SOME ASPECTS OF ABSORPTION, TRANSLOCATION AND METABOLISM OF TWO FOLIAR APPLIED AUXIN HERBICIDES IN GORSE (*Ulex europaeus* L.)

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ABSTRACT

Since *Ulex europaeus* is a problem scrub weed in New Zealand hill country, glasshouse and laboratory experiments were undertaken to study factors relating to the absorption of 2,4-D and picloram and the translocation and metabolism of picloram. Any one of these parameters may be limiting the effectiveness of picloram and other auxin herbicides in the field.

Absorption was studied using *in vitro* and *in vivo* methods. Cuticular surfaces were studied using scanning electron microscopy and the contact angles of droplets were photographed using an optical microscope. Translocation patterns were observed using autoradiographs as well as by sectioning the plant and counting the distribution of radioactivity in each section. Metabolites were examined for by a radio chromatogram scan of tissue extracts seporated by descending paper chromatography.

The results obtained suggest that absorption is poor due primarily to the presence of a thick smooth layer of cuticular waxes. Environmental conditions at, and subsequent to, spraying are likely to affect absorption in the field. Drying of the spray deposits inhibits absorption; re-wetting a dried deposit with mist enhances absorption.

Additives, especially surfactants and the inorganic salts KCN and Na_2HPO_4 enhanced *in vitro* absorption. Information on the mechanism of absorption was gained from the response to light, temperature and concentration of herbicide.

Picloram was readily translocated from the treated stem to the roots. Very little re-distribution of picloram occurred. Source-sink relationships occur.

No metabolites could be detected from spines and stems. Stem tips and roots were not tested.

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ABBREVIATIONS AND HERBICIDE COMMON NAMES

ai	active ingredient
amiben	3-amino-2,5-dichlorobenzoic acid
ATP	adenosine 5'-(tetrahydrogen triphosphate)
CEPA	2-chloro-ethyl-phosphoric acid
cpm	counts per minute (ie the number of cpm recorded)
2,4-D	2,4 - dichlorophenoxyacetic acid
2,4-DB	2,4-dichlorophenoxy butyric acid
2,4-DP	2,4-dichlorophenoxy propionic acid
DEF	s,s,s-tributyl phosphoro-trithioate
DCMU	3-(3,4-dichlorophenyl)-1,1-dimethylurea
dicamba	3,6-dichloro-2-methoxybenzoic acid
dichloroprop	2-(2,4-dichloro phenoxy) propionic acid
diquat	1:1'-ethylene-2',2-dipyridylium dibromide
DMSO	dimethyl sulfoxide
DNP	2,4-dinitrophenol
dpm	disintergrations per minute (ie cpm x efficiency of counting/100)
ethrel	2-chloroethyl phosphoric acid
GA	gibberellic acid
IAA	indole-3-acetic acid
IBA	indole-3-butyric acid
MCPA	4-chloro-2-methylphenoxyacetic acid
NAA	1-naphthalenacetic acid
piclorem	4-amino-3,5,6-trichloropicolinic acid
ppm	parts per million
2,4,5-T	2,4,5-trichlorophenoxyacetic acid
2,4,5-TP	2,4,5-trichlorophenoxy propionic acid (silvex)
TCA	trichloroacetic acid
ULV	ultra low volume

CHAPTER 1: INTRODUCTION

The "invasion of New Zealand by Ulex europaeus" is described by Little (1960) as being "more spectacular than that of any other plant". Its introduction was made early on in the European colonization of New Zealand, since Charles Darwin reported seeing gorse hedges when he visited the Bay of Islands in 1835 (Allan 1940).

In fact gorse was an important agricultural species for the early New Zealand pioneers. It was planted as fences, and grown as shelter for stock, especially during winter snowfalls. As stock fodder, it found favour, especially in the Nelson district. Merry (1952) reported that "gorse seed has been broadcast up to comparatively recent years on poor (Nelson) hill country and by many farmers is regarded with considerable favour". Other farmers found gorse beneficial to soil fertility. Crisp (pers.comm.) described how Southland farmers in the early 1900 s renovated moss-bound pastures by growing a crop of gorse.

Even today, if a cheap and reliable chemical method for control/eradication of gorse existed, the plant could become valuable in erosion control. This is because of its low fertility requirements, its ability to fix atmospheric nitrogen and its high interception of rainfall; 70-80% (Aldridge 1968) compared with 20-40% for most forest species.

However the control of gorse is difficult. The usefulness of Ulex as an agricultural plant ensured its wide distribution in an environment that seemed adequate to meet its requirement. Unfortunately, varying economic conditions and labour supplies resulted in gorse hedges being neglected and areas sown as stock feed were not adequately controlled. As a result "gorse undoubtedly remains New Zealand's most widespread and aggressive introduced brushweed problem" (Mason 1973). Its importance as a weed is accentuated by its fire resistance and its free seeding habits. Further, some of the seeds remain dormant in the ground for long periods of up to 30 years (Moss 1959).

Luxton (1970) estimated that the total area in New Zealand infested with gorse was 600,000 ha of which 80% was considered to be farmable. It is not only in grassland farming, especially hill country, that gorse is a problem but also in forestry. During forest establishment, young trees may be overwhelmed by rapid regrowth of gorse following burning. Regrowth of gorse with the tree crop usually precludes pruning and increases the difficulty of non-commercial thinning.

1.1. The Biology of Gorse

1.1.1. Taxonomy

There are twenty species of gorse (genus Ulex) although Millener (1961) describes a situation of "taxonomic chaos" as existing. This is no doubt a function of the plant's extreme plasticity, being highly variable, both genetically and in response to the environment.

In New Zealand the species is Ulex europaeus L., although Allan (1940) stated "that U. galli planch occurs at Tauranga where it is apparently hybridising with U. europaeus".

In a review of studies on Ulex europaeus L. Egunjobi (1967) describes the existence of two sub species, namely ssp. borealis (2n=96) and ssp. Latebracteatus (2n=64). The former is the commonest type in Britain and has three varieties, while the latter is endemic in Portugal. Although it is most likely that borealis exists in New Zealand, no chromosome work has been published.

1.1.2. Morphology

A number of text books describe the morphology of mature gorse e.g. Priestley et al (1964) and Munscher (1955). A brief description of this legume scrub weed will suffice:-

Gorse is a shrubby perennial; woody stems 1-3m high, densely branched with many stiff, green, leafy thorns. During flowering (twice annually in New Zealand) the shrub is abundantly covered with yellow flowers.

1.1.3. Anatomy

Skipper (1922) studied transverse sections of gorse spines and "young accessory branches", while Dormer (1945) makes mention of the orthostichies in the vascular system of Ulex europaeus.

1.1.4. Ecology

Skipper (1922) in the United Kingdom made a detailed study of gorse and Egunjobi (1969 and 1971) in New Zealand measured dry matter production, the cycling of nutrients and accumulation of nitrogen in pure gorse and gorse in mixed scrub stands of various ages.

Small (1968) notes that in New Zealand, gorse thrives on a great variety of soils. Egunjobi (1967) reports that gorse grows best in acid soils pH 4-5 and has been found to grow poorly on calcareus soils of high pH. Small also points out that gorse has a "fairly narrow climatic range". It is rarely found above 500 m a.s.l. in New Zealand.

Gorse appears to be a serious weed only in a few places in the world where the climate is suitably temperate, including New Zealand, South-Australia, especially Tasmania, the Iberian Peninsula, the western coast of North America and Ireland.

It has not always been appreciated that gorse is well adapted to burning regimes. Mature gorse plants resprout after burning (Little 1965) while considerable numbers, up to 1 million per ha (Rolston and Sineiro 1973) of seeds in the soil may germinate.

1.1.5 Physiology

Early investigations at the turn of the century have been followed up by Millener (1961) and Bieniek and Millington (1967 and 1968) who have studied the physiological factors influencing thorn formation and the change from the juvenile growth form.

1.1.6 Phenology

The phenology of gorse in New Zealand was studied in detail as part of a wider programme relating to biological control of gorse. The results of these studies are reviewed by Miller (1970). Moss (1959) has made further studies on the gorse seed problem.

In conclusion the biology of gorse has received scant attention, especially in New Zealand where a knowledge of the plant's anatomy, ecology and physiology of growth, development and reproduction should be the basis of any control/eradication programme.

1.2 The Control of Gorse

Numerous writers have discussed the control of gorse. Research papers generally cover experiments with chemicals (see Section 1.2.1.). Since it is difficult and expensive to carry out replicated experiments on general management techniques, most reports on management control of gorse are based on either trial results and or personal experience e.g. Duncan (1953), Fiest (1956), Currie (1959), Bell (1961), Suckling (1965), Small (1968), Batten and McDonnell (1969), Walton (1972), Gooding (1973), Wilson (1973), Bascand (1973) and Sineiro (1974).

Booklets on gorse control have also been published e.g. "Hill Country Development" (Ferens, undated) and more recently "Gorse Eradication" (Anon.1972).

A wide range of practices have been used in gorse control including cutting, crushing, burning, cultivation (root raking, blading, giant discing), chemicals and stocking coupled with oversowing and topdressing. Biological control has also been attempted (Miller 1970). Hopes of eliminating seed production with the weevil Apion ulicis have not been realized, despite the fact that the insect has become established throughout New Zealand. The percentage seeds destroyed varies with season and locality, with pod infestations as high as 99% and as low as 10% being recorded.

1.2.1. Chemical Control

Nearly all gorse control programmes utilize herbicides. Only on flat land can cutting or cultivation completely substitute for chemicals. However even on hill country where cultivation is impossible, chemical control must be integrated with stock management, although in forestry herbicides may be the only management tool used in gorse control.

Chemical control of gorse in New Zealand has been reviewed by Matthews (1951), Little (1965) and Mason (1973).

Until 1948 chemical control of gorse was limited to inorganic salts. In that year, 2,4,5-T was introduced and has remained as the most commonly used herbicide.

2,4,5-T and the most frequently used herbicide additives (picloram and dicamba) all have physiological properties of the plant hormone group the auxins. The 2,4-D/2,4,5-T herbicide mixtures commonly used on woody weeds in the northern hemisphere were ineffective on gorse.

The major method of application has been "high-volume gun spray application" (Mason 1973). The most commonly used formulations of 2,4,5-T have been the emulsified low volatile esters (butoxyethanol or iso-octyl) or the mixed butyl esters. 2,4,5-T is commonly applied at a rate of 4 kg ai in not less than 4000 l per hectare of infested gorse up to 2m high, although Meeklah (1967) suggests that doubling this rate (8 kg ai) "may be necessary for really effective control". The amine salts of 2,4,5-T have been proven considerably less effective (Mason 1973).

Following spraying, stem "brown off" is slow, taking at least six months. Whether or not the plant has been killed cannot be finally evaluated for upward of 18 months following spraying. The evaluation of the effectiveness of a chemical treatment is therefore difficult and needs to be improved. The most commonly used system being a regression analysis of collected data (visual estimate of regrowth suppression on a 1-10 scale) for a time period up to 30 months (Upritchard 1973).

There is scope for the experimentation of new techniques, chemicals and new formulations. In forestry Chavesse and Davenhill (1973) have successfully experimented with the use of "multiple spray treatments" where an initial spraying is followed 12 months later with a second treatment e.g.

Initial Treatment	Second Treatment
2,4,5-T (20% ai) Picloram (5% ai) (9 l ha)	2,4,5-T (11.2 l/ha)

Although this is satisfactory in forestry, the tolerance of clovers to auxin herbicides (Matthews 1965) and the persistence of these chemicals can create problems especially where blanket spray applications for regrowth control is required. Thompson (1973) has looked more closely at the effects of herbicides on gorse and pasture species. The use of gramular herbicides have been experimented with (Mason 1973). Carriers other than water for U.L.V. spraying are being investigated (Naish 1973), while the use of foam techniques needs to be investigated. With the phenoxy herbicides being derivatives of the petrochemical industry, chemicals derived from other sources need to be evaluated.

(b) Time of Application

Timing of chemical application is generally related to the growth state of the plant. General recommendations are that gorse should be sprayed during November/February, "the period of maximum soft terminal shoot growth" (Moffat 1973).

Matthews (1951) reported that control is more difficult when gorse is flowering profusely as the flowers absorb the spray and drop off a few days later.

The pressure of other farm management practices and the limited availability of spray contractors at this time make it desirable to improve spray results outside the optimum time period. Results of out-of-season treatments of gorse with mixtures of 2,4,5-T with diquat or paraquat have been reported by Williams and Palmer (1969) and with picloram/2,4,5-T, 2,4,5-T ester alone or in combination with diquat by Moffat (1973). While the former treatment gave the best results, it is unfortunate that Moffat did not try a range and higher rates of 2,4,5-T as recommended for winter growth by Matthews (1951).

(c) Stage of Growth

Matthews (1951) also reports that gorse is quite susceptible to 2,4,5-T, but becomes increasingly resistant with age. Further, he mentions that "there is some indication with mature gorse, that even if a complete foliage and stem check is obtained, regrowths will occur." This could be due possibly to the limited translocation of 2,4,5-T within problem brush weeds. As evidence for this in gorse, Mason (1973) cites that complete spray coverage is demanded of 2,4,5-T, which consequently has limited the use of aerial applications of this chemical.

In an attempt to overcome this problem 2,4,5-T has been formulated with picloram and dicamba, both of which are claimed by their respective manufacturers to be highly translocated. However in aerial spraying even with these mixtures, failures still occur (Plate 1.1).

The causes of herbicide failures are unknown and there is also a general lack of information on the absorption, translocation and metabolism of herbicides in gorse. For these reasons this investigation was initiated.

PLATE 1.1

Gorse bush 12 months after an aerial application of a picloram/2,4,5-T (1:4) spray mixture.

CHAPTER 2: REVIEW OF LITERATURE

An extensive literature of factors affecting the absorption, translocation and metabolism of herbicides has evolved. This review is approached in three sections, each separately covering the above physiological processes. Factors affecting herbicides with auxin properties will mainly be considered. However, the review will not be limited merely to brushweeds since considerable research on, and information about herbaceous species exists, which may have relevance in solving problems relating to brushweeds.

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2.1 ABSORPTION

2.1.1. Introduction

In addition to the roots, plant foliage can absorb both organic and inorganic compounds. The aerial parts of the plant, unlike the roots, are covered by the cuticle, which certainly restricts entry of herbicides, but does not prevent absorption entirely.

The foliar absorption of herbicides and other compounds, has been reviewed by Currier and Dybing (1959), Foy (1964), Sargent (1966), Franke (1967), Hull (1970) and Bayer and Lumb (1973).

There is no clear definition of absorption. It is debatable whether a substance by penetrating the cuticle has entered the living plant. Before it can interact with systems within the cytoplasm of the cell, the herbicide must first penetrate the plasmalemma (Sargent, 1968). In an attempt to distinguish between herbicide which had penetrated the cuticle, but not the plasmalemma, Wanthana et al (1972) first washed the surface with distilled water and then with chloroform. The bulk of the activity (70 percent) was recovered in the chloroform wash.

Another approach has been to wash the leaf to remove un-sorbed material. The adsorbed herbicide is then recovered by soaking the leaf in distilled water or in a "cold" herbicide solution, especially if labelled material was used. However, most workers estimate absorption by applying a known amount of chemical. The amount of chemical recovered in the wash water (a wide variety of washing methods have been employed by different authors), is considered to be unabsorbed. Conversely,

herbicide that is not washed off (estimated indirectly by difference, or directly by extraction) is considered to be absorbed.

2.1.2. Plant Factors Affecting Absorption

(a) Physiochemical Factors

To reach the leaf tissue, herbicides must penetrate the cuticle and epidermis including the plasmalemma (i.e. cell membrane). The cuticle and epidermis are multilayer, non-living outer surfaces on all aerial parts of the plant. Detailed reviews on the cuticle have been written by Martin and Juniper (1970) and on the epidermis by Hull (1970).

(i) The Cuticle

Epicuticular wax is not present on all leaves (Bayer and Lumb, 1973). When they are found waxes may be classified in terms of their physical structure and chemistry. Hull (1970) gives the following structural classification:-

1. simple granular wax crust
2. wax rods and filaments
3. wax plates and scales
4. wax layers and crusts
5. aggregate wax coatings
6. liquid or viscous wax coatings.

Although Martin and Juniper (1970) point out that structural groupings do not define precise chemical entities, Nesting and von Wettsteinknowles (1973) in a study of wheat (Triticum aestivum) mutants, showed that wax tube (rod) formation is closely related to the presence of β-diketones. The chemistry of cuticle wax is discussed in detail by Martin and Juniper (1970) and Mazliak (1968).

The effects of wax (a function of its physical structure and chemical nature) is to markedly influence the contact of impinging droplets and consequent wetting. The quantities of long chain ketones and paraffins are most important, making the surface difficult to wet.

That the epicuticle waxes offer a major barrier to penetration has been demonstrated in numerous experiments, e.g. Shama and Vanden Born (1970) showed that partial removal of wax with chloroform resulted in up to a four-fold increase in penetration of picloram and 2,4-D by the leaves of aspen and poplar (Populus spp).

On the other hand, Bayer and Lumb (1973) are uncertain to what extent that surface waxes actually impede penetration. As evidence, they consider that the rapid decline in spray contact angles on ageing mature leaves is not accompanied by increased penetration.

Cutin consists of a polyester of long chain fatty acids and other substances. Single chains may be linked and this gives a three-dimensional framework to the cuticle, in which the waxes are embedded. Since a portion of the polar groups remains free during polymerization, cutin is semilipophilic in nature and thus represents a junction between the lipophilic waxes and the hydrophilic epidermis.

(ii) The Epidermis

The cell wall contains cellulose and pectin integrated with the cutin and waxes. The cellulose layer often shows lamellation, interspersed with pectin substances. The amount of pectin per layer increases with increasing distance from the lumen. Thus the boundary between the cellulose layer and the cutin may be sharply defined by a pectin sheet.

Within the free space of the cell wall, proteins including enzymes occur, (Hull 1970). In addition to any roles these enzymes may have in the biosynthesis of the cell wall, it is postulated that they could act on exogenous substances penetrating the cell wall.

Trichomes are epidermal hairs of which numerous types exist. They appear to be similar to epidermal cells but have higher metabolic rates. Structurally, they have thinner walls or less cutinization near their bases.

In foliar absorption, the presence or absence of trichomes may be important. In some species, they may be an important route of entry. This may be a consequence of the collection of spray liquids around them, (Hull 1970). On the other hand, the dense mat of hairs on the surface of Salvinia and Pistia prevent wetting in these species.

Depending on the nature of the molecule, it may or may not gain entry to the symplast. Some compounds are excluded by the plasma membrane and are further transported only within cell walls, via the apoplast. Still others are transported to varying extents in both systems (Crafts, 1964). The terms symplast and apoplast were introduced

by Münch in 1930: symplast to describe the total interconnecting living protoplasm including phloem, and apoplast to include the non-living continuous cell wall phases around the symplast. The xylem stream is often included in the apoplast.

The nature of the plasma membrane (a lipoprotein complex) has recently been reviewed by Harris (1972). Although considerable information exists on the uptake of inorganic ions by membranes, the information on the uptake of herbicides is sparse. Further consideration of the role of the plasmalemma is given in Section 3.1.5. The Mechanism of Absorption.

(b) Physiological Factors

The influence of water stress on foliar absorption is unclear. Crafts (1964) in a theoretical discussion considers that as water recedes from cuticular pores with increasing water stress, the resulting air bubbles act as a barrier to the water continuum. The aqueous route of entry then becomes unavailable for penetration.

There have been very few studies on the effect of water stress; Davis et al (1968 a) used two woody species over four levels of moisture stress. The uptake of foliar applied 2,4,5-T was not affected, but in one species, picloram uptake was significantly affected. The same group found no striking differences in the absorption of 2,4-D, 2,4,5-T and picloram in bean plants subjected to water stress.

Leaf age and development affect herbicide penetration. The absorption of most organic and inorganic solutes is generally greater in relatively young leaves. However, variations with development occur and may have important practical implications. As an example, Hull (1970) quotes work on cotton (Gossypium) plants, where maximum herbicide absorption occurs between the ages of 27 and 59 days due to superficial cracks resulting from secondary growth of the cork cambium.

Factors affecting translocation in theory may alter the concentration gradient of the herbicide across the absorption pathway. Currier and Dybing (1959) consider that this may be important. However, there appears to have been few attempts to examine this hypothesis.

2.1.3. Environmental Factors Affecting Absorption

(i) Temperature

A number of workers have reported Q_{10} values of about two: e.g. Greene and Bukovac (1972) with NAA, and Sharma and Vanden Born (1973) with picloram.

The Q_{10} values obtained need not be evidence of active uptake. Norris and Bukovac (1969) comment that the values obtained are related to the lipoidal nature of the cuticle membrane. A relatively high energy barrier has to be overcome in order that the penetrating molecules can reach the activation energy so that the herbicide may pass into or out of the cuticular membrane. Martin and Juniper (1970) also discuss this phenomenon, which they consider is consistent with the existence of a labile wax layer susceptible to temperature within the cuticle.

In vitro absorption studies using incubation solutions are unlikely to indicate the temperature response that would occur in the field. Crystallization and even volatilization of the herbicide may occur at higher temperatures.

(ii) Humidity and Rain

Although a few experiments suggest that exceptions exist, foliar abosrption of both organic and inorganic substances (especially water soluble chemicals) is generally facilitated by a high humidity (Hull, 1970). The effects of a high relative humidity are:-

(a) a slowing in the rate that spray deposits dry. It has been shown that penetration ceases on crystallization of phenoxy acids in spray droplets (Currier and Dybing 1959).

(b) by influencing the degree of hydration of the leaf cuticle, thus extending the water continuum in the plant towards the surface.

(c) favours stomatal opening.

Several of the auxin herbicides show enhanced absorption by high humidities, e.g. picloram (Sharma and Vanden Born 1970), 2,4-D and benzoic acid (Crafts 1961). The failure of Morton (1966) to detect any humidity influence in the foliar absorption of ^{14}C-2,4,5-T in Prosopis juliflora was considered to relate to the fact that Prosopis is a relatively xerophytic species.

Rewetting of the herbicide application area under low humidity has been shown to enhance absorption. Similarly, it is thought that dew

or a light rainfall insufficiently heavy to cause foliar runoff, may enhance absorption of spray deposits (Hull, 1970).

(iii) Light

Hull (1970) comments that reports relating to light effects on the absorption of pesticides and various other organic and inorganic substances continue to be conflicting. Sargent and coworkers have demonstrated with Phaseolus a light dependent relationship for several auxin herbicides. The effect of light on the penetration of 2,4-D was complex. Below 800 f.c. light has little effect, (although stomata open at an intensity of 250 f.c.). Further, this phenomenon occurred only in young leaves on the abaxial (lower surface) and is sensitive to temperature and metabolic inhibitors e.g. DCMU . . which blocks electron flow during photosynthesis. Similar results have also been obtained with dalapon and picloram (Sargent and Blackman, 1970).

Conversely, Davis et al (1968a)found that absorption of picloram in Quercus sp. (oak) and other woody species was inversely related to light intensity.

The absorption of NAA . in Pyrus communis (pear) is light dependent and is inhibited by DNP, another uncoupler of oxidative phosphorylation (Greene and Bukovac, 1972). They also found that inhibitors of the Hill reaction depressed penetration, thus further implicating the involvement of light-derived energy in penetration.

There is little information on light quality. Davis et al (1968a) found that absorption of picloram and 2,4,5-T by oak was not significantly affected by light wavelengths from three different portions of the visible spectrum. However, Sands and Bachelard (1973) showed that far-red light stimulated uptake of picloram by Eucalytus viminalis but not in E. polyanthemos. The effect of far-red was better than any other single light quality tested, as well as white light. There was "no evidence of phytochrome involvement in these far-red effects." Unfortunately they give no details of the method or results that led them to this conclusion. The results in the above species correlated with increased stomatal opening in far-red light in E. viminalis and lack of stomatal opening in E. polyanthemis.

2.1.4. Spray Factors

(a) The Active Ingredient

It has been established by many workers that the growth regulatory and herbicidal properties of substituted phenoxyacetic and benzoic acids are markedly affected by the number and position of halogen substituents. While progressive chlorination of the phenoxyacetic acids leads to an increase in the rate of penetration in both light and darkness, it depresses uptake of the benzoic acids (Bukovac et al 1971).

While the position of the chloro-substitution is not important, increasing chlorination increases the lipid solubility of the phenoxyacetics, while in the benzoic acid series, chlorination is coupled with a decrease in the pK value, (Sargent et al, 1969). Thus at a given pH the chloro-substituted benzoic acid is less lipid soluble. It could be expected that the highly polymerized nature of the outer portion of the cuticle, and its high wax content would favour relatively non-polar compounds having semi-lipophilic characteristics.

While this is generally true, there may not be a strict relationship between chemical structure and partitioning into an organic solvent. Robertson et al (1971) using diphenyl acetic acid and three derivatives, found that although absorption varied threefold, there was little difference in the partitioning into either chloroform or oleic acid.

Structural changes in the side chain markedly influence absorption of the phenoxy alkyl acid herbicides. Various formulations of 2,4-D showed that penetration of the ester was greater than that for the amine, which was superior to that for the salts, (Currier and Dybing 1959).

However, the enhanced penetration of the ester is offset by reduced translocation in Prosopis and Acer, (Hull 1970).

Within a certain range, penetration is a function of concentration, e.g. Greene and Bukovac (1972) showed that NAA penetration into pear leaves was proportional to concentration from 10^{-6}M to 10^{-3}M.

Hull (1970) however, points out that herbicides with a high contact toxicity or when used at excessively high concentrations, may cause injury to the phloem and thereby inhibit further transport of the herbicides e.g. in Typha a threshold concentration for 2,4-D occurs above which excessive contact kill results.

(b) Adjuvants

A wide range of compounds may be mixed with the herbicide to improve absorption (and/or translocation) properties of the active ingredients.

Surfactants (surface active agents) including emulsifiers, detergents and wetting agents are more consistently effective in enhancing foliar absorption than any other types of adjuvants, (Hull 1970). Chemically, a surfactant may be described as having two opposing characteristics - i.e. hydrophilic and lipophilic tendencies (Behrens 1964). Surfactants may be classified as non-ionic, cationic or anionic. The former group have a compatibility with many chemicals and considerable insensitivity to the presence of hard water. It has generally proved desirable to create physical mixes of various surfactants, (Behrens 1964).

The action of the surfactant is to influence one or more of the following factors:-

1. To improve wetting and spreading of the water solutions on wax surfaces by lowering the surface tension of the solution.

2. To improve the solubility of the herbicide, (Temple and Hilton 1963).

3. Jansen (1964) offers a speculative role for surfactants. It is considered that they may promote humid conditions causing the cuticle to swell due to imbibition of water by the hydrophilic portions of the cuticle cutin. This swelling action would result in separation of the wax platelets and extend the water continuum, promoting absorption of polar (water soluble) compounds.

4. Behrens (1964) considers that the surfactant may help prevent the solution from becoming a high-viscosity liquid or a crystal, which would reduce its effectiveness.

5. Some surfactants have phytotoxic characteristics.

As an example of surfactant enhancement, Hull (1970) quotes an experiment where saplings of Populus could be killed as readily with sprays containing 500 ppm of picloram plus one percent of a non-ionic blended surfactant as they could be with 2000 ppm of picloram without the surfactant. Esters of 2,4-D and 2,4,5-T were as effective at 1000 ppm with the surfactant, as at 2000 ppm without surfactant.

Although there are numerous reports in the literature of such surfactant-induced enhancement, there have also been reports indicating that in a particular surfactant-plant system, surfactants are without

effect or may even inhibit penetration of the active ingredient.

Oil solvents and oil-water emulsions can increase the penetration and phytotoxicity of herbicides. Welsh (1973) found in uptake experiments that there was at least a twofold increase in rate and amount of 2,4,5-T taken up by Chrysanthemoides sp. using isoparaffinic oil as a carrier rather than water.

