffen als selektieve herbiciden im grasland en akkerbouwgewassen. Gent Rijkslandbouwhogsch. Rep. 100. Cited by Gressel *et al* (1982).

Taylor, R.L. (1977). Control of winged and slender winged thistles. Proceedings NZ Weed and Pest Control Conference 30: 38-41.

Taylor, R.L. (1981). Weeds of lawns, pasture and lucerne in NZ. Caxton Press, Christchurch. 136 pp.

Thompson, A. (1983). Pasture weed control by ropewick applicator. Proceedings NZ Weed and Pest Control Conference 36: 96-98.

Thompson, A. and Saunders, A. (1984). A combination of 2,4-D and MCPA, alone and in combination, for the control of ragwort. Proceedings NZ Weed and Pest Control Conference 37: 33-36.

Thompson, A., Saunders, A.E. and Martin, P. (1987). The effect of nodding thistle (*Carduus nutans*) on pasture production. Proceedings NZ Weed and Pest Control Conference 40: 222-225.

Thompson, L.M.L., Sanders, G.E. and Pallett, K.E. (1986). Experimental studies into the uptake and translocation of foliage applied herbicides. Aspects of Applied Biology 11: Biochemical and physiological techniques in herbicide research, pp 45-54.

Wapshere, A.J., Delfosse, E.S. and Cullen J.M. (1989). Recent developments in biological control of weeds. Crop Protection 8: 227-250.

Wards, I. (1976). New Zealand atlas. A.R. Shearer, Wellington. 292 pp.

Webb, C.J., Sykes, W.R. and Garnock-Jones, P.J. (1988). Flora of New Zealand Vol IV Naturalised pteridophytes, gymnosperms, dicotyledons. DSIR, Christchurch. 1365 pp.

Weete, J.D. (1977). Herbicide analysis by chromatographic techniques. In Truelove, B. (ed): Research methods in weed science, pp 97-108. Southern Weed Science Society, Alabama.

Whitehead, C.W. and Switzer, C.M. (1963). The differential response of strains of wild carrot to 2,4-D and related herbicides. Canadian Journal of Plant Science 43: 255-262.

Whitworth, J.W. (1964). The reaction of strains of field bindweed to 2,4-D. Weeds 12: 57-58.

Whitworth, J.W. and Muzik, T.J. (1967). Differential response of selected clones of bindweed to 2,4-D. Weeds 15: 275-280.

Wiese, A.F. (1977). Herbicide application. In Truelove, B (ed): Research methods in weed science, pp 1-13. 2nd Ed. Southern Weed Science Society, Auburn, Alabama.

Worthing, C.R. and Walker, S.B. (1983). The pesticide manual: a world compendium. 7th Ed. British Crop Protection Council, Croyden. 695 pp.

Zama, P. and Mumma, R.O. (1983). Amino acid conjugates in 2,4-D treated soybean and red oak. Weed Science 31: 537-542.