The beneficial effects of oil on penetration is thought to result because evaporation from a falling oil or oil emulsion, droplet is slower than from a water droplet of the same size. This difference perhaps assumes greatest importance in aerial sprays carried out under arid conditions, (Hull 1970). Some oils are phytotoxic. Diesel can give poor kills by rapidly killing conductive tissue (Currier and Dybing 1959).

In the treatment of woody plants especially, a carrier containing at least a small fraction of oil, sometimes proves beneficial, probably due to improved penetration of the bark. Crafts (1964) notes that sprays of about five percent chlorophenoxy herbicide in diesel oil is widely used in killing trees and heavy bush.

Humectants are generally added to a spray mix to improve penetration by preventing drying of the spray deposit, thus ensuring an extended penetration time. Glycerol, various glycols and carbowax have generally been used at rates ranging from 0.5 to 2 percent. Hull (1970) quotes work carried out by his group and by others using humectants with phenoxy herbicides. Enhancement is observed with some humectants.

Hughes (1968) found that hydroscopic humectants e.g. poly-propylendiol at five percent by volume gave enhanced absorption of silvex (2,4,5-T.P.) under conditions of high temperature and/or low humidity.

However, there has been insufficient experimental work carried out on humectants and they have not gained wide acceptance in the practical application of herbicides.

Several classes of growth regulators have experimentally been shown to enhance penetration of some organic and inorganic compounds. Hull (1970) however notes that "any advantages gained for practical field application of pesticides have apparently been rather slight or at least have not gained wide acceptance."

Sharma and Vanden Born (1973) reported that G.A. caused a significant increase in picloram absorption by barley (Hordeum vulgare)

cotyledons, while Davis et al (1968a) reported that 2,4,5-T similarly increased picloram absorption in Prosopis. However 2,4,5-T uptake was inhibited by picloram.

The inclusion of certain inorganic salts in the herbicide formulation may increase uptake and biological activity of the active ingredient. Hull (1970) notes that salt-enhanced selectivity activity of phenoxy herbicides on broadleaved weeds in monocotyledonous crops such as maize (Zea mays). However, salt-induced enhancement of phenoxy herbicides with woody plants has been difficult to demonstrate. As an example, Hull (1970) quotes the work of Tschirley (1968) who applied several formulations of 2,4,5-T, 2,4,D and MCPA to five woody species with up to 100 g/ha of ammonium thiocyanate, and did not significantly alter herbicidal activity. However, this rate is exceedingly low in comparison with other experiments.

Szabo and Bukhottz (1961) compared the absorption of 2,4,-D by sunflower and bean leaves at different pH values with organic ion additives. At pH 5, PO_4^- as well as NH_4^+, Zn, Mn, and Co as nitrates gave enhancement in sunflower. While they attributed the enhancement to NH_4 and the metallic ions, Brady (1970) suggests that the effect was due to the nitrate.

Brady (1970) observed the effects of phosphatic acid (0.1 percent) and ammonium nitrate (0.1 percent) and found increases in absorption of isooctyl ester 2,4,5,-T in four woody species of 35 - 180 percent for the former and 77 - 280 percent for the latter. Unfortunately Brady did not buffer his solutions and the effects are difficult to separate from pH effects:-

2,4,5-T in:	pH
1. Water control	5.6
2. 0.1 % NH_4NO_3	4.0
3. 0.1 % H_3PO_4	1.0

Urea enhances the foliar absorption of ions (Franke 1967) although no effect has been shown with herbicides. Ammonium salts are known to enhance uptake of the auxin NAA, (Horsfall and Moore 1962). However, of the numerous ammonium salts investigated, they found that only those of relatively strong acids enhanced uptake. Again, the response is most likely to have been one of pH enhancement, since they did not buffer their solutions to constant pH.

Organic phosphates which injure the cuticle or epidermis, especially DEF increased the effects of picloram, 2,4,5-T and mecoprop salts on four woody species experimented with, (Turner 1973). He noted that the effect did not occur with esters of the herbicide and concluded that "there is evidence that DEF facilitates the entry of water soluble growth regulators herbicides into leaves."

(c) Other Spray Factors

There are at least two distinct systems within the leaf where hydrogen ion concentration may exert an influence on overall foliar absorption; the cuticle itself and the plasmalemma of underlying cells. Many experimental techniques do not clearly differentiate these two systems which may respond somewhat differently to changes in pH (Hull 1970).

Using isolated cuticle, Norris and Bukovac (1972) showed increased absorption as the pH of an NAA solution was lowered. It is generally considered that weak acids penetrate at low pH values when the molecule is largely undissociated. e.g. NAA; pKa 4.2.

pH	penetration (dark)	percent undissociated
3.2	506 cpm/disk	90%
5.2	77 cpm/disk	15%

(From Greene and Bukovac, 1972)

At lower pH values, the undissociated herbicide will often partition more readily into a lipid phase in a lipid/water mixture.

Similar types of results have been obtained with 2,4-D (Ashton and Crafts 1973). In contrast it has been shown that an adjustment of pH on the acid side has no effect on cuticular penetration of picloram (which is a strong acid). However, penetration of the plasma membrane of potato (Solanum tuberosum) tuber tissue by picloram is enhanced by lower pH values, (Baur and Bavey 1970).

Hull (1970) concludes that in spite of numerous demonstrations of pH enhancement of absorption in laboratory experiments, relatively little benefit of pH control has been shown in the field.

Droplet size is related to herbicide retention by the plant and therefore affects absorption. Hartley (1966) and Holly (1964) have reviewed these aspects.

Another aspect is the direct influence of droplet size on absorption. Leonard and Yates (1959) working with Ulex europaeus demonstrated that the application of the same volume as three droplets was more effective than one droplet. Similarly Behrens (1957) obtained a greater percentage kill of Prosopis from smaller droplets than larger droplets using constant volume and active ingredient per unit area. However, the opposite effect occurred if he kept droplet number and herbicide concentration per unit area constant. This would suggest that larger droplets may increase absorption if an increased volume of water is applied (rate of drying reduced). Where constant volume is used, the amount of surface area covered is important.

Davis et al (1968b) demonstrated a significantly increased absorption of picloram by the small leathery leaves of Ilex vomitoria when paraquat (4 kg/ha) was included in the formulation.

Dimethyl sulfoxide (DMSO) has a remarkable ability to deeply penetrate biological tissue. Enhancement effects on pesticides range from no effect to extreme effects. Concentrations of DMSO seem to be an important factor in this relationship.

Hull (1970) quotes a number of examples of DMSO enhancing the absorption of various auxin herbicides in herbaceous and woody species. The formulation of the herbicide is important. In Prosopis, the foliar absorption of water-soluble formulations of 2,4,5-T is considerably facilitated in the presence of DMSO while the inherently superior absorptive characteristics of ester formulations are not significantly altered. Burns et al (1969) found no DMSO enhancement of 2,4-D in Carex cherokeinis.

It has been further observed that DMSO is phytotoxic in concentrations greater than five percent, while the mode of action in enhancing absorption, or of its phytotoxicity is unknown.

In addition to DMSO there are certain other organic solvents which have exceptionally strong penetrability e.g. -butyrolactone, but their effect on herbicide absorption is still under study.

2.1.5. Mechanism of Absorption

(a) Routes of Absorption

Earlier workers and reviewers placed considerable emphasis on the importance of stomatal penetration. Although Currier and Dybing (1959) comment that "the importance of sunken stomata in foliar penetration cannot be assessed at present", they make continual reference to the likely importance of stomata. Franke (1967) comments that stomatal penetration would be of some advantage

because the absorbing surface is enlarged. The internal cuticle may be thinner and because of the higher humidity of the substomatal cavity the risk of spray solutions drying down is reduced.

The conception of stomatal penetration arose from experimental work which showed that the lower leaf surface had a much greater (up to ten-fold) rate of penetration than the upper surfaces, which either have no stomata or very few stomata. Franke (1967) notes that the uptake of hydrophilic compounds is considered by many authors to occur only through stomatal pores .

In a theoretical and later experimental study of stomatal penetration, Schonherr and Bukovac (1970) consider penetration of the stomatal pore will occur when the surface tension of the spray liquid is equal or less than the critical surface tension of the leaf surface. This value for most cuticular surfaces is in the region of 30 dynes/cm. They found that only a few available commercial surfactants will reduce the surface tension of aqueous solutions below this value.

Sands and Bachelard (1973) found that surfactants which enhanced absorption in Eucalytus, had surface tensions ranging from 29 to 35 dyne/cm.

There are two other hypotheses in addition to stomatal absorption to explain enhanced absorption by lower leaf surfaces. The differences may be due, in part, to differences in wax structure between upper and lower surfaces. This has not been seriously considered in the literature, although Martin and Juniper (1971) comment that as a general rule the adaxial (upper) cuticle is more substantial than the abaxial (lower) cuticle. Alternatively, the guard cells which also have a high concentration of ectodesmata, may serve as preferential pathways of entry. This may be particularly so when synthetic auxins are used, since these compounds cause stomatal closure after a time lag of 30 to 90 minutes (Mansfield, 1967). The effect of 2,4-D could not be reversed by CO_2 free air, suggesting a change in the membrane permeability of the guard cell.

Martin and Juniper (1970) state that "the consensus of opinion now, is that stomatal penetration of aqueous solutions is unimportant." They consider the guard cells are important. However, the more recent work on surface tension required for stomatal penetration helps to clarify the situation.

Considerable controversy surrounds the subject of whether the cell wall and cuticular layers are traversed by pores (ectodesmata) and whether there are pathways through the extracellular layers along which water and other polar molecules preferentially traverse. Crafts and co-workers favour a scheme involving two routes of penetration; a lipoidal route and an aqueous route. Supporting evidence for this, is the enhancement of penetration at high humidities which allows extension of the water continuum from the inner symplast to the outer surface (Crafts, 1964). Lipophilic substances are thought to penetrate by a "solution process" within the lipophilic components of the cutin and the waxes of the cuticle.

The ectodesmata exist in particularly high concentrations at the base of the hair cells, in epidermal cells, surrounding the veins, along the anticlinal walls and in the walls of guard cells. Franke (1967) favours the ectodesmata as entry routes since preferential absorption through the above areas has been demonstrated. Bayer and Lumb (1973) conclude that since with one exception ectodesmata have not been shown to extend to the surface of the cuticle, "it is obvious that physio-chemical characterization of the pathway must await its further delineation."

(b) Mechanisms Involved in Cuticular Penetration

The initial reactions during cuticular penetration involve a reversible adsorption, followed by diffusion through the surface layers, at a net rate proportional to its concentration gradient across these layers (Sargent, 1965). The magnitude of the layer is a function of:

1. Availability of the applied regulator. This is influenced by physical properties of the applied solution (e.g. extent of dissociation) and the physio-chemical properties of the leaf surface.

2. The internal supply of "free" herbicide, which is influenced by rate of metabolic conversion or movement to other parts of the plant leaf,

3. The thickness of the surface layers.

(c) Penetration of the Plasmalemma

Bayer and Lumb (1973) in reviewing this topic, comment that little is known of the mechanism involved in movement of pesticide molecules across the plasmalemma.

In the process of uptake, adsorption of anionic herbicides (e.g. phenoxy groups) to positively charged amine and quaternary ammonium groups which are present on proteins and lipoids, will result in less

herbicide being available for translocation, (Brian, 1968).

As well as passive movement of molecules, a metabolic component of uptake may occur. The existence of a metabolic component of uptake is shown by a number of criteria:

1. A temperature coefficient (Q_{10}) of greater than two,
2. Uptake inhibited by anaerobic conditions, inhibitors of respiration and other metabolic inhibitors,
3. Adding compounds of similar structures may result in competitive inhibition,
4. The intercellular concentration is greater than the external solution.

Bayer and Lumb (1973) conclude that while there is considerable evidence that the accumulation of some organic pesticides depends on metabolic energy, it is not apparent whether the energy is required to maintain adsorption sites associated with the plasmalemma or whether the energy is required for transport across the membrane.

2.2. TRANSLOCATION OF HERBICIDES

Translocation, in this review, will involve a discussion of transport over distances greater than from cell to cell; i.e. long distance movement of systemic herbicides.

Herbicides may be grouped into four categories depending on their translocation characteristics:

1. Herbicides that move primarily in the apoplast,
2. Herbicides that move primarily in the symplast,
3. Herbicides that move freely in both the apoplast and the symplast,
4. Herbicides that have restricted translocation in both systems.

Bayer and Lumb (1973) present a reprinted table classifying herbicides into the above groups. The auxin herbicides are primarily translocated in the symplast (amiben, 2,4-D, dichloroprop, M.C.P.A., 2,4,5-T) while compounds such as dicamba and picloram move freely in both the symplast and the apoplast.

Bayer and Lumb (1973) also note that symplastic movement is usually of greatest importance when herbicides are applied to the soil. For this reason, the review will concentrate on symplastic movement.

To understand the factors influencing the movement of herbicides in the symplast, a knowledge of phloem structure, physiology and translocation mechanisms is necessary. A brief description only, will be given in this review.

2.2.1. Intracellular Movement

Following absorption, intracellular movement of the herbicide to the phloem occurs. This has been described by Hull (1970) and Robertson and Kirkwood (1970). The actual pathway is not known and it may differ for different species. Three possibilities have, however, been considered for assimilate movement:

1. Translocation via cell plasma membranes,
2. Translocation via plasmodesmata,
3. Translocation via intracellular free spaces.

2.2.2. Phloem Loading

Kursanov (1963) considers that phloem loading is an active process in which A.T.P. enhancement of assimilates can be demonstrated. Furthermore, the loading process is selective. Both active loading and discrimination between herbicidal compounds is considered to occur, (Milthorpe and Morley 1969). Phloem loading is often considered as part of the translocation process and not as a separate physiological process. Thus factors which are reported to affect translocation (see Sections 2.2.5. and 2.2.6.), may have acted on the loading processes rather than on the process of phloem translocation.

2.2.3. Mechanism of Translocation

The phloem is a complex tissue representing a distribution system serving the whole plant. Its structure has been described in detail by Esau (1969) and Crafts and Crisp (1971). The nature and condition of the sieve plate pores and the arrangement of P-protein is most important with regard to translocation mechanisms.

The actual mechanism of translocation has been the subject of considerable controversy. The various hypotheses have been critically examined by MacRobbie (1971). There are two major concepts. The first involves independent movement of the solutes and water while the second considers that solutes are moving "en masse" with the water. It is impossible to say which of these mechanisms are involved, although there is considerable support for mass flow concepts.

2.2.4. Physiology of Assimilate and Herbicide Translocation

To be effective, a herbicide must be translocated to sites where it is lethal. In perennial herbaceous plants and woody shrubs it is essential that sufficient herbicide reaches the lower stem and root system to prevent regeneration of the plant from basal parts, (Crafts and Crisp, 1971). With this thought in mind, many workers have measured the effectiveness of treatments in terms of an alteration in distribution patterns in favour of increased translocation to the root system. When a treatment enhances absorption, it will generally result in greater radioactivity or concentration of the herbicide recorded in the various sinks. The influence of the treatment is considered to have altered translocation if the percentage translocated as a percentage of the amount absorbed is changed. To obtain a direct measure of the influence of treatment on translocation without the complicating effects of absorption, Basler et al (1970) injected herbicides into the stem.

Finally, there is a paucity of information on translocation in adult woody shrubs in the field. As a result of the difficulties of sampling large plants, coupled with a lack of environmental control, much of the data that has been collected is from young glasshouse-grown material. This must place limits in relating such results to the field.

(a) Distribution Patterns

Detailed reviews on herbicide distribution have been made by Crafts and Crisp (1971) and Foy et al (1971).

Various plant tissues (roots, stems, meristems, flowers, fruits and seeds) constitute sinks for assimilates in plants. Growth, metabolism and storage by these structures require organic substrates that are photosynthesized in the leaves. That the size of the sink influences translocation, has often been demonstrated, e.g. Peel and Ho (1970). Tissues differ in their demand for assimilates. The mechanisms that regulate this variation in "sink-strength" and inter-sink competition are not well understood.

Not only is assimilate distribution influenced by physiological sinks, but the distribution of auxin herbicides is similarly influenced. Both Robertson and Kirkwood (1970) and Crafts and Crisp (1971) present evidence of this, while more recent reports confirm the importance of sinks. Chang and Vanden Born (1971) showed accumulation of dicamba in "actively developing" young leaves at the shoot apex in two broadleaf weeds, while Binning et al (1971) found that the bulb of wild garlic

(Allium vineale) acts as a sink for dicamba during development. Quimby and Nalewaja (1970) using autoradiography demonstrated that dicamba moved symplastically in Polygonum convolvulus and the highest concentrates were in meristemmatic tissue.

Working with picloram, Sharma et al (1971) considered that the pattern of distribution in Cirsium arvense was in harmony with a sink source pattern. Guenthrer (1971) working with the same species observed that while absorbed picloram remained in the leaf, some activity was evident in young leaves, growing tips and roots.

The susceptibility of honeyvine-milkweed (Ampelanus albidus) to early, but not late, applications of 2,4-D was found by Coble et al (1970) to be due to increased translocation of 2,4,-D into the roots, which was more closely related to new root growth than to the amount of top growth. Sutton (1970) concluded that the basipetal translocation of 2,4-D in Myriophyllum basiliense occurred principally in the symplast and followed a source-sink relationship.

Schmutz (1971) in a study on the translocation of 2,4,5-T in woody plants in the field found that maximum basipetal translocation coincided with the summer period of high susceptibility of herbicides and reached a peak about 30 days after the start of effective summer rains.

Crafts and Crisp (1971) discuss redistribution patterns of inorganic and organic compounds. Redistribution into xylem of dicamba, has been reported in several species, while Field and Peel (1971) comment that the radial movement of chlorophenoxy herbicides between phloem and xylem has been demonstrated by several authors. They found that the radial movement of MCPA was greater than that of 2,4-D, which was greater than that of 2,4,5-T in willow (Salix sp.) stem. Redistribution into the xylem is likely to be important when basipetal translocation of the herbicide in the phloem is dominant.

(b) Rate of Translocation

The rate of translocation can be measured either as a velocity (cm/hr) or as the specific mass transfer (g dry wt/ cm^2 phloem/hr). Crafts and Crisp (1971) give a sample of velocity values quoted in the literature. These range from 1.5 cm/hr to 7,200 cm/hr. Most values, including those for herbicides are in the range of 10-120 cm/hr.

There are numerous difficulties in estimating velocity of translocation from radiotracer data and these have been discussed by Canny (1960).

Several of these problems arise because the distribution profile of activity along the stem is generally logarithmic. Determination of the herbicide front is therefore a function of the limits of detection of the measuring instruments. Thus, a situation may arise where phloem loading is slow. This, coupled with rapid translocation, would result in a quick distribution of a small amount of herbicide. Detection of the herbicide by techniques and instruments of varying sensitivity would result in different interpretations about the initial distribution patterns of the herbicide.

2.2.5. Environmental Influences on Translocation

The phloem, being a complex osmotic system, is regulated in its function, by the availability of water. In addition, water stress, also affects the assimilate source. Crafts and Crisp (1971) have reviewed the literature on the effect of water stress on translocation and cite three instances where herbicide translocation has been impaired. In one woody species (Quercus marilandica) they quote the amount of ^{14}C - 2,4,5-T translocated (as a percentage of the amount absorbed) increased from 16-37 percent as the soil moisture increased from 2-16 percent.

Willis and Basler (1971) also reported that soil moisture had little effect on 2,4,5-T absorption, but markedly influenced translocation in elm,(Ulmus sp.).

A number of workers have demonstrated enhanced translocation at higher relative humidities (RH) and this has been demonstrated by Crafts and Crisp (1971).

Burns et al (1969) reported a two-fold increase in absorption coupled with a nine-fold increase in translocation of 2,4-D when 40 percent RH was compared with 100 percent RH. However the response may have been due to the higher temperatures they encountered using a polyethylene bag system to produce high humidity. By injecting 2,4,5-T to avoid absorption, Basler et al (1971) "confirmed conclusions of others" that an increase in basipetal translocation at high RH occurs. Sharma et al (1971) found a similar change in the distribution of picloram at high RH and concluded that reduced transpiration under high RH may partly account for a shift towards basipetal translocation.

Considerable literature exists on the influence of temperature on assimilate transport, although even here much work has been done at low 0°C temperatures. However, Wardlaw (1968) cites an experiment where pretreatment of roots at 23°C resulted in much greater transport than did 8°C.

Crafts and Crisp (1971) cite two cases of the influence of temperature on translocation. In one case, the distribution of 2,4,5-T in Prosopis juliflora was basipetal at 21°C and became increasingly acropetal as temperatures were increased to 38°C. An increase in temperature from 20°C to 30°C is reported to have enhanced translocation of 2,4-D in Phaseolus vulgaris.

There is indirect and direct evidence that increasing light intensities result in greater translocation because of the influence on assimilate production. Crafts and Crisp (1971) cite an example where 2,4-D is readily translocated from photosynthesizing leaves of Phaseolus but very little movement occurs from leaves held in the dark, until starch reserves had been depleted.

Brady (1969) found that increasing light intensity increased absorption in four woody species, but only two of these showed increased translocation of 2,4,5-T to the roots.

2.2.6. Spray Factors

A number of organic and inorganic additives have been shown to alter either the distribution pattern or the rate of translocation of herbicides. The practice of using herbicide mixes is becoming increasingly important and one herbicide may influence the translocation of another. Agbakoba and Goodin (1970) found that cold picloram enhanced translocation of ^{14}C-2,4-D without changing the distribution, while cold 2,4,-D diverted ^{14}C-picloram away from the growing tips and increased picloram accumulation in the roots.

Davis et al (1968a) found that the uptake and transport of picloram by Prosopis juliflora increased in the presence of 2,4,5-T, while picloram depressed the absorption and transport of 2,4,5-T.

Devlin (1970) is reported in Weed Abstracts, to have found greater 2,4,5-T residues in berries of poison ivy, (Rhus toxicodendron) when 100 ppm of IAA or GA was added to the spray. It was not clear whether the effect was due to enhanced absorption, translocation or both. Brady (1970) found that ammonium nitrate increased translocation of 2,4,5-T to the roots of Quercus stellata but had no effect in three other woody species.

Ethylene-releasing compounds influence translocation, probably by stimulating a metabolic sink. Hull (1971) found that ethrel increased ^{14}C assimilate translocation two fold in Johnson grass (Sorghum halepense). The basipetal translocation of aminotriazole and dicamba was not increased, but the concentration of the former increased in the growing bud. However, Binning et al (1971) recorded a five to eight-fold increase in basipetal movement of dicamba when the plants were treated with ethrel at 1,000 ppm. Similarly, pretreatment with C.E.P.A. (2-chloro-ethyl-phosphoric acid) increased basipetal translocation of dicamba. This had important practical applications in the control of wild garlic (Allium vineale) which sets underground bulbs early in the growing season. Once formed, they became dormant and no longer acted as sinks. Control was then difficult.

Herbicide formulation can have a marked effect on translocation. Norris and Freed (1966 a and b) published a series of papers on the absorption, translocation and metabolism of chlorophenoxy herbicides by Acer macrophyllum. Although the 2,4-D ester was more readily absorbed (20.8 percent) compared to 2,4-D acid (2.9) percent), the amount of herbicide reaching the roots was greater when the acid form was used. However, while 2,4,5-T showed similar absorption characteristics, the ester formulation also resulted in more herbicide arriving in the roots. With the ammine formulation, poor absorption offset superior translocation characteristics.

When esters of 2,4-D and 2,4,5-T and their propionic acid analogues were compared, 2,4-DP resulted in nearly three times the herbicide accumulation in roots than did 2,4-D, due both to enhanced absorption, and a two-fold enhancement of translocation. On the other hand, while the propionic derivative of 2,4,5-T was more readily absorbed, it was not as well translocated to the roots as 2,4,5-T itself.

The butyric derivatives of 2,4-D esters while having poor absorption characteristics, was highly translocated to the roots. Since the leaves and stems of Acer have little capacity to oxidize 2,4-DB, efficient transport to the roots occurs (2,4-D is known to damage and thus inhibit phloem transport). Further the roots of Acer were shown to have a high capacity to oxidize 2,4-DB. Considerably more work is required on the translocation of herbicides with different formulations.

2.3. HERBICIDE METABOLISM

A study of herbicide translocation with radioactive labelled herbicides would be incomplete without determining whether radioactivity measured is due to the herbicide or to some metabolite. Auxin herbicides may be inactivated by two types of metabolism, degradation and conjugation. The metabolism of herbicides by some species and not others has resulted in a wide range of selective herbicides being developed. However, even when non-selective control is required metabolism may reduce the effectiveness of the herbicide.

2.3.1. Degradation

A number of pathways for degradation of auxin herbicides in plants exist, including decarboxlyation, ring hydroxylation and B-oxidation of the aliphatic side chains. These pathways have been reviewed by Loos (1969) and Robertson and Kirkwood (1970).

Decarboxylation of 2,4-D is important in resistant species e.g. some varieties of apple (Primer, 1968), Ribes rubrum (red current) and Syringa vulgaris (Eidel'nant and Trublaevich 1968). Sargent et al (1969) reported that decarboxlyation of 2,4,5-T and a number of chlorobenzoic acids including dicamba, occurs in Phaseolus.

The only report of substantial decarboxylation of picloram was by Sargent and Blackman (1970) in young leaves of Phaseolus. They cite the work of Meible et al (1966) who reported limited picloram decarboxylation by cotton (Gossypium sp.).

Picloram, dicamba and to a lesser extent 2,4,5-T are reasonably stable in many plant species (Loeffler and van Overbeck 1971). Zea mays and Hordeum vulgare however, degrade dicamba to 5-hydroxydicamba. Hydroxylation of dicamba has also been reported to occur in other members of Poaceae (Gramineae) by Swanson (1969) and Chang and Vanden Born (1971). Ring hydroxylation has also been reported for the phenoxyacetics, (Robertson and Kirkwood 1970).

Detoxification can be blocked in some species. For instance, detoxification of M.C.P.A. by cleavers (Galium aparine) could be blocked by an α-methyl substitution in the side chain which prevented β-oxidation occurring. In McIntosh apples , replacement of the para-chlorine in 2,4-D with fluorine makes the molecule resistant to degradation. Thus, blocking must inhibit the sterospecificity of the degrading enzymes.

2.3.2. Conjugation

Conjugation of IAA. and NAA is well documented in plants. Glucosides are formed immediately following auxin uptake while after a two to four hour lag, conjugates with amino acids, especially aspartic acid appear (Gorem and Bukovac 1973).

Robertson and Kirkwood (1970) review the earlier work on conjugation of phenoxy acid herbicides. Earlier workers found evidence of protein-lipoprotein adsorption of M.C.P.A. at physiologically mature sites in resistant species (Brian 1958), while a number of Russian workers are described in Weed Abstracts as reporting similar results; e.g. Chkanikov and Pavlova (1968) found 2,4-D bound with proteins of molecular weight 50,000 in cereals, while no bonding could be found for susceptible beans (Phaseolus) and sunflower (Helanthus annua). Hallman and Eliasson (1972) also obtain protein complexes from cereals treated with 2,4-D. Further, biologically active 2,4-D could be recovered by hydrolysis of protein precipitate with NH_4OH. They also found that a considerable proportion of the 2,4-D (45 percent in 24 hours) formed water soluble complexes from which 2,4-D was released by hydrolysis.

While the nature of the complex was not determined, other workers have reported a glucose ester of 2,4-D, while amiben (2,4-dichloro-3-amino-benzoic acid) readily forms glycoxides with glucose and ribose (Loffler and van Overbeck 1971). Swanson (1969) suggests that poor translocation of amiben in many species is presumably due to conjugation. Loffler and van Overbeck (1971) report that 2,4-D has been shown to react with aspartic acid, while ten other ninhydrin positive metabolites of 2,4-D have been reported.

Picloram has been reported to form conjugates in several species. Maroder and Prego (1971) note that most plants do not apparently possess enzymatic mechanisms to modify picloram. They did identify complex formation of picloram with ethanol extraction in two species, Prosopis ruscifolia and Diplotaxis tenuifolia. Although neither complexes were identified, hydrolysis did release free picloram.

Wheat leaves rapidly conjugate considerable amounts of picloram as a water-soluble complex (57 percent in 24 hours), while only 3.4 percent was complexed in a T.C.A. insoluble fraction (protein precipitate). Further, the water soluble complex was very labile and could not be detected when ethanol was used as the extractant, (Hallmen and Eliasson, 1972).

Sharma and Vanden Born (1973), using ethanol extraction reported soluble conjugates of picloram from barley leaf (but not barley coleoptile)

and from the leaf of Canadian thistle (Cirsium arvense). The conjugates gave a positive reaction for sugar with benzidine and a negative reaction for amino acid with ninhydrin. Acid hydrolysis released free picloram. Note that ninhydrin is specific for the aliphatic or alicyllic amino group; thus the amino group of picloram (an aromatic compound) will not react with ninhydrin.

The labile nature of some conjugates has not always been appreciated, and thus may have gone undetected in some experiments.

CHAPTER THREE MATERIALS AND METHODS

3.1. PLANT PROPAGATION

3.1.1. Propagation from cuttings

Gorse can be easily propagated from cuttings. Secondary stems about 10cm in length were prepared by cutting off the lower spines and trimming the upper spines. The cut surface was dipped in 2 percent IBA. powder and the prepared cutting placed upright in a coarse pumice:peat (80:20) mixture. Bottom heat (21°C) and a moist environment, obtained by misting was used. Roots were visible within 14 days.

Cuttings from one adult plant only were taken to form a single clone. Gorse is highly variable and this has the advantage of reducing variation due to genotype and the disadvantage of limiting the interpretation of results.

3.1.2. Transplanting

One week after root appearance the cuttings were transplanted into 15 cm square plastic pots containing a mixture of thoroughly wet pumice and peat (50:50). No adjustment of the mixture pH was necessary. The plants, (Plate 3.1) were grown in a glasshouse and watered daily. Inorganic nutrient solution (Appendix 1) was applied at one monthly intervals.

3.1.3. Innoculation

Nodules may be important sinks for auxin herbicides (Coaldrake 1970). Rhizobium (strain 5042), suitable for Ulex europaeus were supplied by Mr. R.M. Greenwood (D.S.I.R., Palmerston North). With a heat sterilized loop, rhizobia were transferred to McCartney bottles containing sterilized bactonutrient agar. After 48 hours at 27°C, the rhizobia were rinsed into distilled water (one bottle to 500 ml) and 25 ml of solution added to each pot.

3.1.4. Pollination

As seed pods may act as sinks for herbicides it was necessary to ensure adequate fertilization. Initially hand pollination was used, but pollination by Bombus spp was later found to be efficient. Flowering occurred from August to November on cuttings that were six months old.

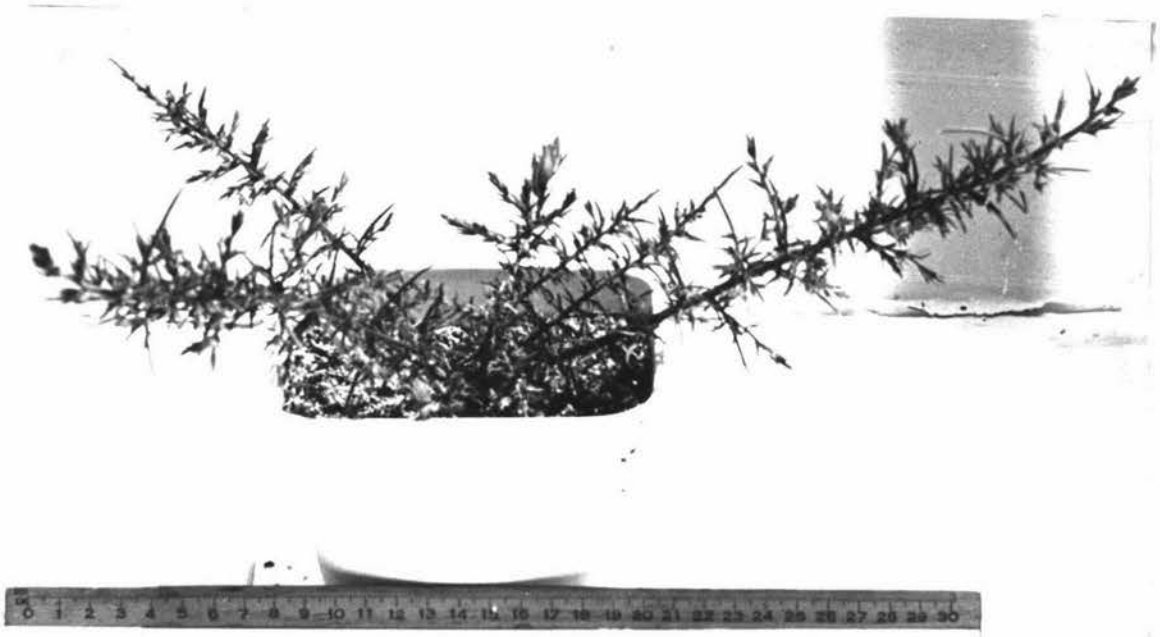

PLATE 3.1

Gorse cutting at six months. This plant is typical of the cuttings used in the study.

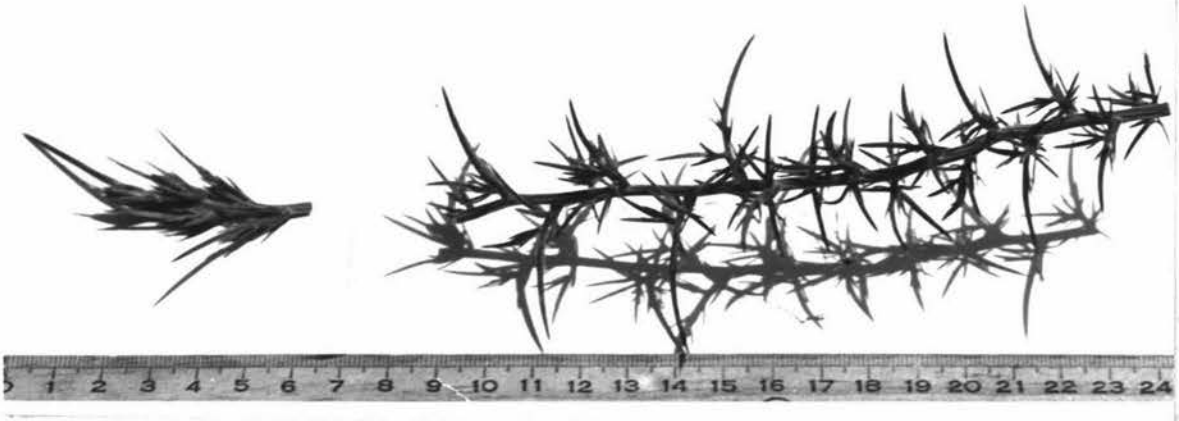

PLATE 3.2

Stem of gorse showing the terminal 6 cm excised in preparation for an <u>in vitro</u> absorption experiment.

3.2. THE HERBICIDES

3.2.1. 2,4-Dichlorophenoxy-acetic acid (2,4-D).

Ten uc of the dimethylamine salt of 2,4-D-1-^{14}C, specific activity 34.0 mc/m mole were donated by Prof. R. Behrens. This was mixed with 40 percent w/v 2,4-D formulated as the dimethylamine salt, and diluted to 750 ppm. This concentration is similar to concentrations commonly used in weed control.

3.2.2. 4-amino-3,5,6-trichloropicolinic acid (Picloram)

Carboxyl-^{14}C picloram (105 uc) was donated by the Dow Chemical Company (U.S.A.). A paper chromatogram scan (See section 3.5) was used to check the purity of the ^{14}C -picloram. No contaminants were detected (Figure 4.14). The picloram was formulated as the potassium salt by reacting with a 1:1 molar ratio of 1 N KOH solution. This was diluted with a commercial formulation of picloram* and is referred to as the picloram mixture in this text.

An oil based mixture was prepared by mixing the K salt of ^{14}C-picloram with alkane 60, (supplied by I.W.D., New Plymouth).

3.2.3. Detection of ^{14}C-Herbicides

The ^{14}C-herbicides were detected using liquid scinitillation spectrometry and the Channels Ratio method of quench correction, (Herberg 1965) was applied. Detection was normally made either using a 1 ml water subsample added to 10 ml of the scintillation fluid (Appendix II), or by digesting the oven dried, ground plant material in a perchloric acid/hydrogen peroxide mixture (Appendix III), before adding the scintillation mixture.

Volatility of picloram and loss of ^{14}C label during digestion was checked by placing 5 µl of the herbicide mixture on a coverslip and placing it in a forced draught oven (80°C) for various time intervals, or in the digestion mixture at 80°C for 20 minutes.

All glassware was decontaminated (Appendix IV) before re-use. Clean vials were selected at random and their levels of radioactivity checked against a standard background vial.

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* The commercial formulation of picloram used in the experiments was Tordon 520 Brushkiller (5% w/v picloram as the potassium salt plus 20% w/v 2,4,5-T as the butyl ester, in the form of an emulsifiable concentrate) diluted 1:250 with distilled water.

3.3. ABSORPTION

Two different in vitro techniques and one in vivo method were used to estimate the absorption of the ^{14}C labelled herbicides.

3.3.1. In vitro absorption

Both methods involved placing a known amount of plant material in an incubating solution for a set time period. Generally the terminal 6 cm of an actively growing branch was excised (Plate 3.2) and weighed. To reduce variation, the segments were cut to fall within the fresh weight range of 450-550 mg. The cut surface of the segments was sealed with paraffin wax to prevent absorption through the cut. Each segment was placed in a test tube containing a known amount of radioactive herbicide buffered to a known pH. The tubes were placed in a waterbath at 27°C ± 1°C (unless otherwise stated) under a light bank of 12 cool white fluorescent tubes and 4 incandescent bulbs (7000 lux).

Initially, absorption was calculated by difference. The concentration (dpm. $m.^{-1}$) of ^{14}C-2,4-D at the beginning and end of the run was measured (1 ml sample). The latter sample is the concentration unabsorbed and hence the difference is the concentration absorbed.

While this method is quick, it lacks the sensitivity (see Section 4.1.2) of methods based on determining the amount of herbicide absorbed, directly. In the latter case, the plant tissue was washed twice after incubation. The first wash was a rinse in distilled water; the second, agitation in 10 ml distilled water with 0.25 percent w/v (1 drop) Triton X-100 for 30 seconds on a whirlmixer. The plant tissue was then dried (80°C for 24 hours), weighed, ground, subsampled (50mg) and digested in the scintillation vials. The radioactivity of the sample was then determined.

It was hoped that preliminary, unreplicated experiments using absorption by difference method would indicate likely areas to investigate. However, the results were too variable to be of any use.

3.3.2. In vivo absorption

Experiments with picloram were performed on whole plants in the glasshouse or in a growth chamber. Unless stated otherwise, the following environmental conditions prevailed in the growth chamber; light (12 cool white fluorescent and 4 incandescent bulbs) 7000 lux; temperature 22 ± 2°C; relative humidity 70 ± 10%. In both cases, temperature and relative humidity were continuously recorded by a thermohydrograph. The plants were initially hand sprayed with Tordon 520 Brushkiller (1:250 dilution) to the point of runoff. Five µl (30,000 dpm) of the labelled herbicide were applied by microsyringe to a selected

area, usually the branch tip. At the end of the experimental period, the treated area was excised and placed in a 25 ml vial containing 10 ml water with 0.25 percent w/v Triton X-100. The vial was capped and shaken on a Whirlmixer for 30 seconds. A 1 ml volume was then syringed out and the absorption determined (See Section 3.3.1).

3.4. SCANNING ELECTRON AND OPTICAL MICROSCOPY

The scanning electron microscopy study was made in conjunction with Miss K. Card (D.S.I.R.). Leaf, stem and spine samples of material grown indoors and outside were sectioned, freeze dried and double coated with carbon followed by palladium-gold. The surfaces of the samples were scanned and the micromorphology observed and photographed. Attempts to detect spray deposits by X-ray probe for Cl^- and K^+ were unsuccessful probably because of the low concentration of picloram when it is applied at normal rates.

Droplets (1 and 2.5 µl) of water and the spray mixture, applied to gorse surfaces by microsyringe were observed by optical microscopy and photographed using an Olympus-Tokyo FH microscope with an interchangeable microphoto unit (model PM-7).

3.5. TRANSLOCATION

Translocation was studied by autoradiography and by examining the distribution of radioactivity in sections. Studies were made in both the glasshouse and growth chamber. Temperature and relative humidity were recorded by a thermohydrograph. The plants were sprayed with the picloram mixture and the labelled picloram mixture applied to a point 6cm below the branch tip.

Plants were prepared for autoradiography by treating with 0.5 µc of the picloram mixture, applied by microsyringe to the stem which had been scraped to facilitate absorption.

At the end of the experimental period the treated stem and roots were excised from the rest of the plant. The roots were thoroughly washed to remove the adhering potting mixture and all plant sections were frozen in liquid air. After thawing, the plants were pressed for 14 days, the papers being changed after days one and seven.

The pressed plants were mounted onto light-weight cardboard 20cm x 25cm using Plastipad adhesive glue and placed in light proof sachets containing 20cm x 25cm Kodak X-ray Film (stock Item No. 8853). After 7 days exposure the X-ray film was developed using Kodak X-ray Developer.

A total of 0.042 µc (60,000 dpm) was applied to each plant. At the end of the experimental period the treated area was excised, washed and the degree of absorption determined. The rest of the plant was then sectioned. The treated stem was normally cut into 2.5 or 3 cm lengths. 0+ branches were removed from the main stem as single units, while the roots were cut off and washed to remove any adhering potting mixture. Sketches were made of each plant to determine the relationship of various branches.

The plant material was oven dried (80°C for 24 hours), weighed, ground to a fine powder in a "Krups 75" coffee grinder, subsampled, and digested in scintillation vials, (Appendix IV). The amounts of radioactivity were determined by liquid scintillation counting.

3.6. METABOLISM

It is necessary in translocation studies involving radioactive labelled material to determine whether degradation of the compound occurs as the translocation pattern obtained may be due to a metabolite and not to the herbicide being studied. Alternatively, conjugation with amino acids or simple carbohydrates may occur and reduce the amount of translocation occurring.

For metabolism studies of ^{14}C-picloram, segments (1.0g FW) of gorse were incubated in 0.1 µc of the potassium salt of ^{14}C carboxyl labelled picloram made up to 15 ml with the picloram mixture.

After 24 hours incubation at 27°C the samples were ground with fine sand and 5 ml 0.1 M NH_4HCO_3, pH 7.3 (adjusted with CO_2) in a pestle and mortar. A further 20 ml of buffer were used to transfer the slurry to centrifuge tubes. The extracts were centrifuged for five minutes at 3,000 rpm and the supernatant collected. The pellet of cell wall material was oven dried, ground, subsampled (50 mg) and the radioactivity determined in the normal manner, following bleaching. (See Appendix III).

The supernatant was divided into two equal portions. In one fraction the soluble proteins were precipitated using 4 ml of 5 percent $ZnSO_4 \cdot 7\ H_2O$ titrated with 0.3N $Ba(OH)_2$ using phenolphthalein as an indicator. (Dawson et al, 1969).

The precipitated soluble protein solution was centrifuged (10 minutes at 3,000 rpm). The radioactivity of the pellet was determined as before, while the supernatant collected from the centrifuging was reduced by freeze drying in preparation for separation by chromatography.

Descending chromatography on Whatman No. 1 paper in the upper phase of an n-butanol:acetic acid:water (40:12:50) mixture was used. The bottom phase was placed in the chromatagraphy tank and allowed to saturate the atmosphere.

The chromatogram ran overnight and after drying, the ^{14}C distribution was detected with a Packard Radiochromatogram Scanner.

The method used differed from those reported in the literature. For example Hallmen and Eliasson (1972) extracted with 0.1M phosphate buffer pH 7.2, and precipitated the soluble proteins with T.C.A. The NH_2HCO_3 buffer was used in this study to facilitate the freezedrying process, while T.C.A. was avoided in case acid labile conjugates were present. Several authors have used ethanol to extract picloram with (e.g. Sharma and Vanden Born 1973) but in wheat, Hallmen and Eliasson (1972) found that conjugates so formed were ethanol labile.

3.7 STATISTICAL ANALYSIS

The conversion of recorded cpm (counts per minute) to dpm (disintegrations per minute) by Channels Ratio method, was initially done by electronic calculator and later by computer. A fortran programme using the regression lines derived in Figures 4.1 and 4.2 were used as the basis of these calculations.

The experiments in this study were simple comparisons between treatments generally in relatively controlled conditions. For this reason, t-tests were used in the probability calculations.

CHAPTER FOUR RESULTS

The results are presented briefly in the form of tables, with mean values and their standard errors and statistical significance based on the t-test calculated with 2n-2 degrees of freedom. Absorption results from *in vitro* experiments are expressed as absorption per unit weight; either fresh weight (FW) or dry weight (DW), depending on the method used. Photographs taken during the study have been included and where necessary, data are presented graphically. The results are divided into four sections; techniques, absorption, translocation and metabolism.

4.1 TECHNIQUES

4.1.1. Calibration Curves for Channels Ratio

Calibration curves for counting water-based samples and digested samples were derived using standard quenched samples supplied with the scintillation counter. The curves are presented in Figures 4.1 and 4.2. The equations describing the curves were derived by linear and curvilinear regression analysis and the resulting equations used to correct, by computer, the counts recorded by the counting channel.

The digested samples were a pale yellow colour due to a reaction between the scintillation fluid and the bleaching agent. As a consequence of this colour quenching, high gain settings (25 percent) were required when samples of low activity (approaching background) were being counted e.g. root samples in translocation experiments. The results of counting low activity samples at three different gain settings are shown in Table 4.1.

TABLE 4.1 Detected ^{14}C levels of digested samples counted for 20 minutes at three gain settings. (Background counts at 500±20 dpm).

Sample			Counting Channel (20 min)		
Total DW (mg)	Subsample (mg)	Weight Correction Factor	9% Gain	20% Gain	26% Gain
83.5	55.0	1.52	495	689	875
331.0	69.0	4.80	520	2059	4523
161.0	52.5	3.07	486	1350	2544
115.0	53.0	2.17	531	661	862

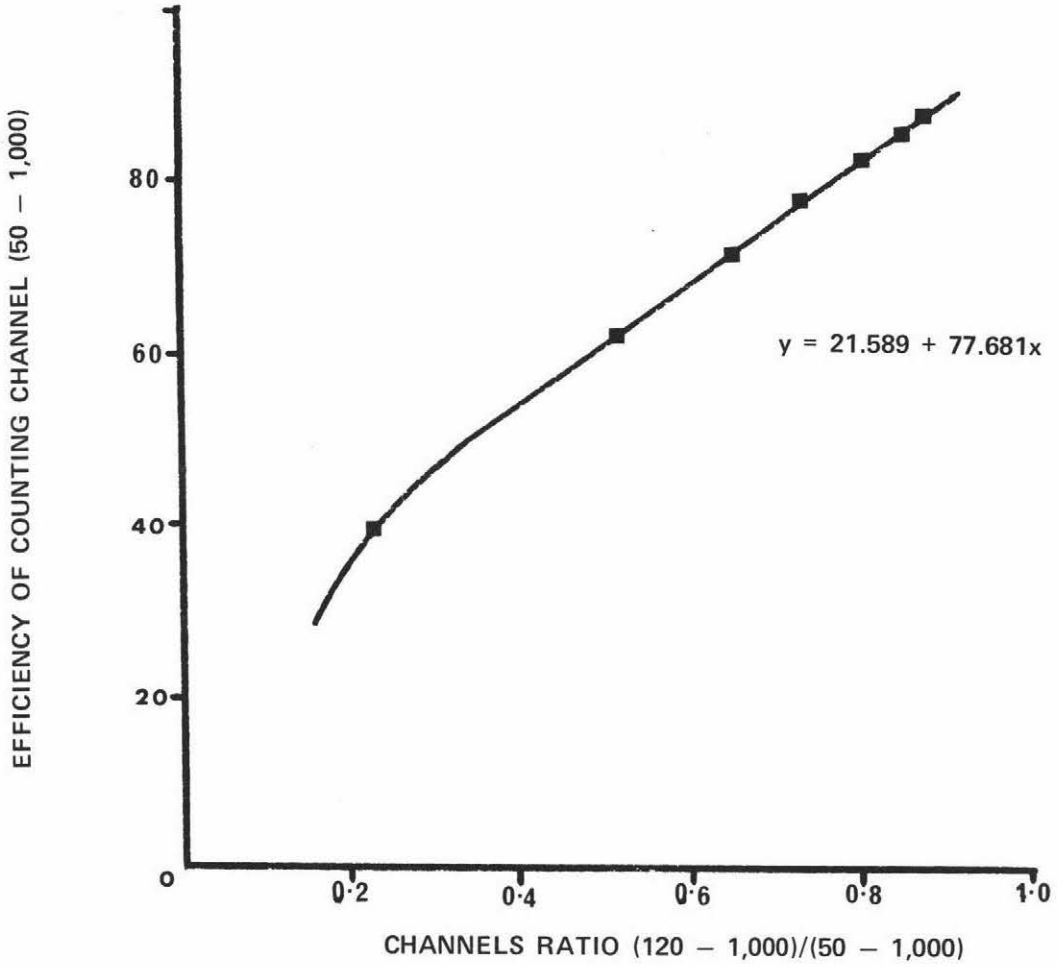

FIGURE 4.1: Calibration curve derived for counting 14_C in aqueous samples (gain 9.0%).

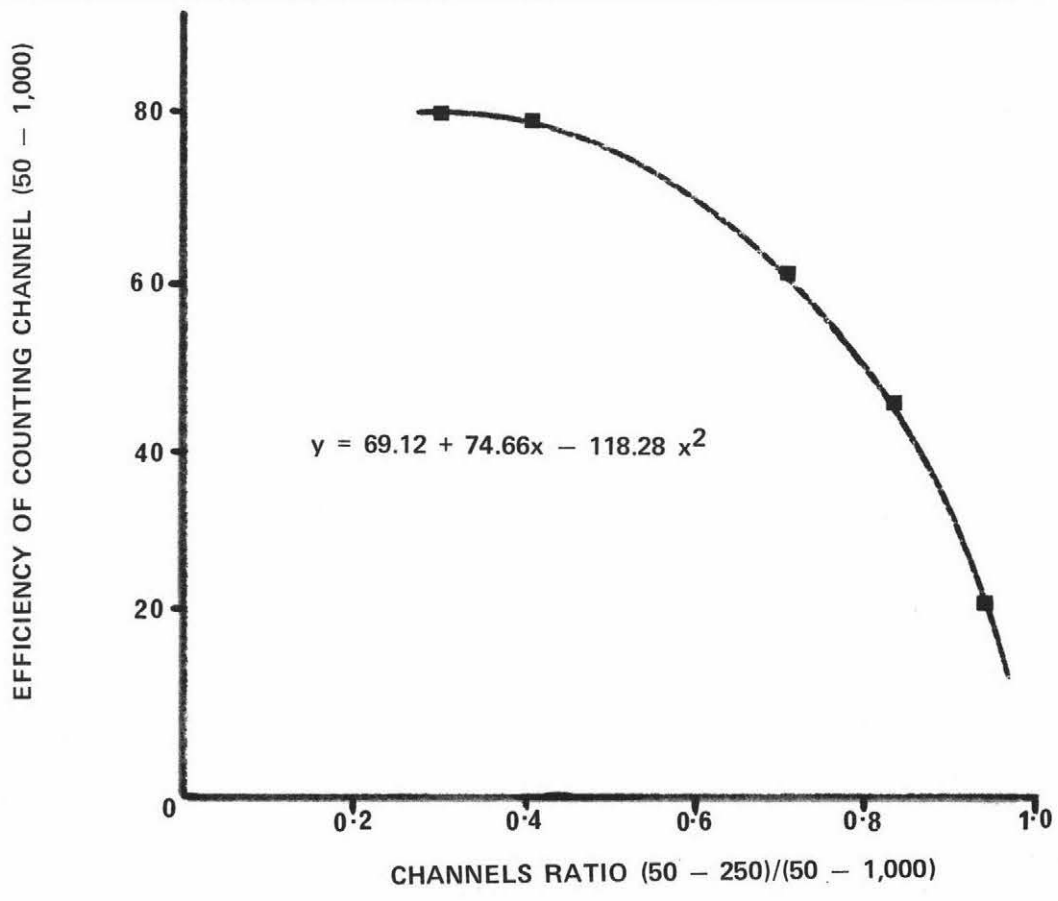

FIGURE 4.2: Calibration curve derived for counting 14_C in digested samples (gain 25.0%).

At 9 percent gain it is doubtful whether the counts recorded are different from background. However, at 20 percent and 26 percent gain, the counts are clearly greater than background.

4.1.2. In Vitro Absorption

Two in vitro methods were used. The quicker, difference method, (see 3.3.1.) was not as sensitive in detecting absorption, when compared with the digestion method. When absorption of picloram over a time period was studied, the two methods gave considerably different results, as shown in Table 4.2.

TABLE 4.2 In vitro absorption of the K-salt of ^{14}C-picloram at two time periods using two methods. (Temperature, 27°C; light, 7000 lux).

Time (hours)	Method	Amount ^{14}C-picloram absorbed (dpm.g FW^{-1} x 10^{3})
0.5	Difference	4.30*
	Digestion	17.70
16.0	Difference	4.05*
	Digestion	52.40

* Although three samples were used in each determination absorption was detected only in one sample. The figure given is the one value recorded in each case.

4.1.3. Leaf weight and Area Relationships

With in vitro absorption, the amount absorbed must be related to a plant parameter such as leaf area or leaf weight. While absorption per unit leaf area would be desirable, it is difficult to measure the leaf area of gorse but on the other hand, fresh weight and to a less extent, dry weight, are readily measured. The relationship between fresh weight and the area, determined with an automatic leaf area meter (Daichi Boeki Shokai Co.) is presented in Figure 4.3. There is a close relationship between leaf fresh weight and leaf area, and hence leaf weight measurements were used.

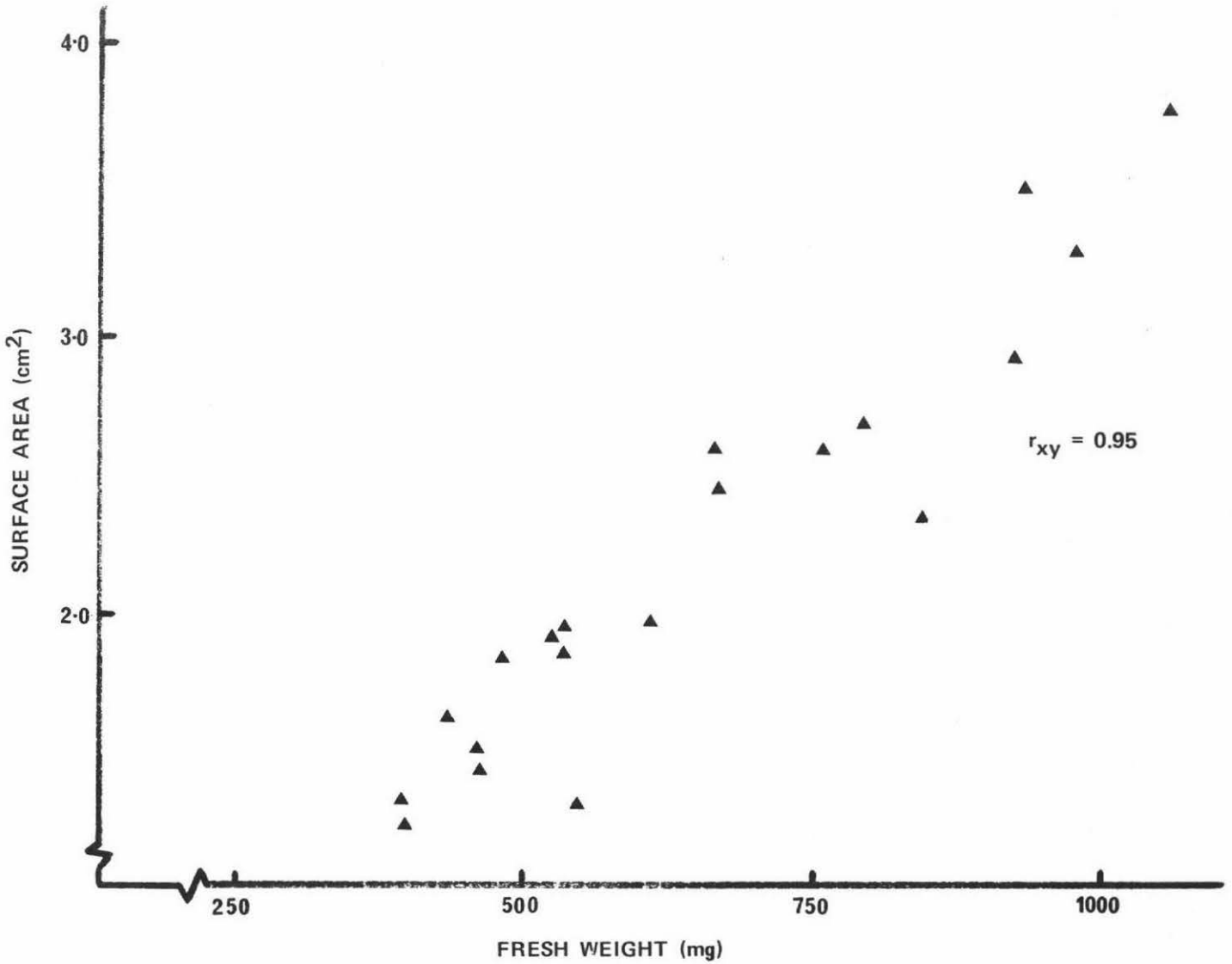

FIGURE 4.3: Correlation between fresh weight and surface area of gorse stem tips.

4.1.4. Plant Age Effects

The in vitro experiments were carried out over a period of several months. During this period, the plants were continually producing new stem tips. Tips which appeared of similar maturity were selected for the experiments and during the course of the work there was no apparent change in the nature of the tips. An experiment was run to test whether any change in the absorption capacity of newly produced stem tips occurred at two different plant ages. The results are presented in Table 4.3.

TABLE 4.3 Comparison between newly produced stem tips from plants of two different age groups to absorb ^{14}C-2,4-D dimethylamine.

Age of plant (weeks)	Sample size	% absorbed	t
25	9	11.30 ± 0.69	
			NS
39	12	9.65 ± 0.77	

Experimental conditions. 2,4-D concentration 760 ppm, temperature 27°C; 0.25% w/v Triton- X100, light 7000 lux. percentage data analysed using angular transformation (Snedecor and Cochran, 1969).

While the stem tips produced at 39 weeks absorbed less than those at 25 weeks, the difference was statistically non-significant.

4.1.5. Precooling Influence on Recorded Counts

With any instrument, correct operating procedure is important. For instance, pre-cooling of the tubes for 40 minutes in the chamber of the scintillation counter was found to be necessary, as shown graphically in Figure 4.4. Details relating to this figure are to be found in Appendix V.

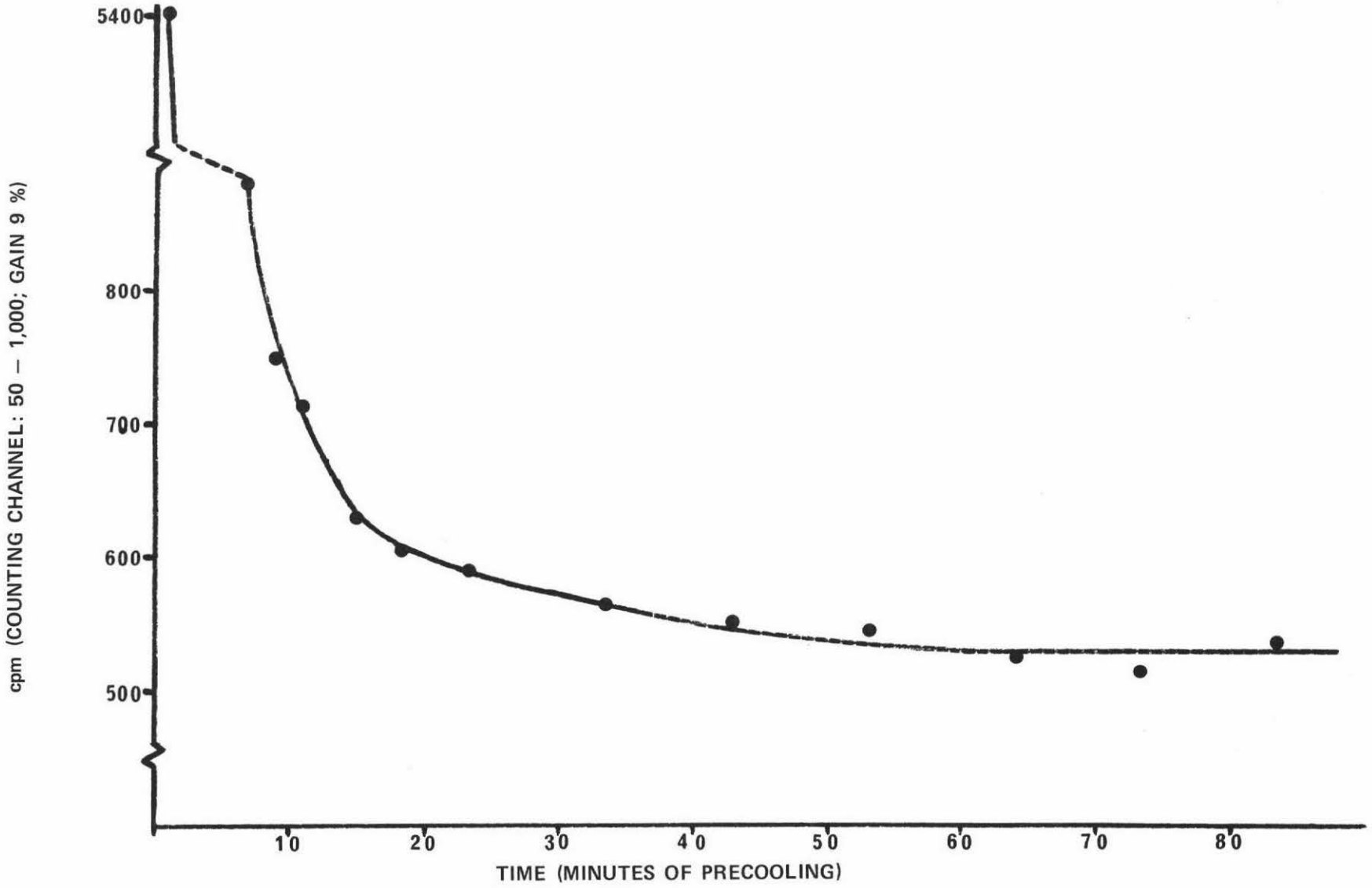

FIGURE 4.4: The influence of precooling on the recorded counts.

4.1.6. Estimation of ^{14}C Losses

During the experiments ^{14}C-picloram could be lost due to volatilization of the herbicide from leaf surfaces and especially during oven drying. Decarboxylation and loss of label during digestion may also occur. An experiment was run to estimate whether loss of ^{14}C label was occurring. No losses were detected. The results are presented in Table 4.4 and 4.5.

TABLE 4.4 Estimates of the volatilization loss of ^{14}C picloram (K-salt)

Temperature (^{o}C)	Time (hours)	^{14}C Remaining on Slide (dpm x 10^4)
33	0.0	2.57 ± 0.12
	2.0	2.69 ± 0.11
	6.0	2.56 ± 0.06
	12.0	2.71 ± 0.05
	24.0	2.63 ± 0.03
82	0.3	2.73 ± 0.02
	24.0	2.54 ± 0.10

TABLE 4.5. Loss of ^{14}C- picloram (K-salt) due to oxidation during perchloric acid/hydrogen peroxide digestion.

Treatment	^{14}C remaining in vial following digestion (dpm x 10^3)	t
Control (not heated)	14.13 ± 0.28	a*
Normal	13.92 ± 0.65	a
Without cap (simulates leaking cap)	14.32 ± 0.56	a
Rapidly cooled after digestion by cold water rinse and placed in cool (20°C) room.	14.07 ± 0.59	a

* Data followed by same letters are non-significant at $p > 0.1$.
Samples heated for 20 minutes at 80°C, with caps screwed down firmly.

4.2. ABSORPTION

4.2.1. Environmental Influence of Absorption

A number of *in vitro* and *in vivo* experiments studying environmental influences on absorption were carried out.

(a) Temperature

The influence of temperature on the absorption of 2,4-D and picloram is presented in Tables 4.6 and 4.7. In both tables greater variability occurred at higher temperatures. With 2,4-D, a second experiment was run, with increased sample size to overcome the variability. The two herbicides respond in a different manner to an increase in temperature. While 2,4-D has a constant Q_{10} value, picloram has a reduced Q_{10} value at higher temperatures.

TABLE 4.6 Temperature effect on the *in vitro* absorption of ^{14}C-2,4-D dimethylamine.

Temperature (^{o}C)	% Absorbed	Amount Absorbed dpm. $ml^{-1}.g\ FW^{-1} x10^{2}$.	t	Q_{10}
Expt. (a)				
17	6.5	4.27 ± 0.30	$P > 0.001$	1.80
27	11.4	7.67 ± 0.51	$P > 0.001$	1.79
37	20.4	13.75 ± 1.87	$P > 0.1$	
Expt (b)				
27	11.3	7.53 ± 0.23	$P > 0.001$	1.96
38	24.8	16.51 ± 0.16		

Experimental conditions: Time 12 hours; light 7000 lux; 764 ppm 2,4-D + 0.25% w/v Triton X-100, temperature ± 0.5°C.

TABLE 4.7 Temperature effect on the in vitro absorption of ^{14}C-picloram (K-salt) from a picloram: 2,4,5-T (1:4) mixture.

Temperature (°C)	Amount absorbed ($dpm.gDW^{-1}.x10^4$)	t	Q_{10}
17	1.34 ± 0.12 (A)	P > 0.01	2.13
27	2.85 ± 0.07 (B)	P > 0.01	)
37	3.45 ± 0.40 (BC)	NS	) 1.21

Experimental Conditions: 0.25% w/v Triton x-100; time 8 hours, light 7000 lux; temperature ± 0.5°C. Data followed by the same letters are significant at P > 0.01.

The difference between the absorption of 2,4-D and picloram is further demonstrated using an Arrhenius plot (Appendix VI) of the data. This is a technique commonly used to study the effect of temperature on enzyme kinetics (Fruton and Simminds 1953). The activation energies for herbicide uptake, calculated from the Arrhenius plot are presented in Table 4.8.

TABLE 4.8 The activation energies for uptake of ^{14}C-2,4-D dimethylamine and the K-salt of ^{14}C-picloram.
(Appendix VI contains full details)

Herbicide	Temperature Range (°C)	Activation energy (kj/Mole)
2,4-D	17-27	40.3
2,4-D	27-37	47.0
picloram	17-27	52.4
picloram	27-37	34.4

(b) Light

The influence of light on absorption and the interaction of the respiratory inhibitor KCN on the absorption of 2,4-D in the light and darkness is presented in Table 4.9.

TABLE 4.9 In vitro absorption of ^{14}C-2,4-D dimethylamine in darkness (tubes wrapped in aluminium foil), and in the light, with and without the respiratory inhibor, KCN.

Treatment	% Absorbed	AmountAAbsorbed ($dpm.ml^{-1}.gFW^{-1}x10^2$)	t
Expt. (a)			
Dark	9.1	5.69 ± 0.76	P > 0.1
Light	11.5	7.69 ± 0.51	P > 0.01
Dark + 10^{-2}M KCN	17.3	10.91 ± 1.14	
Expt. (b)			
Dark	5.5	1.75 ± 0.26	
Light (control)	9.2	2.95 ± 0.33	P > 0.02
Light (24 hour dark pretreatment)	10.2	3.27 ± 0.34	NS
Light (24 hour dark pre-treatment + $1-^{-2}$M KCN)	12.4	3.98 ± 0.48	P > 0.1

Experimental Conditions: Temperature 27°C; time 12 hours; light 7000 lux; 764 dpm 2,4-D + 0.25% w/v Triton X-100.

The KCN was observed to cause a chlorophyll discolouration of the incubation solution, indicating a change in membrane permeability. This is associated with a 92 percent increase in absorption. Darkness during the experiment reduced absorption by 25 percent (Experiment a) and 40 percent (Experiment b), although a dark pretreatment itself does not reduce absorption.

The influence of light on the absorption of picloram was also studied and the results are presented in Table 4.10. Darkness caused a 23 percent reduction, which was statistically non-significant.

TABLE 4.10 In vitro absorption of the K-salt of ^{14}C-picloram in darkness and in the light. Time 24 hours; temperature 27°C; light 7000 lux; 0.25% w/v Triton X-100.

Treatment	Amount Absorbed ($dpm.ml^{-1}.g\ FW^{-1} \times 10^2$)	t
Light	3.26 ± 0.66	NS
Dark	2.48 ± 0.32	

(c) Environmental Factors Influencing Drying of Spray Droplets

The pattern of picloram absorption over various time periods was studied using both in vitro and in vivo methods. Figure 4.5 presents the absorption patterns recorded for the in vitro method, while Figure 4.6 presents the pattern recorded for the in vivo method. Appendix VII contains the detailed information that Figures 4.5 and 4.6 are derived from.

On the basis of the in vivo absorption pattern in Figure 4.6 where maximum absorption had occurred between 5 and 10 hours after herbicide application, it was hypothesized that environmental conditions may cause the spray droplet to form dry deposits. Absorption from the herbicide deposit would be slight, (less than 20 percent total absorption occurred) and further absorption would occur only if the deposit was re-dissolved.

The hypothesis was tested in several misting experiments. The treated plants were placed in dry conditions with an air stream blowing over them. At various times the plants were re-wetted with a mist, care being taken to avoid runoff. The results of two misting experiments are presented in Table 4.11 and Figure 4.7. (See Appendix VIII for statistical details).

Increases in absorption resulting from misting compared with no misting ranged from 82 - 107 percent, (See Table 4.11). In Figure 4.7, misting increased absorption by 89 percent, while a second misting gave a slight, but statistically non-significant increase over a single misting.

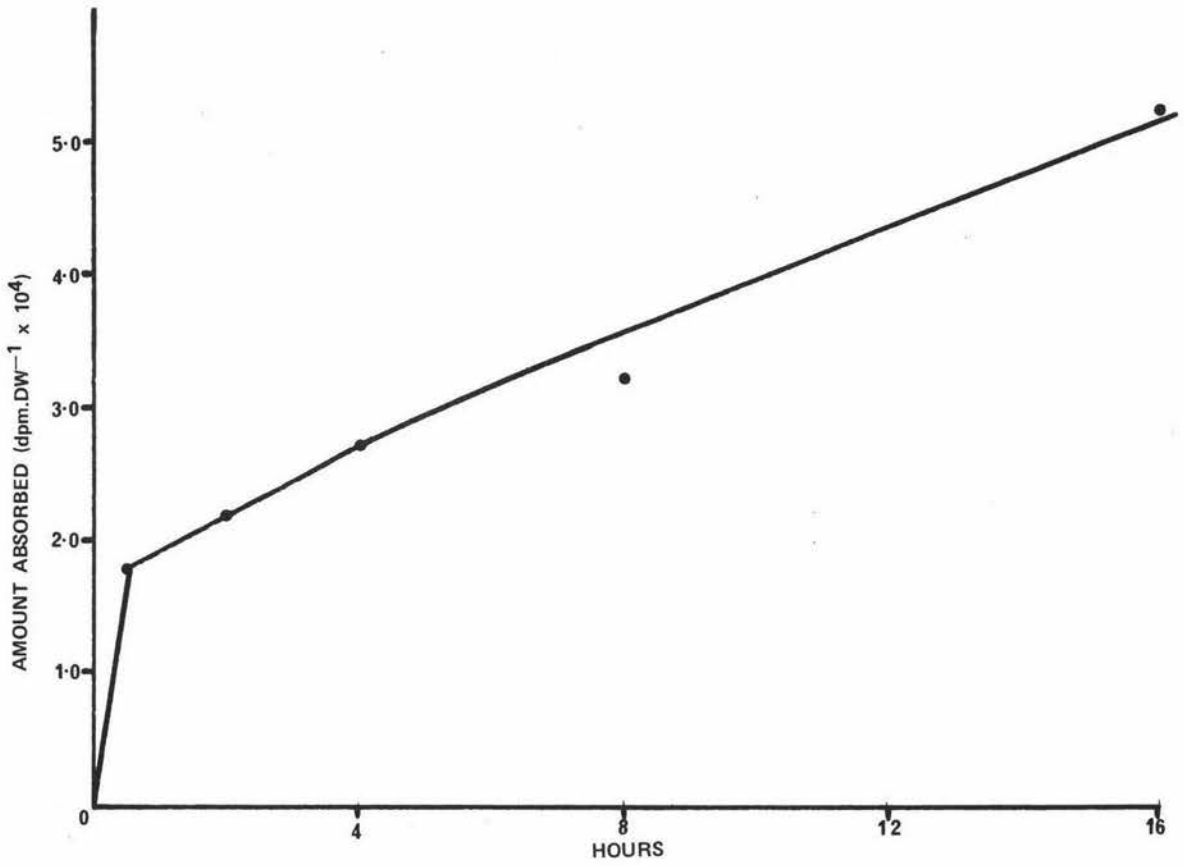

FIGURE 4.5: *In vitro* absorption, over a 16 hour time period, of the K–salt of ^{14}C–picloram from a picloram: 2,4,5–T mixture (1:4).

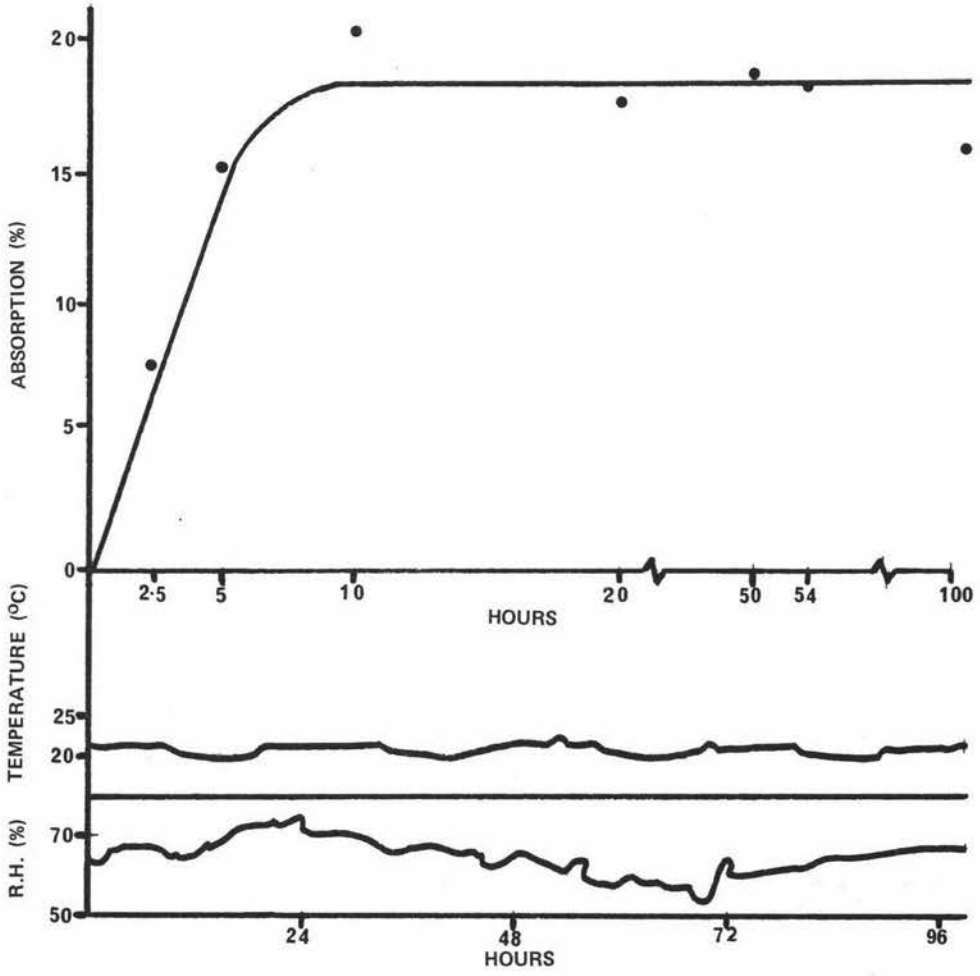

FIGURE 4.6: *In vivo* absorption, over a 100 hour time period, of the K–salt of ^{14}C picloram from a picloram: 2,4,5–T mixture (1:4). A thermohydrograph trace is also presented.

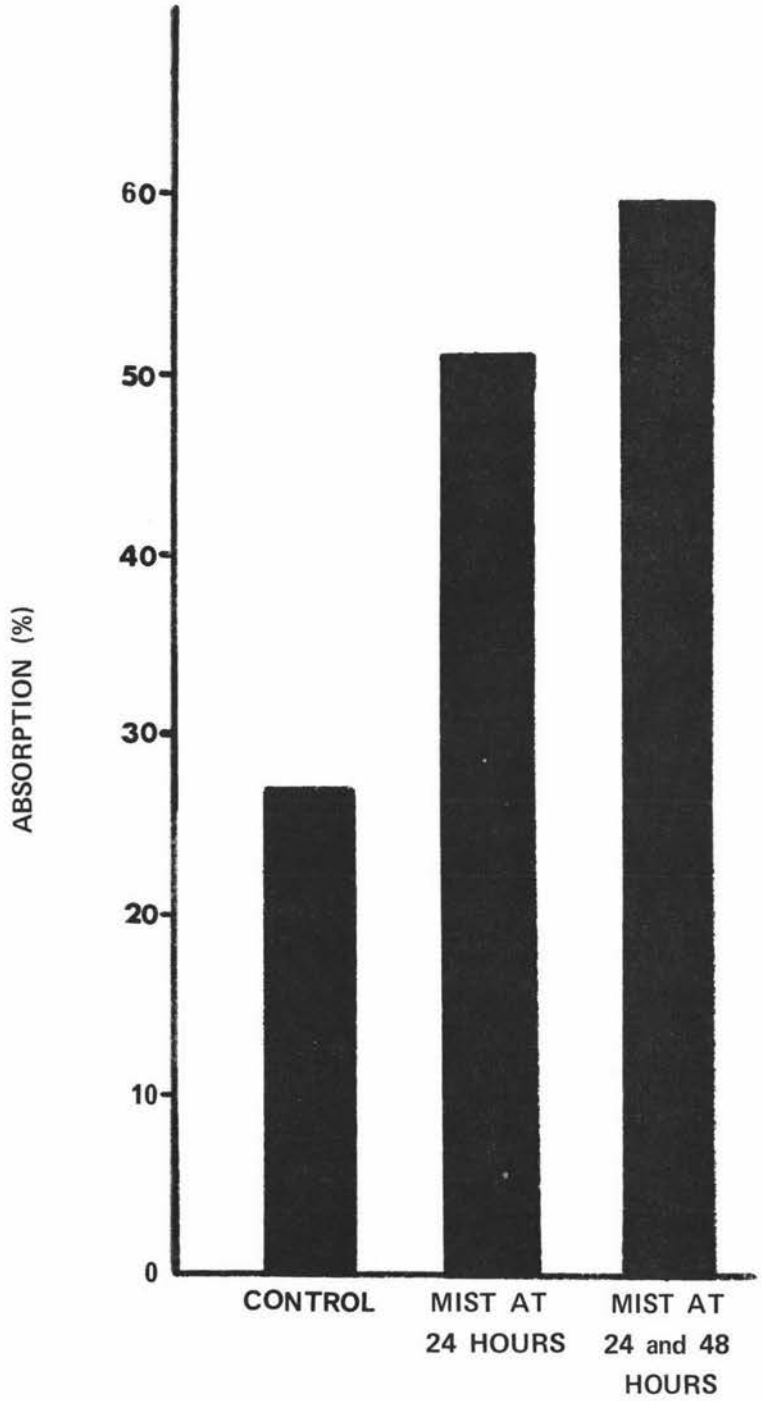

FIGURE 4.7: The effect of misting on the *in vivo* absorption of the K–salt of ^{14}C–picloram from a picloram: 2,4,5–T mixture (1:4). Plants harvested after five days.

TABLE 4.11 In vivo absorption of the K-salt of ^{14}C-picloram formulated in a picloram: 2,4,5-T mixture (1:4), diluted with water 1:250, under dry atmospheric conditions.

Time (Hours) (application to harvest)	Treatment	Herbicide recovered by washing (dpm x 10^3)	t	% Absorbed
2.5	Dry	19.54 ± 0.66 a *	P>0.02	15
	Rewet (at 1.5 hrs)	15.84 ± 0.87 b		31
10.0	Dry	19.11 ± 0.52 a	P>0.001	17
	Rewet (at 5 hrs)	15.83 ± 1.02 b		31
79.0	Dry	18.01 ± 0.80 a	P>0.01	22
	Rewet (at 50 hrs)	13.80 ± 0.96 b		40

* Results followed by different letters are significant at $P > 0.05$.

Experimental conditions: Light 7000 lux, temperature 21-23°C, RH 64-71% with an air stream blowing over the plants.

4.2.2. Spray Factors Influencing Absorption

(a) pH

In vitro experiments were carried out to determine the influence of pH on absorption from the incubating solution. The influence of pH on the absorption of 2,4-D is presented in Figure 4.8. Table 4.12 shows the effect of pH on the absorption of picloram acid and Figure 4.9, the influence of pH on the absorption of picloram formulated as the K-salt. Details relating to Figures 4.8 and 4.9 are to be found in Appendix IX.

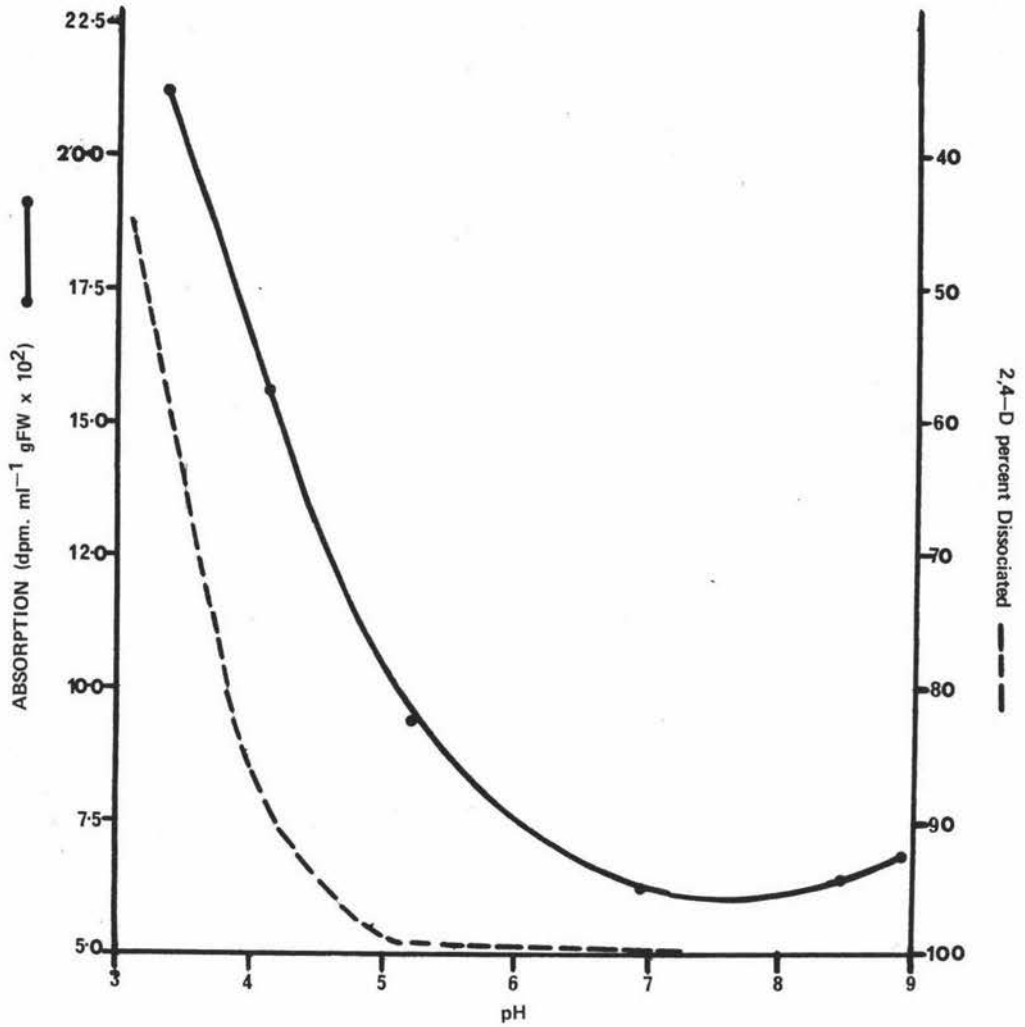

FIGURE 4.8: The effect of pH on the *in vitro* absorption of ^{14}C – 2,4, –D.

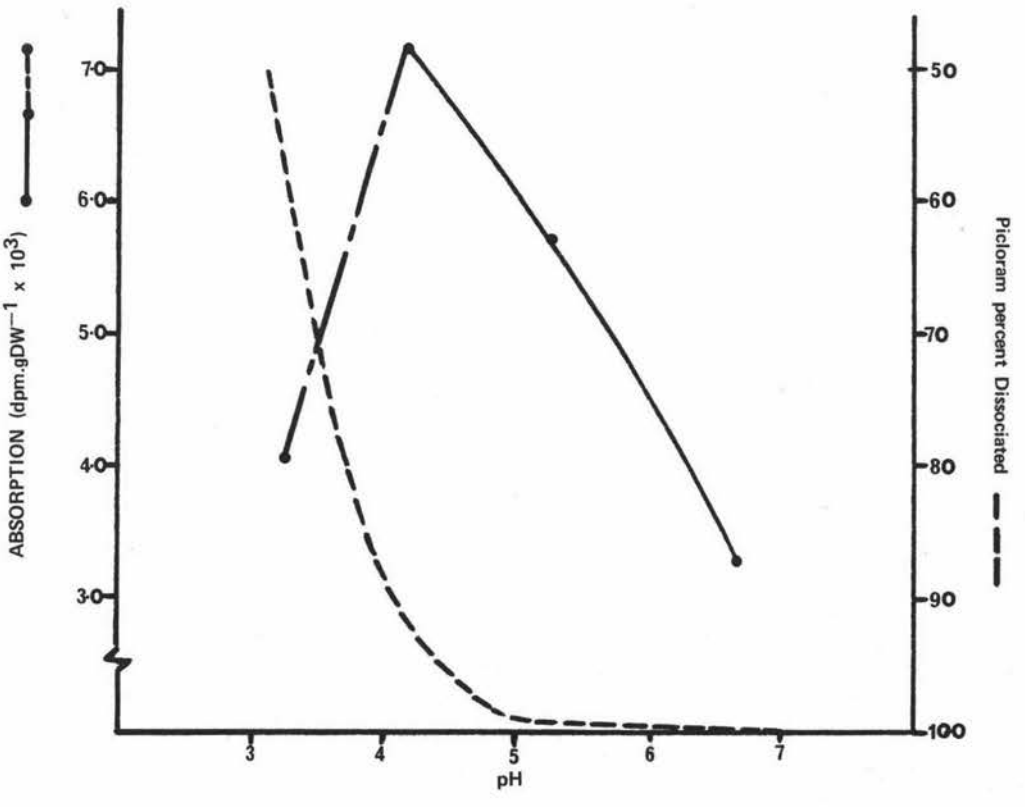

FIGURE 4.9: The effect of pH on the *in vitro* absorption of the K–salt of ^{14}C–picloram from a picloram: 2,4,5–T mixture (1:4).

TABLE 4.12 In vitro absorption of picloram acid at different pH values. Buffered with $NaHPO_4$/citric acid.

pH	% Absorbed	Amount absorbed ($dpm.ml^{-1}.g\ FW^{-1} \times 10^2$)	t
2.82	16.7	4.35 ± 0.30	) P > 0.1
3.59	13.8	3.59 ± 0.30	
4.55	11.1	2.89 ± 0.23	) P > 0.1
5.70	13.9	3.62 ± 0.34	) P > 0.1
7.40	11.2	2.95 ± 0.42	) P > 0.1
8.10	13.7	3.51 ± 0.25	) NS

Experimental conditions: Temperature 27°C.; light 7000 lux; time 12 hours; Triton X-100.

The pH has a marked influence on the absorption of 2,4-D. A three-fold increase in absorption occurred as the pH was lowered from pH 7 to pH 3.35. On the other hand, picloram was not as responsive, especially the acid formulation, although the K-salt of picloram showed a 2.2-fold increase as pH was lowered from pH 6.65 to pH 4.15. The sudden drop off at pH 3.25 (see figure 4.9) is unexpected and statistically significant. Because of the uncertainty of the reality of this low pH response, it has not been drawn as a solid line in Figure 4.9.

The pH effects observed, have been linked in the literature, to the degree of dissociation of the herbicide. The degree of dissociation can be predicted from the Henderson-Hasselbach equation:

$$pH = pKa - \log \frac{base}{acid}$$

The pKa for 2,4-D was taken as 3.22 while that of picloram as 3.10 (Sargent and Blackman, 1970). The calculated dissociation curves of 2,4-D and picloram are also plotted in Figures 4.8 and 4.9. When the relative rates of absorption and the relative changes in dissociation are compared, (Table 4.13) large increases in absorption occur at higher pH values while little change in dissociation percentage occurs. At low pH values the reverse occurs.

TABLE 4.13 Relative changes in absorption of ^{14}C-2,4-D dimethylamine with respect to the relative change in the undissociated form of 2,4-D at different pH levels.
(the table is derived from Figure 4.8)

pH	Relative increase in absorption	Relative increase of undissociated 2,4-D
3.5 - 4.0	26.8	60.0
4.0 - 5.0	41.8	35.7
5.0 - 6.0	22.3	44.3
6.0 - 7.0	8.9	0.6

(b) Concentration

The results of changing the concentration of the incubation solution on the absorption of 2,4-D are presented in Table 4.14.

TABLE 4.14 In vitro absorption of ^{14}C-2,4-D dimethylamine from solutions of different concentrations.

Concentration (ppm)	% Absorbed	Amount Absorbed ($dpm.ml^{-1}.g\ FW^{-1} \times 10^2$)	t
1146	10.4	4.40 ± 0.15	
			$P > 0.05$
746	9.7	3.22 ± 0.32	
			$P > 0.01$
382	7.7	1.21 ± 0.24	
			NS
190	14.9	1.25 ± 0.17	

Experimental conditions: Temperature 27°C, time 12 hours; light 7000 lux; 764 ppm 2,4-D - 0.25% w/v Triton X-100.

(c) Surfactants

The effects on absorption of two nonionic surfactants that were readily available, were tested. The influence of Triton X-100 on the absorption of 2,4-D and picloram is shown in Table 4.15. Figure 4.10 shows the effect of two concentrations of Triton K-100 (iso octyl phenyl polyethnoxyethanol) and Dowfax 9N9 (nonyl phenol ethylene oxide) on the absorption of picloram. (See Appendix X for further details). The surfactants tested caused a marked increase in absorption. Triton X-100 resulted in two-fold increase in 2,4-D absorption. Dowfax was more efficient than Triton X-100 in promoting the absorption of picloram.

TABLE 4.15 The effect of surfactant on *in vitro* absorption of ^{14}C-2,4-D dimethylamine, 764 ppm and picloram acid 440 ppm.

Treatment	% Absorbed	Amount Absorbed ($dpm.ml^{-1}.g\ FW^{-1} \times 10^2$)	t
2,4-D			
No surfactant	6.5	1.98 ± 0.21	$P > 0.001$
0.25% w/v Triton X-100	13.4	4.09 ± 0.43	
Picloram			
No surfactant	8.3	2.13 ± 0.23	$P > 0.1$
0.25% w/v Triton X-100	12.6	3.26 ± 0.66	

Experimental conditions: Time 12 hours; light 7000 lux; temperature 27°C.

(d) Humectants

From the results obtained in the misting experiment (4.2.1:c) it was decided to test the influence of a humectant (Alkane 60) on the absorption of picloram. The results of this experiment are presented in Table 4.16. The humectant appears to inhibit absorption. Alkane 60 was observed to be phytotoxic, causing desiccation of soft foliage.

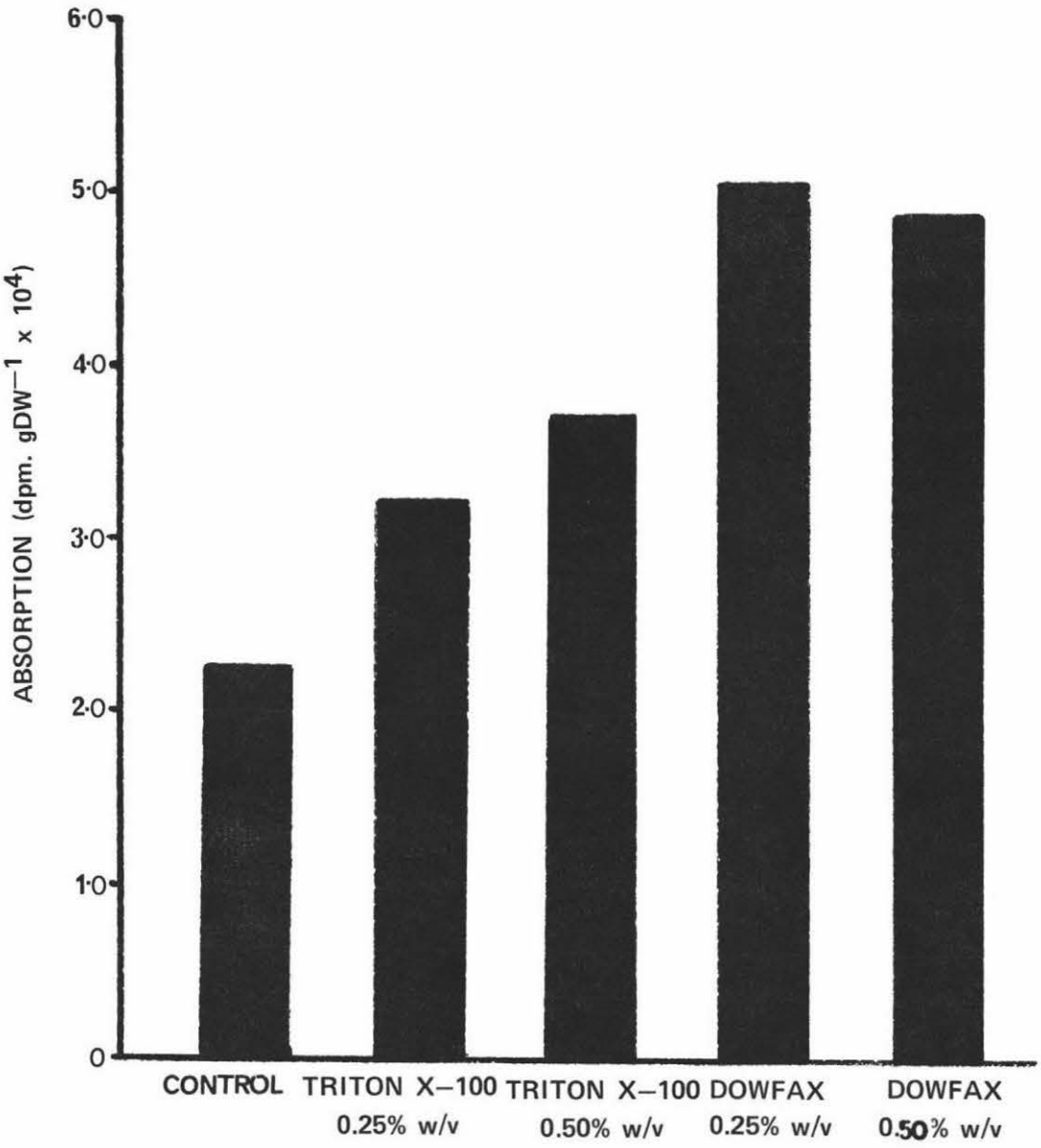

FIGURE 4.10: The effect of surfactants on the *in vitro* absorption of the K–salt of ^{14}C–picloram from a picloram: 2,4,5–T mixture (1:4).

TABLE 4.16 The *in vivo* absorption of the K-salt of ^{14}C-picloram from an alkane based solvent.

Time (hours) application to harvest	Amount of ^{14}C recovered	t
0	8.31 ± 0.12	
		NS
2	8.39 ± 0.19	
		NS
4	8.57 ± 0.17	
		NS
8	8.92 ± 0.17	
		NS
25	8.61 ± 0.54	
		NS
120	7.76 ± 0.54	

Experimental conditions: Light 7000 lux; temperature 19-21.5°C; relative humidity 57-67%.

(e) Additives

In the literature, (See 3.1.4) there have been numerous reports on various additives, organic and inorganic, enhancing absorption. A number of additives were combined with 2,4-D. Generally the additives used were not effective in promoting absorption, with exceptions of KCN and Na_2HPO_4. The results of these experiments are presented in Table 4.17.

TABLE 4.17 The effect of inorganic and organic additives on the *in vitro* absorption of 2,4-D dimethylamine.

Treatment	% Absorbed	Amount absorbed ($dpm.ml^{-1}$. g $FW^{-1} \times 10^{-2}$)	t
Boric Acid (5% w/v of 1M solution, pH 6.8)	21.5	5.79 ± 0.54	P>0.1
Control pH 6.8	26.8	7.52 ± 0.74	
Urea	12.5	3.82 ± 0.38	NS
Control	13.4	4.09 ± 0.33	
Organophosphate (malathion 0.15% ai; pH 6.8)	10.5	3.35 ± 0.31	NS
Control pH 6.8	9.2	2.95 ± 0.33	
KCN (10^{-2}M) pH 9.65	15.5	5.57 ± 0.62	P>0.05
Control pH 6.8	13.4	4.09 ± 0.33	
Sucrose			
0 ppm	11.2	7.66 ± 0.70	
10^2	10.9	7.48 ± 0.65	NS
10^3	12.0	8.36 ± 1.04	NS
10^4	12.0	8.34 ± 0.74	NS
10^5	5.1	3.51 ± 1.16	P>0.01
Na_2HPO_4 (10^{-2}M) (pH 8.17)	17.0	6.16 ± 0.51	P>0.001
Control pH 6.8	13.4	4.09 ± 0.33	

Experimental conditions: 764 ppm; time 12 hours; temperature 27°C; light 7000 lux.

4.2.3. Plant Factors Influencing Absorption

(i) Cuticular Wax.

The influence of the cuticular wax layer on absorption was studied. Gorse leaf surfaces have a considerable amount of wax as shown in Table 4.18. The wax recovered is equivalent to 0.3 mg/cm^2 (See Appendix XI).

The effect of a ten second chloroform wash pretreatment on the subsequent absorption of 2,4-D and picloram is presented in Figure 4.11. The removal of lipids by chloroform markedly increases absorption.

TABLE 4.18 Lipid recoveries from glasshouse grown gorse following a one minute dip in chloroform.

Weight of wax per g DW tissue (mg/g)	% leaf wax recovered on a dry weight basis
25.4 ± 2.6	2.54

A comparison between the rates of absorption of glasshouse and outdoor material of the same genotype, was made, as well as a comparison with another species, blackberry (Rubus fruticosus). The results of these experiments are presented in Table 4.19.

TABLE 4.19 The in vitro absorption of ^{14}C-2,4-D dimethylamine by a gorse genotype grown outdoors and in a glasshouse, and by the leaf of blackberry grown outside.

Treatment	% Absorbed	Amount Absorbed ($dpm.ml^{-1}.\ g.\ FW^{-1} \times 10^2$)	t
Gorse			
Glasshouse	13.4	4.09 ± 0.33	P>0.01
Outside	8.9	2.72 ± 0.28	
Blackberry			NS
Outside	10.1	3.08 ± 0.28	

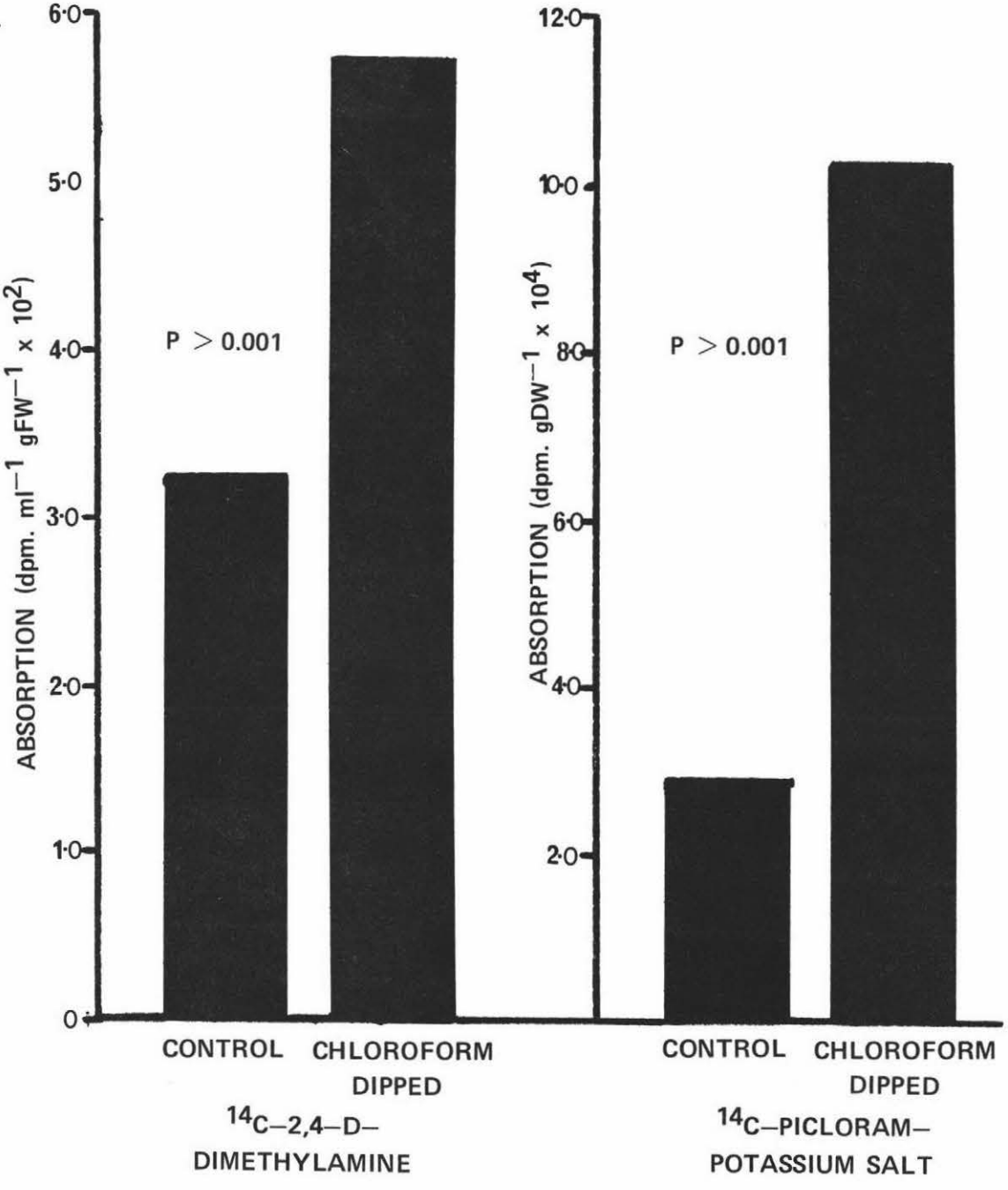

FIGURE 4.11: The effect of a 10 second chloroform dip on the *in vitro* absorption of ^{14}C–2,4–D and ^{14}C–picloram.

(ii) Scanning Electron Microscopy

The surface morphology of gorse spines, leaves and stems is shown in Plates 4.1 - 4.6.

The following observations and calculations were made from the plates:

1. Gorse spines and young stems are convoluted into a series of ridges (ten in number) and hollows (Plates 4.1 and 4.2). Stomata are confined to the hollows, while the hairs arise from the ridges. (Plate 4.3).

2. Hairs. All surfaces of gorse are pubescent, (Plates 4.1 to 4.4). The hairs are long (0.3 - 1.0 mm) and often curved. They are not rigid. Hair densities (centre of one hollow to the next) were estimated to range from 30-40 hairs per mm^2.

3. Surface wax: No clear wax formation was visible except on juvenile leaves. (Plate 4.5).

4. Stomata. The stomata have a "chimney" of cutin. (Plate 4.6) which has been observed in plants adapted to dry habitats, (Meidner and Mansfield 1968). Stomatal density was calculated using the area from the centre of one ridge to the next. Estimates from three photographs gave densities of: 12, 14 and 47 stomata per mm^2. The depth of the hollow was not taken into consideration in these calculations and this would over estimate density. Estimates of the dimensions of the stomatal apparatus were made from several photographs. Dimensions of 22 - 24 μ x 17 μ were estimated. Both the stomatal parameters (density and dimension) estimated from the photographs are low; similar to the lowest values quoted by Meidner and Mansfield (1968) for 27 species.

PLATE 4.1

Scanning electron micrograph of a gorse spine from glasshouse propagated material. Magnification x 100.

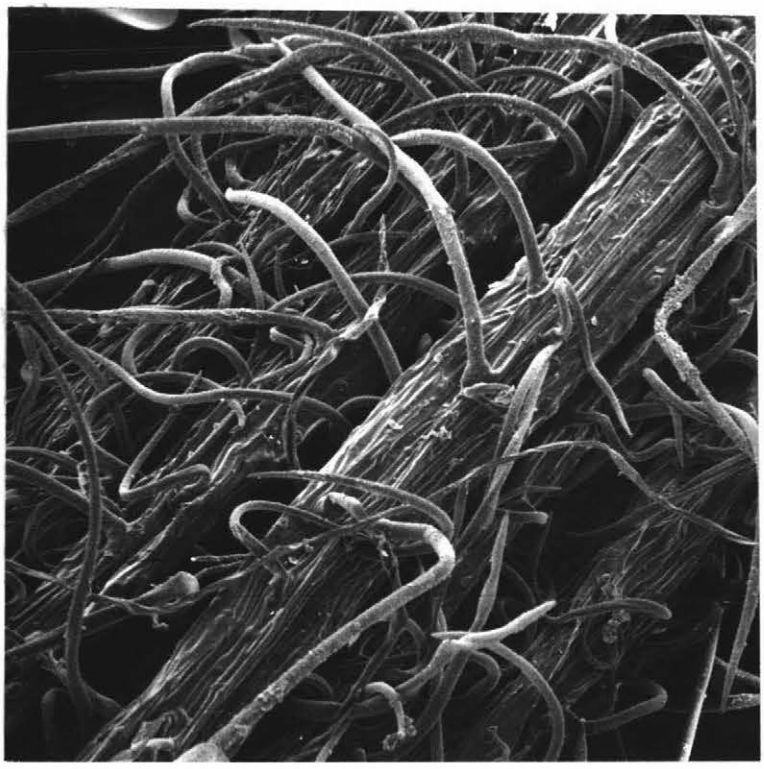

PLATE 4.2

Scanning electron micrograph of an unhardened gorse stem from glasshouse propagated material. Magnification x 95.

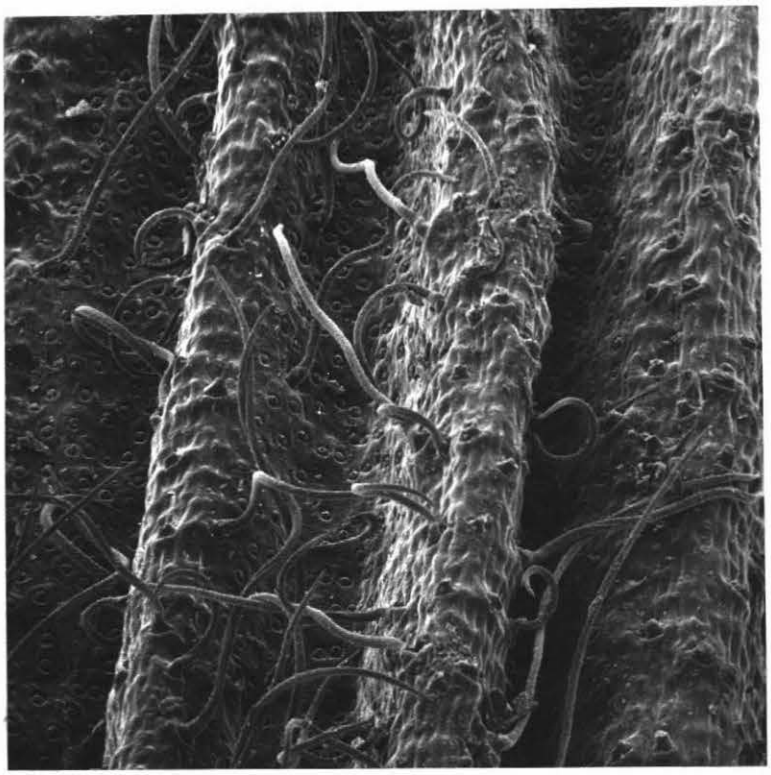

PLATE 4.3

Scanning electron micrograph of a gorse spine from material grown out-of-doors. Magnification x 90.

PLATE 4.4.

Scanning electron micrograph of hairs on a gorse spine from glasshouse propagated material. Magnification x 150.

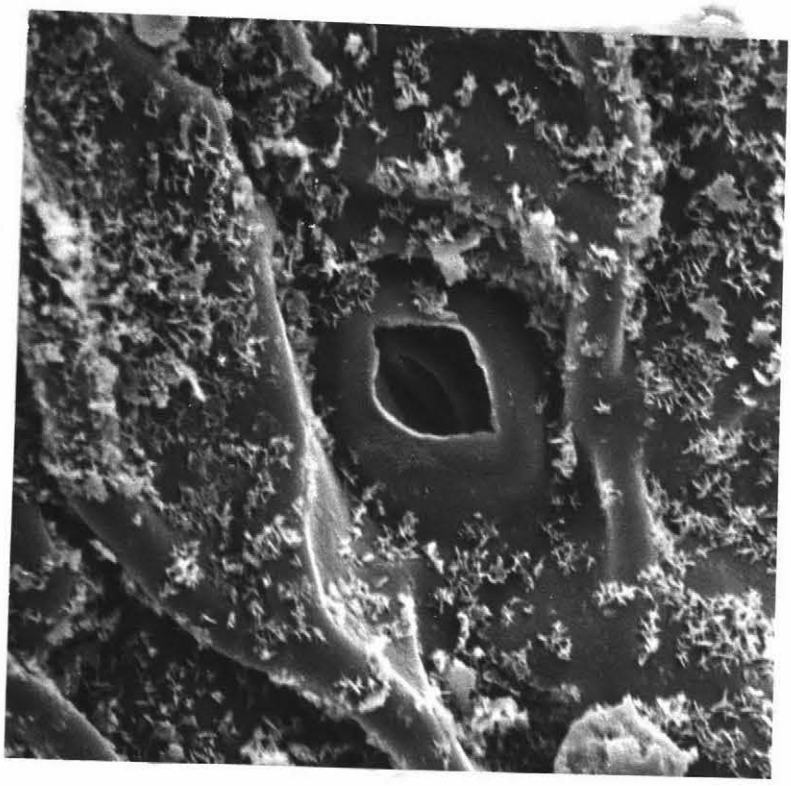

PLATE 4.5

Scanning electron micrograph of wax formation on a gorse leaf from glasshouse propagated material. Magnification x 1900.

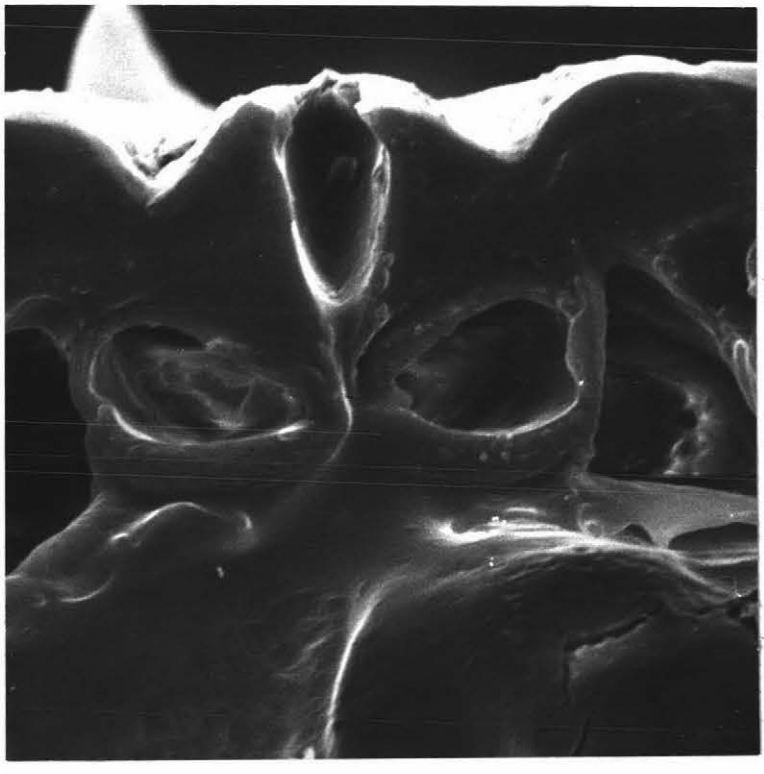

PLATE 4.6

Scanning electron micrograph of a cut, transverse view of a gorse spine from material grown out of doors. The guard cells are clearly visible below the stomatal "chimney". Magnification x 2800.

(iii) Contact Angle

Droplets of water and picloram spray were observed and photographed. Maximum contact angle (78°) recorded by photography (see Plate 4.7) was with small (1 μl) water droplets. Larger (2.5 μl) droplets had lower contact angles. Picloram spray droplets (commercial formulation) and lower contact angles than water. The contact angle decreased with time.

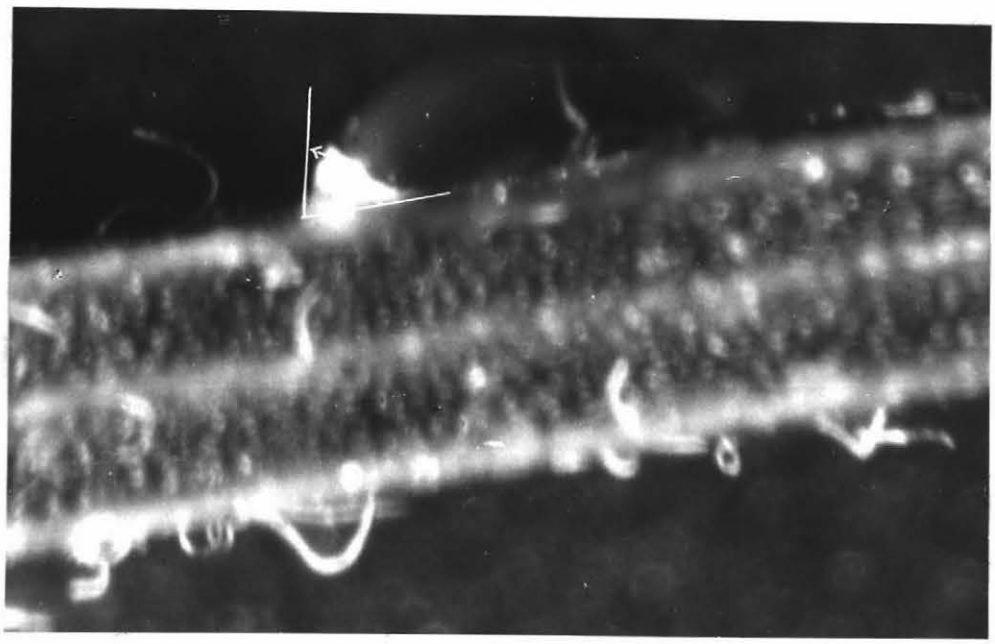

PLATE 4.7

Optical micrograph of a 1 μl water droplet on a gorse spine. Contact angle measured at 78°. Magnification x 150.

4.3 TRANSLOCATION

The translocation and distribution of ^{14}C-picloram in small pot-grown gorse plants was studied. Autoradiographs were prepared of samples treated for different time intervals. In addition, an attempt to quantify the distribution of ^{14}C-picloram by sectioning the plant was undertaken. These experiments included herbicide distribution as a function of time, the influence of misting following spraying, and the influence of sinks on translocation. For a number of reasons, (See Section 5.1) these experiments were only partially successful.

(i) Autoradiographs

The distribution of ^{14}C-picloram in the shoots and roots 1,2,4,7, and 16 days after application of the herbicides is shown in Plates 4.8 to 4.16. To prevent translocation occurring during pressing, the plants were sectioned into a number of units and frozen with liquid air. However, despite this precaution of rapid freezing, herbicide movement during pressing cannot be discounted.

The following observations were made from the autoradiographs:

(1) The roots are rapidly labelled, herbicide arriving within 24 hours (Plate 4.9). However, there appears to be no build-up of label in the roots during the following 16 days (Plate 4.16).

(2) Sink-source relationships occur. The nodules are more heavily labelled than the surrounding roots. (Plate 4.12, 4.13) and there is also preferential labelling of the seed pods (Plate 4.15).

(3) Both acropetal and basipetal translocation occurs. Generally, where acropetal translocation occurs, all the spines and leaves above the point of herbicide application can be clearly distinguished in the autoradiographs, although in Plate 4.11, one side of the stem is more heavily labelled. In contrast, basipetal translocation is predominantly confined to the treated stem (e.g. Plate 4.10), with the roots being labelled in preference to other branches (Plate 4).

(4) The treated zone is heavily labelled, due to the large amounts of unabsorbed herbicide remaining on the stem surface e.g. Plate 4.8.

(ii) Quantitative Analysis of Sections

When the stem tip was treated with ^{14}C-picloram, it retained a considerable proportion of the applied herbicide, as demonstrated in Figure 4.12. Later, it was found that by treating the stem below the tip, greater basipetal translocation occurred. Figure 4.13 shows the short term distribution of picloram when a point 6 cm below the tip was treated. Unlike the stem tip, the treated zone does not retain herbicide. Basipetal translocation is in a series of peaks, rather than one main peak.

(iii) Rate of Translocation

The velocity of translocation of ^{14}C-picloram can be estimated from Figure 4.13. Estimates presented in Table 4.20 are based on the detection of label 20cm from the source two hours after herbicide application. A limitation of this estimation is that

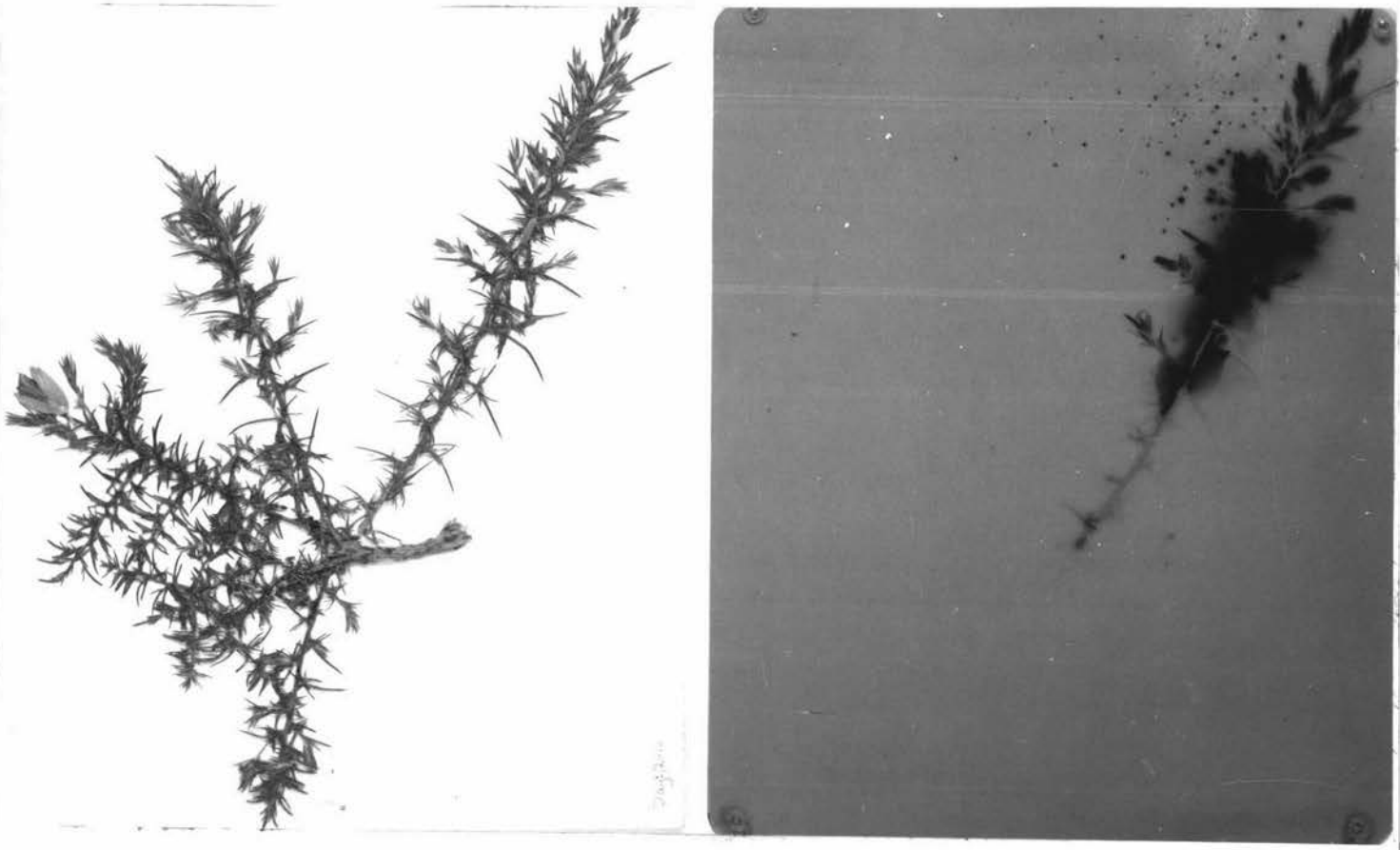

PLATE 4.8

Autoradiograph of gorse plant shoots 24 hours after the application of a K-salt of ^{14}C picloram as a picloram: 2,4,5-T mixture (1:4).

PLATE 4.9

Autoradiograph of the roots of the gorse plant shown in Plate 4.8.

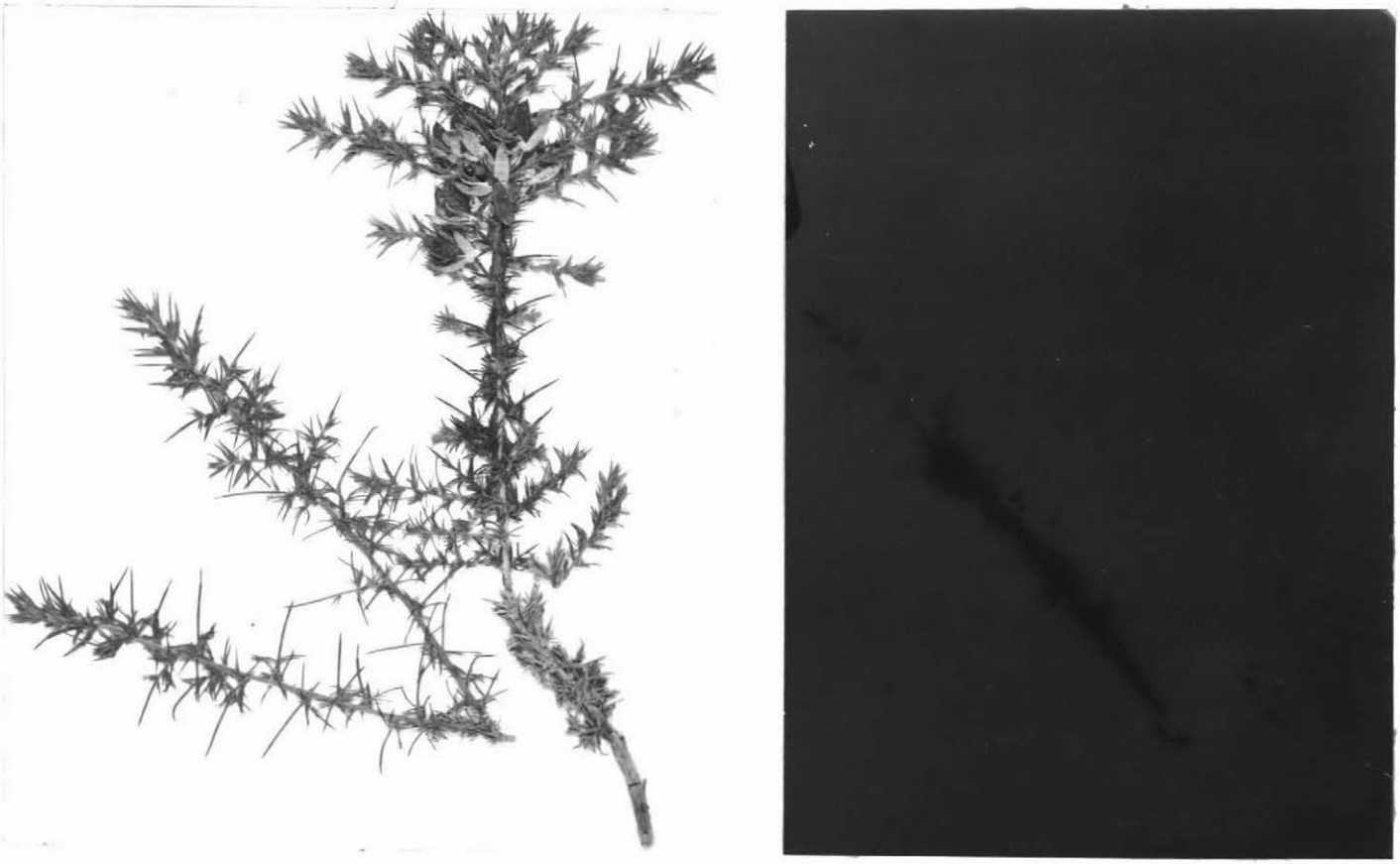

PLATE 4.10

Autoradiograph of the gorse plant shoots 48 hours after the application of the K-salt of ^{14}C-picloram as a picloram:2,4,5-T mixture (1:4).

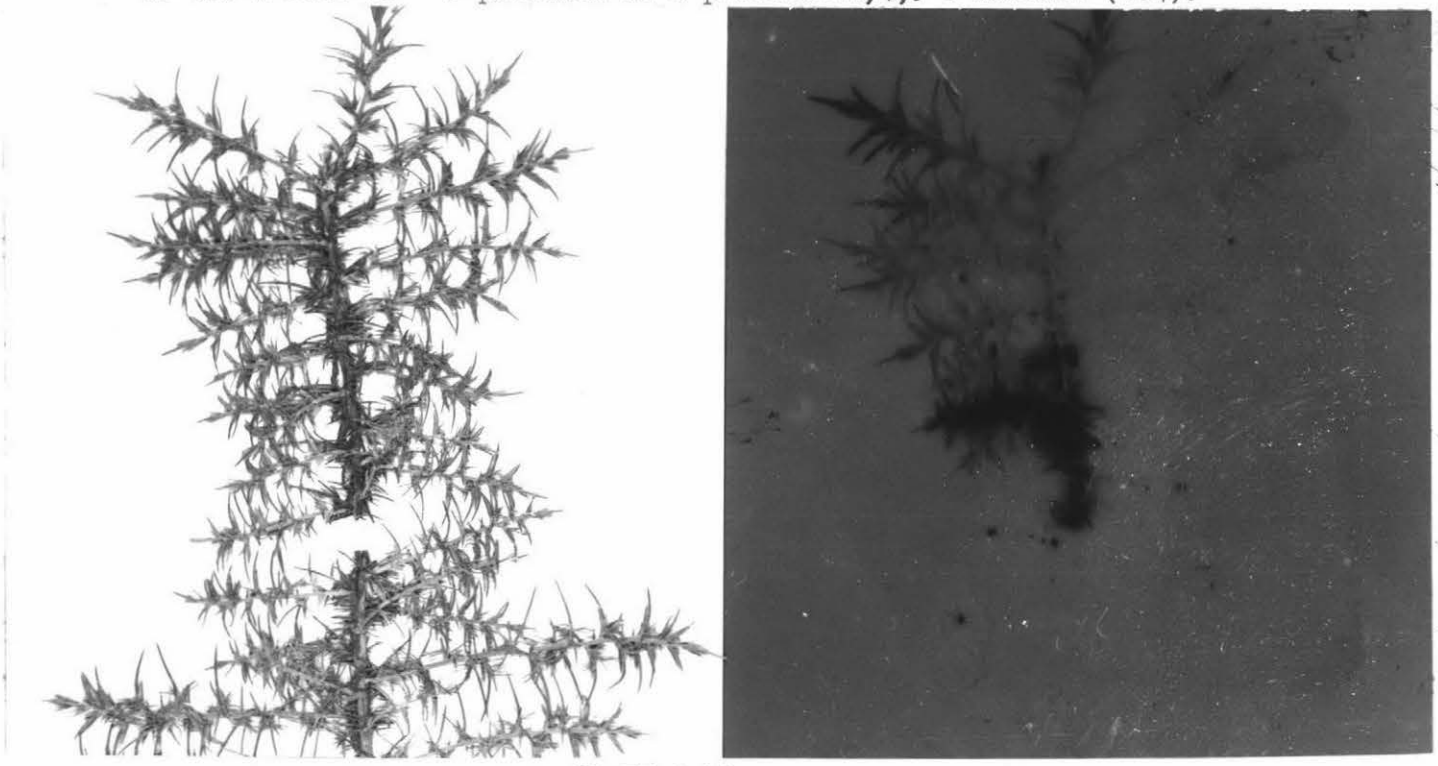

PLATE 4.11

Autoradiograph of the upper shoots of a gorse plant 4 days after the application of the K-salt of ^{14}C-picloram as a picloram:2,4,5-T mixture (1:4).

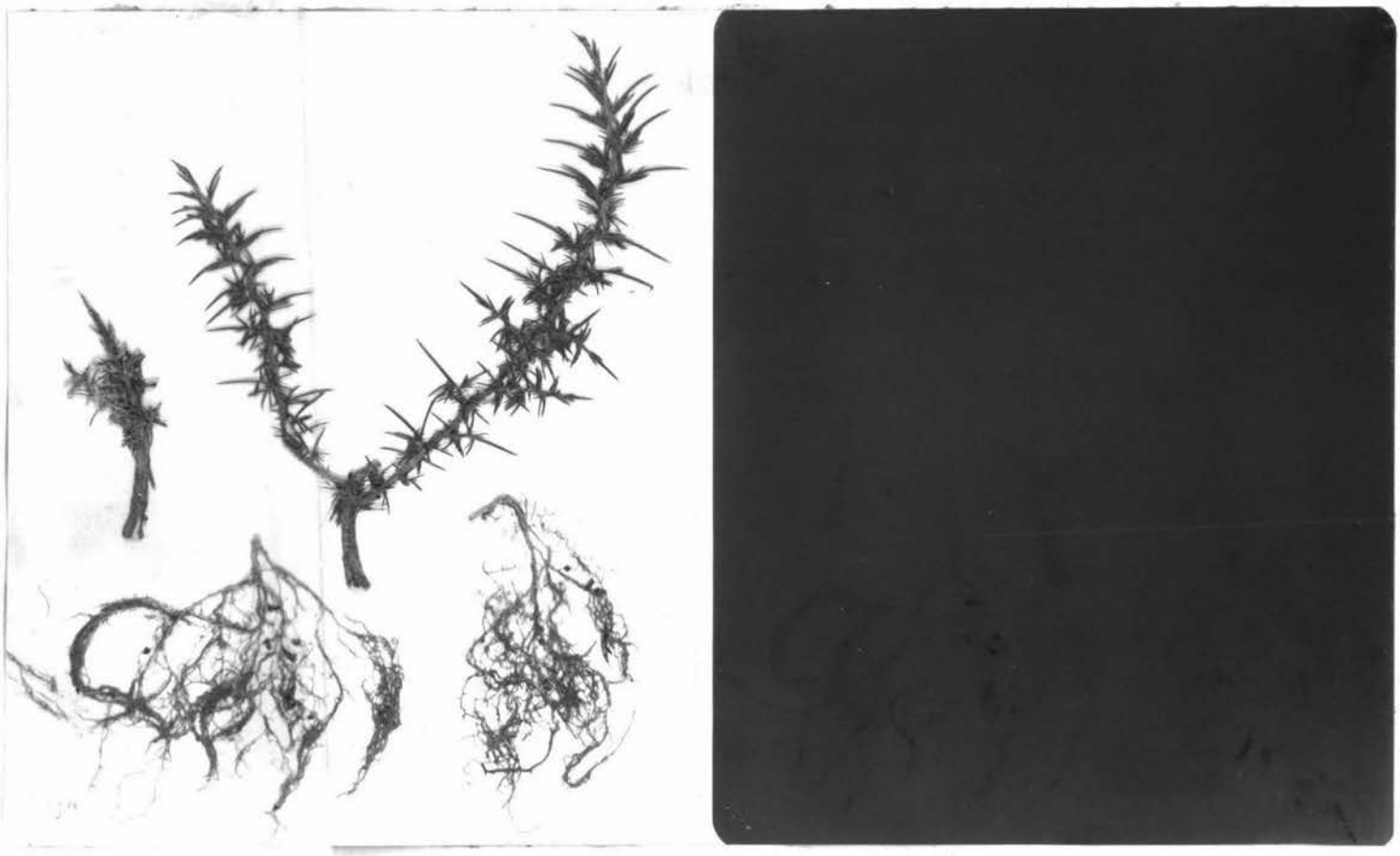

PLATE 4.12

Autoradiograph of the lower shoots and part of the root system of the gorse plant shown in Plate 4.11. Note the preferential labelling of the nodules.

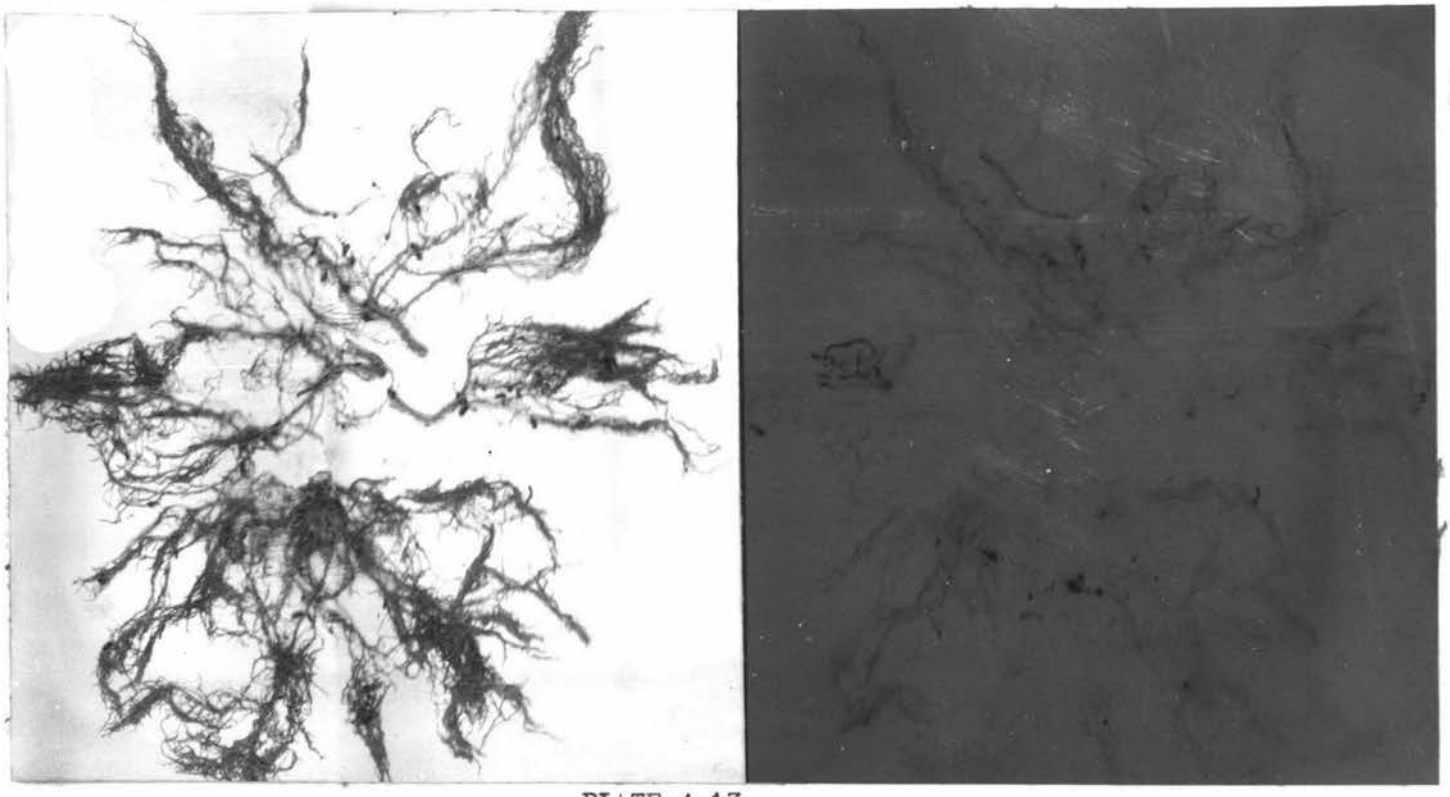

PLATE 4.13

Autoradiograph of the remaining roots not shown in Plate 4.12.

PLATE 4.14

Autoradiograph of the shoots of a gorse plant 7 days after the application of the K-salt of ^{14}C-picloram as a picloram:2,4,5-T mixture (1:4)

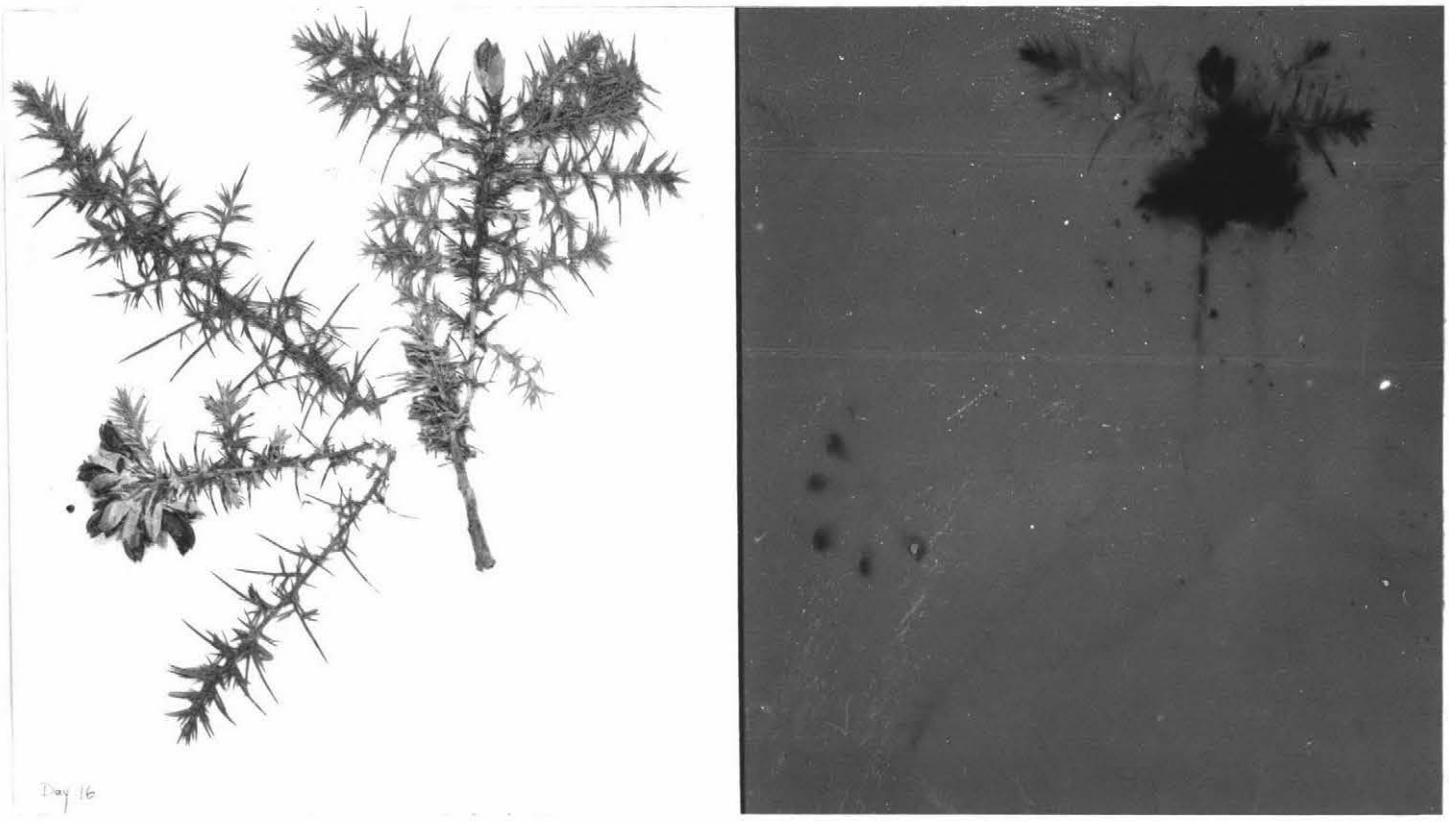

PLATE 4.15

Autoradiograph of the shoots of a gorse plant 16 days after the application of the K-salt of ^{14}C-picloram as a picloram:2,4,5-T mixture (1:4).

Note the preferential labelling of seed pods.

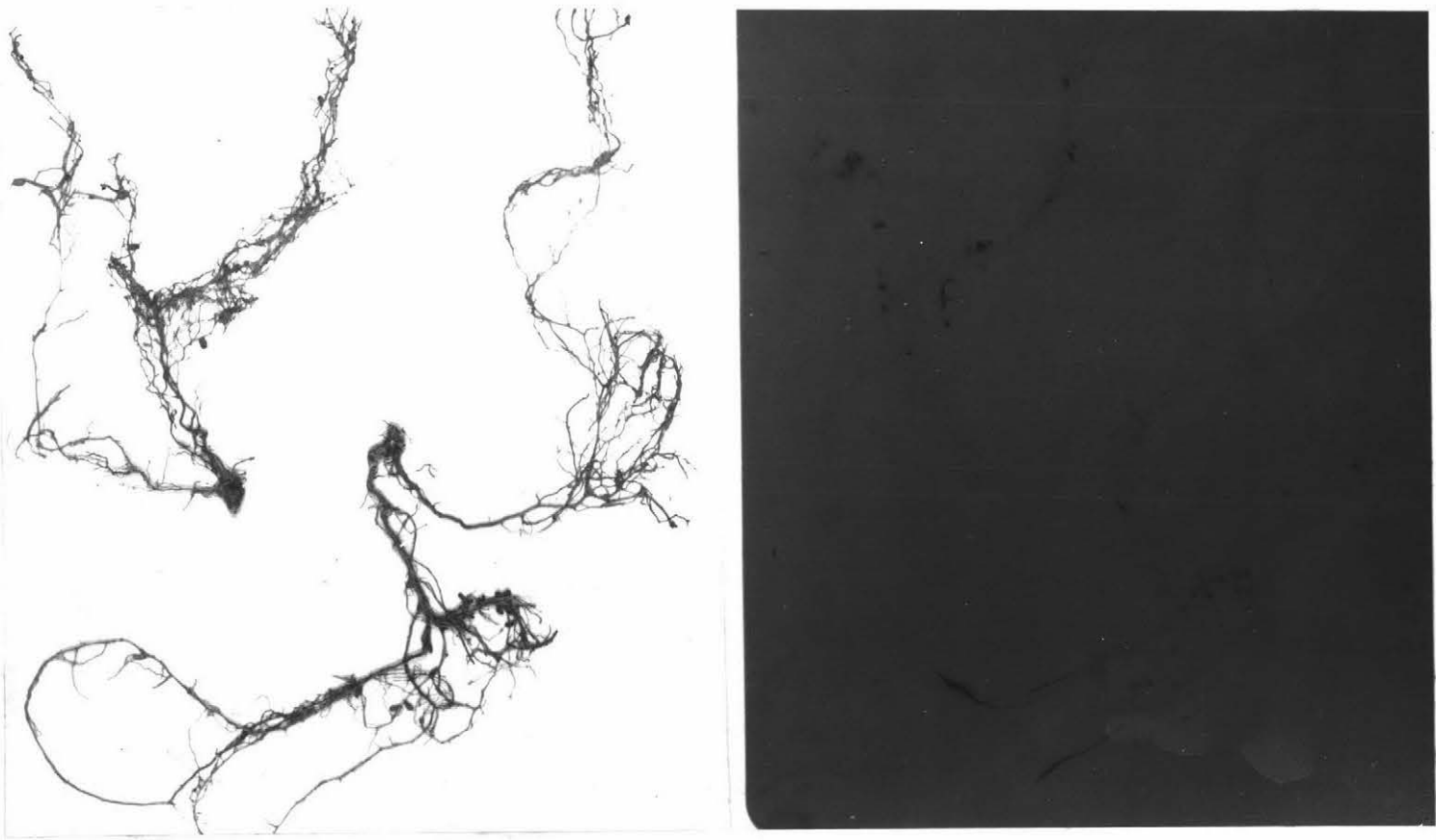

PLATE 4.16

Autoradiograph of the roots of the gorse plant shown in Plate 4.15.

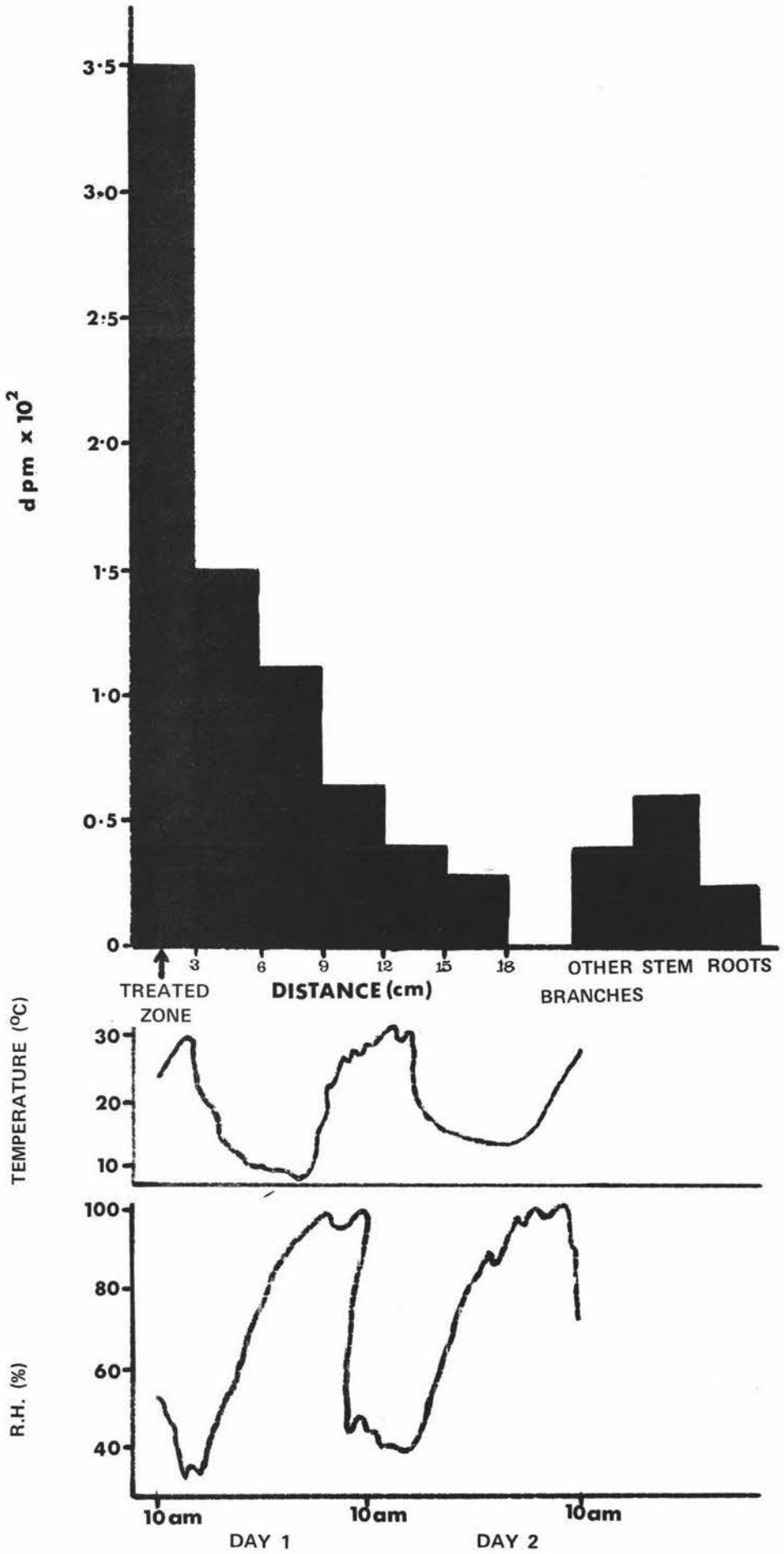

FIGURE 4.12: The distribution of ^{14}C–picloram formulated as the K–salt after 48 hours following application of the herbicide to the stem tip.

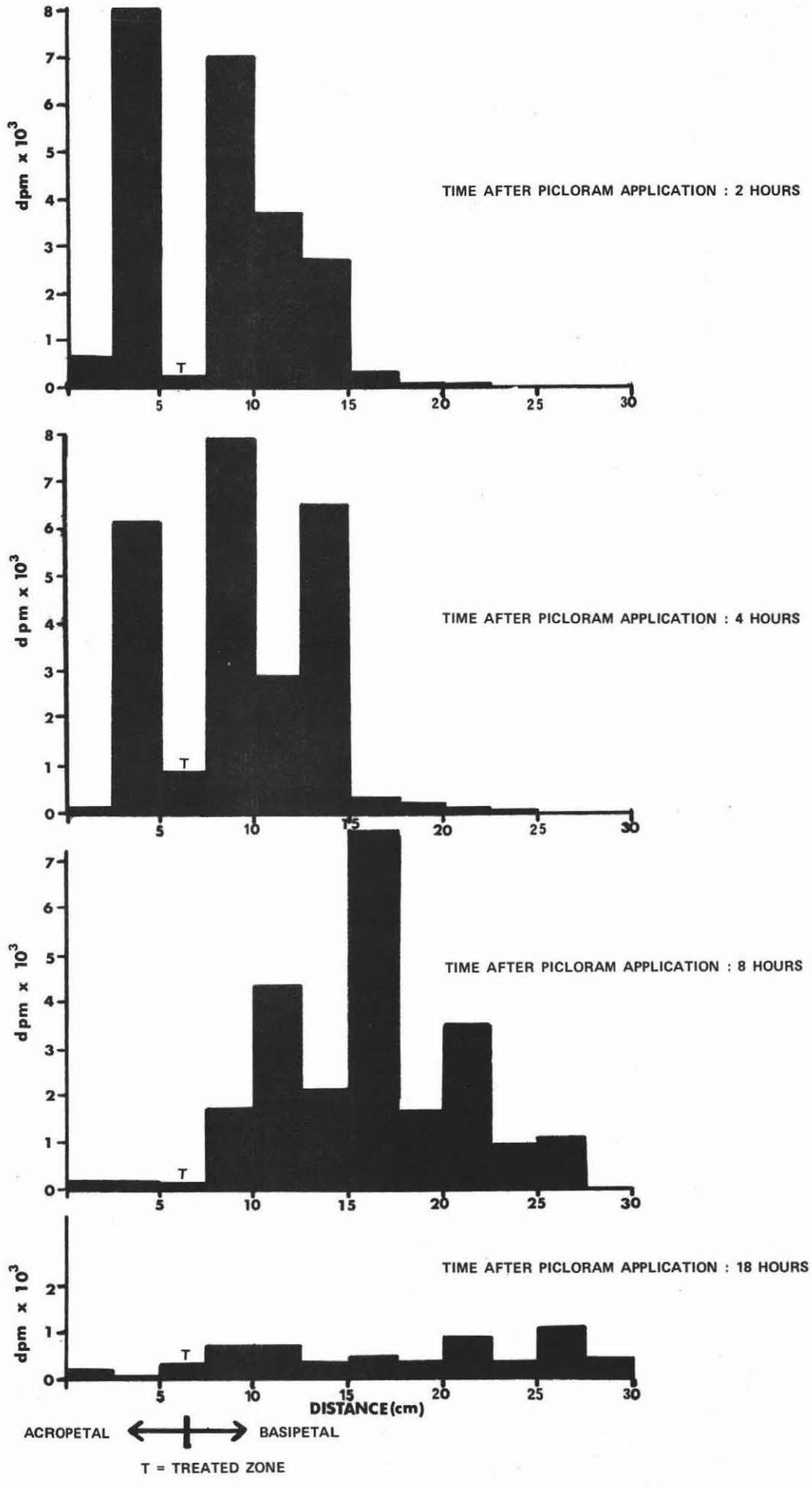

FIGURE 4.13: The distribution of the ^{14}C–picloram formulated as the K–salt along a 30 cm length of stem following application of the herbicide 6 cm below the stem tip.

^{14}C-picloram below the sensitivity level of the detector could have been translocated beyond 20cm. If this did occur, the estimated velocities would be under-estimated.

TABLE 4.20 Estimation of the velocity of ^{14}C-picloram translocation, using three different estimates of the time interval required for absorption and phloem loading.

Total time (hr)	Time for absorption and phloem loading (hrs)	Translocation time (hr)	Distance (cm)	Estimated velocity cm.hr^{-1}
2.0	0.5	1.5	20	12.5
2.0	1.0	1.0	20	20.0
2.0	1.5	0.5	20	40.0

4.4. METABOLISM

Following incubation, most of the radioactivity was recovered in the supernatant (soluble fraction). The soluble protein precipitate contained a very small fraction of the radioactivity. Table 4.21 presents the results obtained during extraction.

The radiochromatogram scan of the supernatant following grinding, and of the supernatant after soluble proteins have been precipitated, have only one distinct peak. This corresponds to free picloram. (See Figure 4.14).

TABLE 4.21. Distribution of radioactivity after 24 hours incubation with the K-salt of ^{14}C-picloram in a picloram:2,4,5-T (1:4) mixture.

Initial activity		$6.47 \pm 0.02 \times 10^5$ dpm.ml^{-1}.
Final activity		$4.81 \pm 0.09 \times 10^5$ dpm.ml^{-1}.
Absorption (by difference)		$=1.56 \times 10^5$ dpm.ml^{-1}.
Percentage absorbed	24%	
Insoluble fraction (cell residue)	1.17%	$2.48 \pm 0.19 \times 10^4$ dpm
Protein precipitate	0.06%	$0.12 \pm 0.01 \times 10^4$ dpm
Soluble fraction (by difference)	98.77%	

Experimental conditions: Temperature 22°; light 20,000 lux.

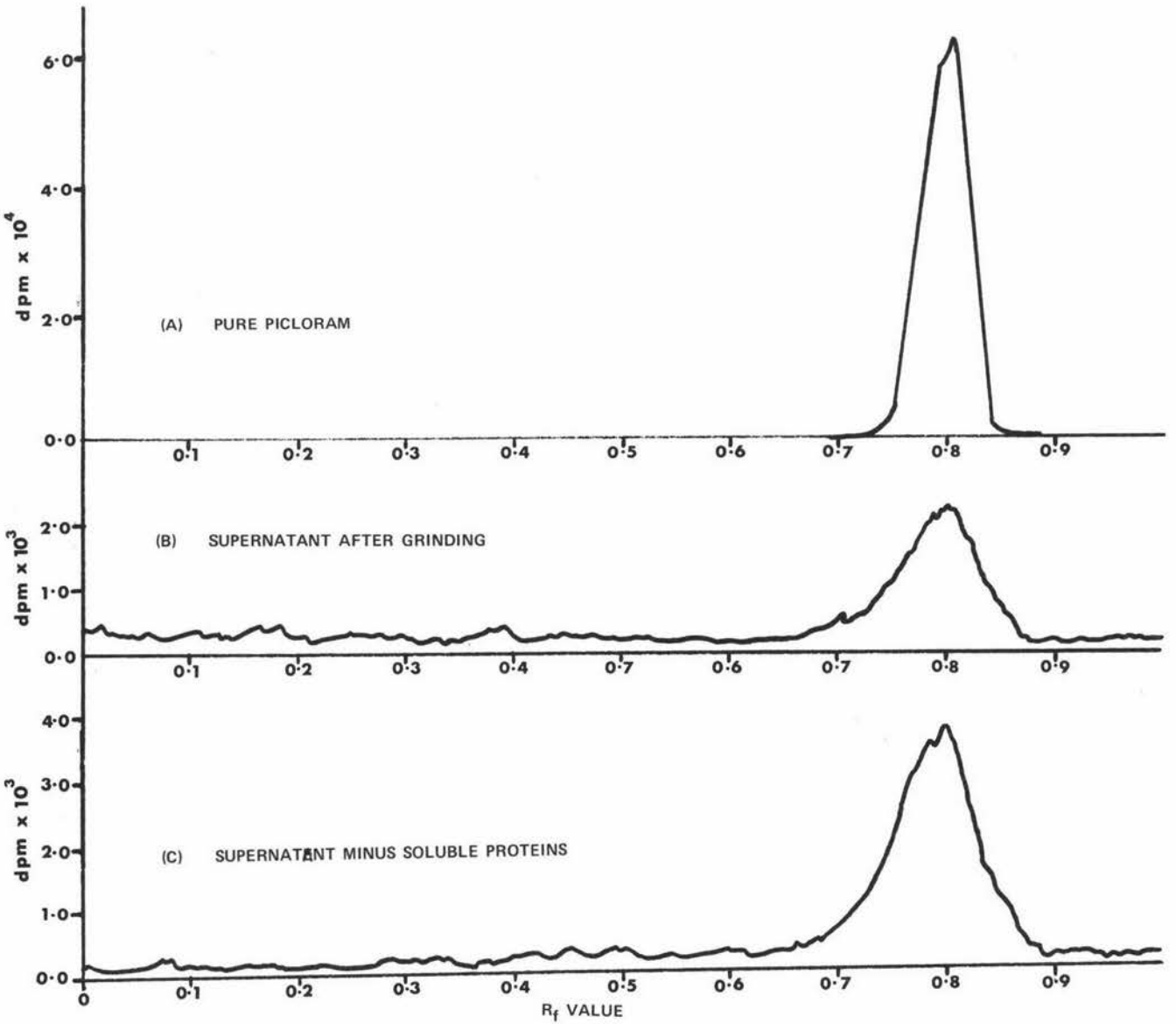

FIGURE 4.14: Radiochromatogram scan for ^{14}C–picloram and metabolic products, following 24 hours incubation.

CHAPTER FIVE DISCUSSION

In discussing and interpreting the results, experimental limitations must be considered. The experiments were performed in the glasshouse and laboratory using small, but not juvenile plants, of one genotype. Thus extrapolation of results to the farm situation is not possible. However the techniques used in the study allow a rapid screening of the many factors which may affect absorption and translocation. The logical extension of this work is to test the most promising possibilities with field trials.

5.1 TECHNIQUES

Water based samples were technically easy to handle with the particular scintillation mixture used in the experiments. On the other hand, digested samples were coloured, due to an interaction between the scintillation fluid and perchloric acid/hydrogen peroxide (bleaching agent). Neither the resulting colour quenching nor the high gain settings required to offset this, were initially appreciated. Consequently, results from some of the translocation experiments could not be interpreted.

Determination of absorption by the difference method is suited to a plant system where a large percentage of herbicide is being absorbed. Absorption in the gorse system rarely exceeded twenty percent of the herbicide present in the incubating solution. Small errors in determining the initial and final concentrations will result in large errors. An example of this is given in Table 5.1.

As a consequence of this insensitivity, trends can be obscured and large changes in absorption are required if statistically significant differences are to be detected. Once this limitation was appreciated, the digestion method was used to measure absorption.

TABLE 5.1 Example of the insensitivity of the difference method for determining absorption when ^{14}C-herbicide concentrations are measured with a two percent error.

Initial concentration of ^{14}C-herbicide	=	20000 ± 400 dpm.ml^{-1}
Final concentration of ^{14}C-herbicide	=	16000 ± 320 dpm.ml^{-1}
Amount absorbed (by difference)	=	4000 ± 720 dpm.ml^{-1}
Percent absorbed	=	20%
Percent error	=	18%

Absorption from an incubation solution is most probably related to the surface area in contact with the solution. The spiny nature of gorse makes it difficult to measure surface area but on the other hand, fresh weight is readily determined and was closely related ($r = 0.95$) to leaf area.

There was no change in the absorption from tips excised from plants of two different ages and this was to be expected, since tips of similar maturity were selected in an attempt to avoid changes with age.

The precooling curve (Figure 4.4) indicates the importance of correct operating procedure when analytical equipment is being used, and this is not always easy when the operator is unfamiliar with the equipment.

Losses of ^{14}C-picloram due to volatilization or digestion could not be detected as shown in Tables 4.4 and 4.5. No, or very little, volatilization was expected because of the low vapour pressure of picloram; 6.16×10^{-7} mm Hg at 35°C (Martin 1968). Similarly, no digestion losses of ^{14}C-picloram were found by Lumb (pers. comm.), with the perchoric acid/hydrogen peroxide mixture.

5.2. ABSORPTION

Throughout the series of experiments on absorption, a prominent feature is the low rate of absorption. The absorption of picloram determined by *in vitro* and *in vivo* techniques rarely exceeded 20 percent of the applied herbicide. Poor absorption must be a limitation in the field to the effectiveness of picloram.

Since 2,4-D also showed poor absorption characteristics in these experiments, and Leonard and Yates (1959) have reported poor absorption of 2,4,5-T, it can be postulated that this is a characteristic of gorse.

Further experiments with different formulations and other herbicides is required to test this hypothesis.

5.2.1. Environmental Factor Influencing Absorption

The decrease in temperature coefficient (Q_{10}) with increasing temperature that occurred with picloram (see Table 4.8) has also been noted to occur with picloram in barley and soybean sections (Sharma and Vanden Born 1973) and with 2,4-D in Phaseolus (Sargent and Blackman 1962).

The activation energies for uptake of ^{14}C-picloram decrease with increasing temperature and are similar in value to those obtained by Sharma and Vanden Born (1973) for soybean hypocotyl. In contrast, the activation energies for uptake of ^{14}C-2,4-D increase with increasing temperature. These changes in activation energy with temperature, suggest that more than one mechanism of absorption is occurring. Sharma and Vanden Born (1973) suggest this indicates the involvement of both passive and active processes in the uptake.

Light appears to have a role in the absorption of 2,4-D and picloram by gorse. Sargent and Blackman (1962 and 1970) report similar light/dark effects for those two chemicals in Phaseolus. The statistically non significant result for picloram (Table 4.10) is similar in magnitude to the significant result obtained for 2,4-D, suggesting that the light/dark effect is biologically important. The enhancement of 2,4-D absorption in light following a dark pre-treatment was also observed by Sargent and Blackman (1962).

There are several different sites at which light may be acting. Light may cause a change in stomatal aperture or membrane permeability of the guard cell. This may or may not be phytochrome mediated. Light may involve photosynthesis directly in absorption by the production of A.T.P. or other phosphorylated compounds that would be required for active uptake or by providing electrons or hydrogen ions for ionic exchange purposes. Alternatively light may indirectly increase the concentration gradient if the herbicide is translocated with the assimilate stream.

Respiratory processes may be involved in absorption. The addition of KCN, an inhibiter of cytochrome oxidase greatly increased absorption. It could be postulated that respiration is required to maintain the integrity of the membrane. The observed chlorophyll discolouration of the incubation solution after KCN treatment suggests that membrane integrity has been affected. However, the effect may be due to the alkaline nature of KCN causing disruption of the membrane components.

Clearly, there is insufficient evidence from these experiments to make any definite conclusions about the role of photosynthesis, phytochrome and respiration in absorption.

The time course pattern for *in vitro* absorption of picloram (see Figure 4.5) has an initial rapid entry, followed by a slower rate of absorption. This has also been reported for picloram by Sargent and Blackman (1970) and Sharma and Vanden Born (1973), as well as for other auxin herbicides.

This is the characteristic pattern for "Type 1" accumulation first reported by Saunders *et al.* (1966) for 2,4,5-T and demonstrated for picloram by Sharma and Vanden Born (1973). A characteristic feature of "Type 1" accumulation is that the material accumulated starts to be lost from the tissues. While a loss of picloram into a non picloram solution was not tested, the hypothesis that "Type 1" accumulation was occurring would explain the absorption pattern observed.

The low rates of *in vivo* absorption of picloram under dry conditions and the marked increase in absorption following misting (See Figure 4.7) support the hypothesis that absorption from spray deposits is slight, and further absorption only occurs if the deposits are redissolved. This result may explain in part the variable results that occur, especially when gorse is aerially sprayed with picloram and other auxin herbicides.

It has been observed by Upritchard (1973) that the effectiveness of 2,4,5-T is improved in areas of reliable summer rains compared to areas where summer droughts regularly occur. This may be due to the formation of spray deposits and hence low rates of absorption under dry conditions, although other explanations are also feasible; e.g. poor growth under dry conditions may result in poor translocation of the herbicide.

The misting effect is important and the result should justify further study in controlled climate rooms on the interaction of the drying potential of the environment (temperature, relative humidity and wind) with spray volume, droplet size and droplet number. Leonard and Yates (1959) using gorse found at 24°C a considerable increase in the absorption of 2,4,5-T at 95 percent R.H. compared to 70 percent R.H.

If the variable results obtained by aerial spraying of gorse are due to the drying potential of the atmosphere, then several changes in spraying methods are necessary. Spraying under very hot conditions should be avoided. Currie (1959) believes that the volumes of water carrier typically used in aerial spraying are insufficient. Greater volumes of water may reduce drying of spray droplets and/or improve coverage (see Section 5.3:

Translocation), or alternatively a carrier with low volatility characteristics may be required.

Attempts to use Alkane 60 in place of water resulted in negligible picloram absorption, (See Table 4.17). This may have been a result of the observed phytotoxity of this chemical. The experiment with Alkane 60 needs to be repeated before firm conclusions can be drawn. Other humectants should also be tested. However with the present world oil supply situation, research efforts should place emphasis on water rather than oils as the herbicide carrier.

The marked increase in 2,4-D absorption at low pH has been demonstrated in Phaseolus by Sargent and Blackman (1962) and Ashton and Crafts (1973). The latter consider this is characteristic of molecules that enter leaves via the lipid route, the undissociated molecules penetrating more readily than the dissociated molecules. The dissociation curves for 2,4-D are plotted in Figure 4.8. While there is a similarity in shape between the absorption and dissociation curve, an analysis of the relative changes between the curves (See Table 4.13), demonstrates that factors other than the effect of pH on dissociation of the herbicide are also involved. One such factor suggested by Van Overbeck (1956) is the repression of ionization at low pH values of the acid residues in the cuticle itself.

The penetration of picloram into gorse at different pH values is difficult to interpret. Picloram acid showed a slight enhancement at low pH (See Table 4.12), although absorption was greater at pH 5.70, compared with pH 4.55 and 7.40. This effect is statistically significant at the 10 percent level, but it is impossible to determine whether there is any biological significance in the response.

The penetration of picloram formulated as the K-salt, is also difficult to interpret. The lowest pH value tested, resulted in a very low rate of absorption and this is in contrast to a trend of increasing absorption with decreasing pH. Although the result is statistically significant, it should not be regarded as being biologically real, until further experiments support or refute this observation. The results on pH effects are not conclusive enough to support or refute the claim of Ashton and Crafts (1973), that "the adjustment of pH on the acid side has no effect on the penetration of picloram".

The effect of concentration of the incubation solution on the absorption of 2,4-D was investigated. Above a threshold concentration (382 ppm), absorption increased linearly with concentration. The results obtained here however cannot be compared with Sargent and Blackman (1962),

who found in Phaseolus that absorption was linear over the range 1 - 200 ppm. The threshold may indicate that above this concentration, sites for active absorption have been saturated and with increasing concentration, passive diffusion would become increasingly important. If this hypothesis is true, then it would be expected that treatments which enhance active absorption, would raise the threshold concentration. Further experimentat is required to test this hypothesis.

The two non ionic surfactants tested markedly enhance the in vitro absorption of both picloram and 2,4-D (See Figure 4.10 and Table 4.16). The control solutions were commercial formulations. The picloram:2,4,5-T mixture (1:4) contains an emulsifier to stabilize the oil soluble components when the concentrate is diluted with water. Any effect this emulsifier has on herbicide penetration, is secondary to its role as a stabilizer. The 2,4-D formulation contains no emulsifier or surfactants.

The magnitude of the surfactant response suggests that further in vitro screening of a wide range of surfactants, both ionic and non ionic, at different concentrations is required. The most promising surfactants could be tested using in vivo absorption techniques with final evaluations being made in field trials.

The only published field trial on the effect of surfactants on the control of gorse (Houlker 1960), showed that 0.125 percent or 0.25 percent of the sodium salt of a secondary alkyl sulphate gave improvements in the initial control of gorse, although long term control was not improved by the surfactant. Mason (pers. comm.) comments on field work with surfactants, and notes that "final kill (of gorse) has never been consistently enhanced by the addition of any surfactant with brushweed herbicides" O'Connor (pers. comm.) recommends the addition of 0.25 percent surfactant for spraying gorse in early summer. This recommendation is based on field experience.

Finally, it is of interest to speculate on the nature of the in vitro surfactant response obtained in this project. It is suggested that a direct effect, perhaps on the cuticular permeability, occurs, rather than an indirect effect on the spray, e.g. improved wetting.

Of the additives investigated, only two enhanced absorption. The KCN enhancement has already been discussed, (See Section 5.21). Unfortunately, the pH curve for 2,4-D does not extend sufficiently, to accurately partition the response between the pH effect itself and other effects of KCN. However, extrapolation of the pH curve suggests that factors

other than pH are involved in the KCN response.

The 50 percent enhancement of 2,4-D absorption by Na_2HPO_4 (pH 8.17) can be attributed as a real Na_2HPO_4 effect, there being no absorption response at pH 8.17 (See Figure 4.8).

Further in vitro and in vivo research on the effect of additives at different concentrations on the absorption of auxin herbicides, expecially those additives that have not been tested with gorse, should provide information that could be tested in the field.

When glasshouse grown gorse was compared with gorse of the same genotype grown outdoors, the latter had a lower rate of absorption. This is consistent with numerous observations, (Hull 1970) that wax development in glasshouse grown material is not as great as that of material grown outside.

As a result of the high wax content and the low rates of absorption an investigation of the cuticular surfaces was made using scanning electron microscopy. No obvious wax structures were present except on juvenile leaves. The wax appears to be present as a thick smooth layer. The small size and the "chimney form" of the stomata would reduce the likelihood of stomatal absorption occurring.

It is not known whether the hairs prevent droplets making contact with the bottom of a groove. However, the length and sparseness of the hairs suggests that this is not a problem. Further, the contact angles of water droplets are less than 90° and this suggests that wetting the gorse surfaces is not a problem. (Martin and Juniper 1970). This is contrary to the claim of Pryor and Dana (1952) that the pubescent spines of Ulex "makes it difficult to wet with chemical sprays", although they produce no evidence to support their statement.

5.3. TRANSLOCATION

The pattern of translocation obtained from the autoradiographs are similar to those described for auxin herbicides by Ashton and Crafts (1973). The translocation of 2,4,5-T in Ulex europaeus was described by Schicke and Linden (1963) to proceed primarily along the main axis between the top and the root, and only to a much lesser extent along the lateral axes. A similar pattern occurred in these experiments, where picloram was concentrated in the treated stem and root, while other branches received only traces of picloram label. Thompson (pers. comm.) has made a field study on translocation of herbicides in gorse. By protecting some parts

of the plant with polyethene, from spray and observing the changes in these unsprayed areas, he can estimate the effectiveness of various chemicals. Differences in terms of changes in the protected areas and regrowth from protected areas indicate that the herbicides gave differing degrees of control. The degree of control is most likely a function of the amount of herbicide translocated to lateral branches. Interim results from Thompson (unpublished) indicate that the effect on protected foliage with picloram/2,4,5-T is greater than dicamba/2,4,5-T, which is greater than 2,4,5-T.

It has long been recognized that complete coverage of gorse bushes is necessary for adequate control. This is easily achieved when high volumes of water carrier are applied. However, the control of gorse in New Zealand hill country whether by aerial spraying, or from the ground, has moved to low volume spraying for economic reasons, where adequate coverage is difficult.

If the hypothesis that there is poor translocation into other lateral branches is correct, then the techniques and equipment required for low volume spraying applications must be improved. The evidence that poor translocation into lateral branches occurs is, at present not conclusive. The autoradiographic evidence obtained for ^{14}C-picloram in this thesis while supporting the hypothesis, has been carried out at one stage of growth on pot plants. The stage of growth does affect the distribution of 2,4,5-T in gorse (Schicke and Linden 1963). Using the daily rate of shoot extension as a parameter of growth, they found that with increasing rates of growth, basipetal translocation increased.

The lack of build up of ^{14}C-picloram in the roots may be related to the stage of growth. Basipetal translocation from the treated stem appears to be greater in the quantitative analysis (see Figure 4.13) than in the autoradiographs (see Plate 4.8). These two studies were made on plants of different ages and at different time intervals and probably represent differences in metabolism and/or growth (e.g. extension or change in dry matter with time). The importance of age and season on the direction and amount of translocation and the relating of these parameters to the physiological condition of the plant would make an important extension of the results reported in this thesis.

If regeneration of gorse from the basal crown is to be prevented then sufficient herbicide must be translocated to the roots. The stage of growth will probably affect picloram distribution in a similar manner to 2,4,5-T distribution. Further experimentation is required to test for this. If poor basipetal translocation occurs when top growth is low, then

the amount of herbicide reaching the roots is reduced. Two questions arise from this; what amount of herbicide is required to kill root tissue? Will increasing herbicide concentration compensate for poor basipetal translocation (if this does occur), when spraying in seasons other than spring?

Coaldrake (1970) has already reported that legume nodules may act as sinks for 2,4,5-T. The source-sink pattern of ^{14}C-picloram distribution (Plates 4.13 and 4.15) supports the hypothesis of Crafts and Crisp (1971) that picloram, like other auxin herbicides is phloem translocated and the rate of distribution is controlled by the nutrient requirements of various sinks. The acropetal distribution is either due to picloram translocation in the xylem and/or phloem translocation to the stem apex. The clear labelling of all spines above the point of treatment is indicative of apoplast translocation, although the nature of translocation (xylem and/or phloem) as shown in Plate 4.11, is open to speculation.

The quantitative analysis points to the importance of the site that labelled herbicide is applied to. The difference in the distribution patterns obtained when the tip is treated (Figure 4.12) compared to a point below 6 cm below the tip, (Figure 4.13) also indicates the importance of experimental technique. Retention of ^{14}C-picloram by the tip would occur if either the tip was acting as a strong sink, or if the tip can conjugate picloram. It can be speculated that the apex has higher levels of natural auxins and thus the enzymes required to conjugate natural and synthetic auxins including picloram.

The short term distribution of ^{14}C-picloram (See Figure 4.13) has several peaks. The latter peaks are thought to have resulted from acropetally translocated label being phloem loaded, and translocated basipetally.

Rates of translocation constitute an area of research full of conflicts (Canny, 1960), thus a range of velocities from 12.5 to 40 cm.hr^{-1} are estimated. These values represent medium to slow rate of phloem translocation (Crafts and Crisp (1971) although Canny (1962) considers 1 to 10 cm.hr^{-1} to be realistic.

5.4 METABOLISM

No metabolites were detected. However, the incubation system used in these experiments is not suited for the detection of decarboxylation. This is a common failing in the design of many herbicide metabolites experiments reported in the literature. Although no conjugates were detected, it is possible that picloram conjugates in gorse are highly

labile. Picloram conjugates detected by Sharma and Vanden Born (1973) were not labile, although Hallman and Eliasson (1972) found evaporation of the solution to dryness caused some release of free acid from picloram conjugates.

The metabolism experiment in this study used spines from young plant tissue. Sharma and Vanden Born (1973) found that in barley, different tissues (coleoptile and leaf) had different abilities to conjugate picloram. A more detailed investigation is required to examine whether different tissues of gorse, e.g. roots and especially stem tips from which picloram is not readily translocated, (see Figure 4.12), have any ability to conjugate picloram. Similarly differences in plant age should be studied as Sargent and Blackman (1970) reported an age effect in the ability of Phaseolus to decarboxylate picloram.

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CHAPTER SIX CONCLUSIONS

The experiments were designed to locate factors relating to poor herbicide kills of Ulex europaeus which occur especially when gorse is aerially sprayed. Factors relating to absorption, translocation and metabolism were studied, as any one of these three factors may be limiting the effectiveness of picloram in the field. The glasshouse and laboratory experiments therefore cover a wide range of factors. Often the experiments have not been performed in depth; one genotype; one formulation; one rate. Nevertheless, several factors which could be limiting the effectiveness of picloram and possibly of other auxin herbicides were detected.

From the results obtained, it is concluded that absorption is poor and this is due primarily to the presence of a thick smooth layer of wax on the cuticle. Environmental conditions at the time of application, especially those causing rapid drying of the spray deposit are important. Similarly it is postulated that environmental conditions following spraying which cause the spray deposit to redissolve are equally important in determining herbicide effectiveness. Further studies in a controlled climate laboratory are necessary to find what environmental conditions are optimum for absorption. Several additives, especially surfactants, enhance absorption, while Na_2HPO_4 and KCN were the only inorganic salts of those tested that enhanced *in vitro* absorption. Additives may give enhanced absorption and possibly better kills in the field. Whole plant studies are needed to test these *in vitro* findings.

Other *in vitro* information provides evidence on the mechanism of absorption. Absorption is stimulated by light. At least two mechanisms (perhaps active and passive mechanisms) of uptake occur. Support for this conclusion comes from the temperature response, especially the influence of temperature on activation energies and from the herbicide concentration response.

The translocation pattern suggests that herbicide is restricted to the treated stem and roots, with very little redistribution occurring over a 16 day period. Therefore poor coverage may be reducing herbicide effectiveness. Source-sink relationships occur with root nodules being preferentially labelled, while developing seed pods on non treated stems also become clearly labelled. There appears to be no build up of herbicide in the roots. It is not known whether a lethal dosage of picloram reached the roots. The distribution pattern obtained by sectioning stems differs markedly in terms of acropetal accumulation

to the pattern obtained by autoradiographs. Growth rate and time of season may cause the differences in translocation patterns. This has previously been demonstrated with 2,4,5-T in Ulex europaeus (Schicke and Linden 1963), and further study of factors affecting the distribution of picloram and other auxin herbicides in Ulex is required.

The stems and spines do not appear to metabolize picloram. Stem tips do not readily translocate picloram and therefore may be capable of metabolizing picloram but this was not tested.

Glasshouse and laboratory research allows many factors to be studied, under relatively constant environmental conditions. This type of study is important and can provide useful leads for further experimentation in the field. It is also important that both the physiologist working in the laboratory, and those scientists engaged in field research appreciate the usefulness and limitations of laboratory experiments on herbicide absorption, translocation and metabolism.

APPENDIX I NUTRIENT SOLUTION

The following nutrient was applied at the rate of 100 ml per plant at one monthly intervals.

10 ml/l	$CaCl_2$	1 M stock solutions
10 ml/l	KCl	
5 ml/l	$MgSO_4$	
4 ml/l	KH_2PO_4	
10 ml/l	Fe. ETDA	$\frac{1}{2}$ M stock solution
2 ml/l	Combined micronutrient mixture containing B, Cu, Mo, Mn, Zn, and Co.	

APPENDIX II: LIQUID SCINTILLATION COUNTING

^{14}C levels were determined using a Packard Tricarb Liquid Scintillation Spectrometer. Scintillation fluid was mixed in the following proportions:

100 mg POPOP (1, 4Di-2-(5-phenyl oxazolyl))benzene.
4.0 g POP (2,5-Diphenyloxazole)
500 ml Triton X-100
1,000 ml Toluene.

Ten ml per vial were used.

During counting the following channels were used:

50 - 1,000/120 - 1,000

both at 9 percent gain for water based samples and

50 - 1,000/50 - 250

both at 25 percent gain was used for digested samples (which were coloured quenched).

Calibration curves using colourless quenched standards were drawn (Fig. 4.1 and Fig. 4.2). Linear and curvilinear equations for the curves were solved so that the efficiency and hence the number of disintegrations per minute could be calculated. Due to the random disintegration of radioactive molecules the natural standard deviation (N.S.D.) of a number of registered counts is equal to the square root of that number (I.A.E.A. 1964). Therefore, where possible, an attempt was made to record 10,000 counts giving a N.S.D. equal to $\pm 10^2$, i.e. 10,000 $\pm$ 1%. This was difficult to achieve in the translocation experiments where the C^{14} levels were low.

APPENDIX III: DIGESTION OF PLANT MATERIAL

Perchloric acid/ hydrogen peroxide digestion to bleach samples for Liquid Scintillation Counting was originated by Mahin and Lofberg (1966). Reid and Hurtt (1969) used this method for picloram. Lumb (pers. comm.) heated vials at 80°C in a forced draught oven. However, in the present study, this procedure was found to cause the welding of some caps to the vials.

The following procedure was found to be satisfactory:

> To each vial was added (seperately), 0.4 ml of 70% $HC1O_4$ and 0.6 ml of 30% H_2O_2 and about 50 mg (accurately weighed) oven dried, ground plant material. The caps were firmly screwed on and the vials heated at 80°C on a sand covered (2 cm deep) hot plate. After 20 minutes the vials were shaken to help the tissue remnants to become soluble and allowed to cool to room temperature. Ten ml of scintillation fluid (Appendix II) were added to each vial causing a pale yellow colouration to develop.

The vials were counted and the efficiency of counting determined.

APPENDIX IV: DECONTAMINATION PROCEDURE

The glassware was heated for four hours at 90°C in a waterbath containing five percent solution of Extran in water. After the solution had cooled, the vials were rinsed in water and dried in an oven.

Background counts were determined on a random sample of the decontaminated tubes and compared with a standard background vial.

APPENDIX V:

THE EFFECT OF PRE-COOLING VIALS ON THE COUNTS RECORDED BY SCINTILLATION SPECTROMETRY

PRE-COOLING (minutes) (Time period over which counts were recorded)	RECORDED COUNTS (cpm) (± Natural Standard Deviation)
0 - 2	5058 ± 50
6 - 8	880 ± 21
9 - 10	760 ± 19
13 - 14	717 ± 19
15 - 16	675 ± 18
17 - 21	630 ± 8
22 - 26	605 ± 9
32 - 36	598 ± 11
42 - 46	555 ± 11
52 - 56	549 ± 10
62 - 66	523 ± 10
72 - 76	514 ± 10
77 - 81	536 ± 10

APPENDIX VI:

CALCULATION OF ACTIVATION ENERGIES FOR THE UPTAKE OF ^{14}C-2,4-D DIMETHYLAMINE AND THE K-SALT OF ^{14}C-PICLORAM AT DIFFERENT TEMPERATURES

Activation energies can be derived from the Arrhenius equation:

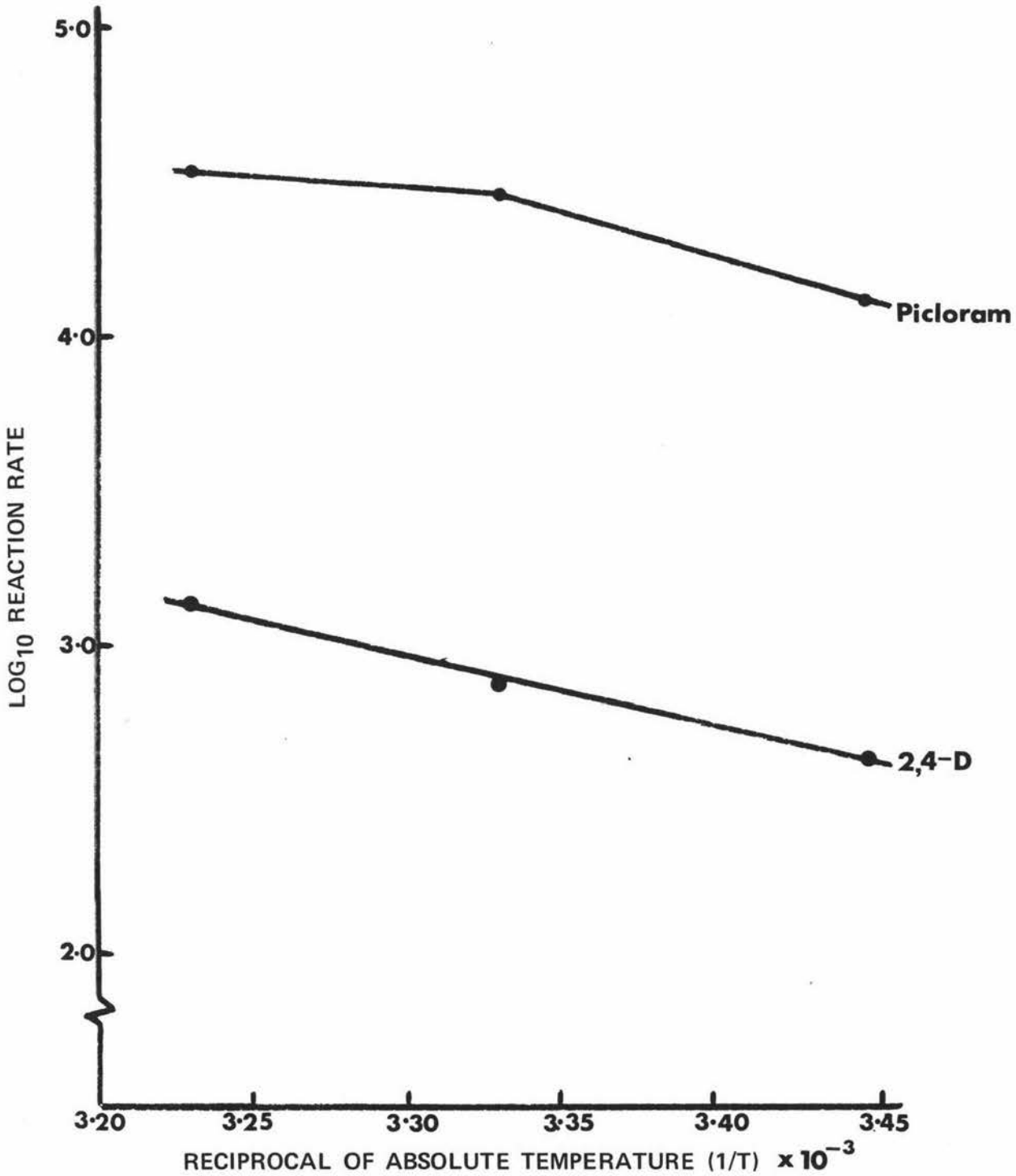
$$\frac{\Delta \text{ in } K}{\Delta \quad T} = \frac{A}{RT^2}$$

Where: K = reaction rate
T = absolute temperature
R = gas constant (1.987 cal/°/mole)
A = activation energy

A can be calculated from the slope of the line obtained on plotting log K against $\frac{1}{T}$ (See Appendix). The slope is equal to $\frac{A}{\ln . R}$ (i.e. $\frac{A}{4.58}$ cal/mole)

Temperature °C	°Absolute	$\frac{1}{T_A}^{\circ} \times \overline{10}^3$	$\Delta \frac{1}{TA}^{\circ} \times \overline{10}^3$
37	310	3.23	0.10
27	300	3.33	0.12
17	290	3.45	

Herbicide	Log_{10} Rate	Change in Rate (Δ K)	Slope $\Delta K/\Delta 1/TA^{\circ} \times 10^3$	Activation Energy Kcal/mole	Activation Energy kJ/mole
2,4-D					
37°	3.1383	0.2535	2.54	11.22	47.0
27°	2.8848	0.2544	2.12	9.71	40.3
17°	2.6304				
Picloram					
37°	4.5378	0.1830	1.80	8.20	34.4
27°	4.4548	0.3277	2.73	12.51	52.4
17°	4.1271				



APPENDIX VI: Arrhenius plot of data on the absorption of ^{14}C–2,4–D dimethylamine and the potassium salt of ^{14}C–picloram.

APPENDIX VII:

ABSORPTION OF ^{14}C-PICLORAM DURING DIFFERENT TIME INTERVALS.

(a) IN VITRO ABSORPTION OF THE K-SALT OF ^{14}C-PICLORAM FROM A PICLORAM: 2,4,5-T MIXTURE (1:4), OVER A TIME PERIOD OF 16 HOURS: TEMPERATURE 27°C; LIGHT 7000 LUX.

Time (hours)	Amount Absorbed (dpm^{-1} g. DW^{-1}. x 10^4)	t
0.5	1.77 ± 0.90	
		P > 0.02
2.0	2.20 ± 0.03	
		P > 0.05
4.0	2.72 ± 0.14	
		NS
8.0	3.21 ± 0.34	
		P > 0.01
16.0	5.24 ± 0.28	

(b) IN VIVO ABSORPTION OF THE K-SALT OF ^{14}C-PICLORAM FORMULATED IN A PICLORAM: 2,4,5-T MIXTURE (1:4), OVER A TIME PERIOD OF 100 HOURS; TEMPERATURE 21-23°C; RELATIVE HUMIDITY 70-80 PERCENT.

Time (Hours)	% Absorbed	Herbicide Recovered (dpm x 10^4)	t
0.0	0	2.30 ± 0.04	
			P > 0.05
2.5	7.8	2.12 ± 0.06	
			P > 0.05
5.0	15.2	1.95 ± 0.07	
			NS
10.0	20.4	1.83 ± 0.08	
			NS
20.0	17.8	1.89 ± 0.09	
			NS
50.0	18.7	1.87 ± 0.13	
			NS
54.0	18.3	1.88 ± 0.05	
			NS
100.0	16.0	1.93 ± 0.10	

APPENDIX VIII:

THE EFFECT OF MISTING ON THE ABSORPTION OF THE K-SALT OF ^{14}C-PICLORAM FROM A PICLORAM: 2,4,5-T MIXTURE (1:4); HARVESTED FIVE DAYS AFTER APPLICATION. TEMPERATURE 18-22°C; RH 57-65 LIGHT 7000 LUX.

Treatment	% Absorbed	Herbicide Recovered by Washing (dpm x 10^3)	t
Not misted	27	16.83 ± 0.68	P 0.001
Misted at 24 hours.	51	11.68 ± 0.81	NS
Misted at 24 and 48 hours	59	9.52 ± 1.00	

APPENDIX IX: THE EFFECT OF pH ON IN VITRO ABSORPTION

(a) IN VITRO ABSORPTION OF ^{14}C-2,4-D DIMETHYLAMINE AT DIFFERENT pH VALUES. BUFFERED WITH $NaHPO_4$/ CITRIC ACID = 0.25% w/v TRITON X-100; TEMPERATURE 27°C; LIGHT 7000 LUX; TIME 12 HOURS

pH	% Absorbed	Amount Absorbed (dpm. ml^{-1}. g FW^{-1} x 10^2)	t
3.35	34.3	21.46 ± 1.16	P > 0.001
4.15	25.2	15.77 ± 0.70	P > 0.001
5.22	15.4	9.64 ± 0.26	P > 0.05
7.05	10.4	6.41 ± 1.20	NS
8.47	10.4	6.43 ± 1.33	NS
8.93	12.3	7.73 ± 1.20	

(b) IN VITRO ABSORPTION OF THE K-SALT OF PICLORAM FROM A PICLORAM/2,4,5-T MIXTURE (1:4) AT DIFFERENT pH VALUES. BUFFERED WITH $NaHPO_4$/ CITRIC ACID + 0.25% w/v TRITON X-100. TEMPERATURE 22°C; TIME 12 HOURS; LIGHT LUX

pH	Amount absorbed (dpm.g DW^{-1}. x 10^3)	t
3.25	4.05 ± 0.34	P> 0.001
4.15	7.20 ± 0.16	P > 0.01
5.25	5.72 ± 0.59	P > 0.01
6.65	3.26 ± 0.32	

APPENDIX X:

THE EFFECT OF SURFACTANTS ON THE IN VITRO ABSORPTION OF THE K-SALT OF ^{14}C-PICLORAM.

Treatment	Amount absorbed (dpm. g. DW^{-1} x 10^4)	t
No surfactant	2.27 ± 0.14	a
0.25% w/v Triton X-100	3.28 ± 0.39	a
0.50% w/v Triton X-100	3.71 ± 0.32	b
0.25% w/v Dowfax	5.07 ± 0.17	b c
0.50% w/v Dowfax	4.90 ± 0.18	b c

P> 0.1

(Values followed by different letters of the alphabet, are significant at P> 0.01 level.)

Experimental conditions: Buffered at pH 5.8 with Sterilized $NaHPO_4$/ citric acid, and 1% sucrose. Temperature 22°C, light lux; Time 12 hours.

APPENDIX XI:

CONVERSION OF WAX RECOVERY DATA FROM mg/gDW TO mg/cm^2.

In the literature, wax yields are normally quoted in mg/cm^2. In gorse, it is most difficult to measure surface area. The wax recovery measured (mg/g DW) can however be converted, if two assumptions are made:

(1) the dry matter percentage of gorse is 10 percent or greater.
(2) the actual surface area is twice that recorded by the automatic leaf area meter.

Both these assumptions are conservative. In Figure 4.3, a relationship between recorded surface area and fresh weight was derived.
Thus, 500 mg Fresh weight = 2 cm^2 (recorded)

Therefore, 500 mg x $\frac{10}{100}$ Dry weight = 2 cm^2 x 2 (actual area)

i.e. 50 mg DW = 4 cm^2

Therefore 12.5 mg DW = 1 cm^2

There are 25.4 mg wax per 1,000 mg DW.

Therefore wax in mg/cm^2

$$= \frac{12.5 \times 25.4}{1,000}$$

$$= 0.3 \text{ mg wax/cm}^2.$$

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