

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

SOME ASPECTS OF ABSORPTION, TRANSLOCATION AND METABOLISM OF

TWO FOLIAR APPLIED AUXIN HERBICIDES IN GORSE

(Ulex europaeus L.)

A Thesis

presented in partial fulfilment of the requirements for the degree

of

MASTER OF AGRICULTURAL SCIENCE

at

MASSEY UNIVERSITY

MAURICE PHILIP ROLSTON

1974

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 separated 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.

ACKNOWLEDGMENTS

I wish to gratefully acknowledge the following people who have made this study possible:

Mr. A.G. Robertson for initiation of the project and helpful guidance throughout the course of study and in the preparation of this manuscript.

Mr. R.M. Richards (Horticulture Department), for teaching me the art of making cuttings and for arranging plant propagation facilities.

Prof. R. Behrens (Minnesota) for his advice and encouragement and for supplying the ^{14}C -2,4-D.

Dr. David Penny and members of the Botany and Zoology Department for use of the liquid scintillation counter.

Dr. G.G. Pritchard, (Chemistry, Biochemistry and Biophysics Department) and Dr. Laurie Kennedy (Applied Biochemistry Division D.S.I.R.) for their help in preparing and scanning the paper chromatograms.

Dr. G.W. Mason (I.W.D., New Plymouth) for his interest in this project.

Mr. R.M. Greenwood (Rhizobium Laboratory, D.S.I.R.) for supplying rhizobia to inoculate the gorse plants.

Miss K. Kard (Physics and Engineering Laboratory, D.S.I.R.) for her cooperation and help in the scanning electron microscopy study.

Dr. David Woolley (Horticulture Department) for his encouragement and help in developing the autoradiographs.

Mrs. M. van Reenen for translating the Schicke and Linden (1963) paper.

Prof. B.R. Watkin, Mr. Alex Chu and other members of the Agronomy Department for their interest and encouragement during the course of the project.

Dr. R.W. Brougham (Grasslands Division, D.S.I.R.) who permitted me to work full time writing this manuscript during the initial months of my employment with Grasslands.

My wife, Shirley Rolston for her assistance during the project and in preparing this manuscript.

Mrs. J. Humphries for typing this manuscript, Miss M.E. Soulsby (D.S.I.R.), for preparation of the plates and the staff of the Massey Printery for the production of the figures.

Ivon Watkins Dow Ltd., New Plymouth, are thanked for the herbicide formulations they supplied; and the Dow Chemical Company, U.S.A. for the gift of 105 μ c 14 C-picloram.

Financial assistance from the N.Z. Weed and Pest Control Society and the Farmers Union Scholarship was gratefully received.

TABLE OF CONTENTS

	<u>PAGE</u>
TITLE	i
ABSTRACT	ii
ACKNOWLEDGEMENTS	iii
TABLE OF CONTENTS	v
LIST OF TABLES	vii
LIST OF FIGURES	ix
LIST OF PLATES	xi
LIST OF APPENDICES	xiv
ABBREVIATIONS USED	xv
 CHAPTER 1	
INTRODUCTION	1
1.1	2
1.2	3
 CHAPTER 2	
REVIEW OF LITERATURE	7
2.1	7
2.1.1	7
2.1.2	8
Absorption	8
(a) Physio chemical factors	8
(b) Physiological factors	10
2.1.3	11
2.1.4	13
2.1.5	18
2.2	21
TRANSLOCATION OF HERBICIDES	21
2.2.1	22
2.2.2	22
2.2.3	22
2.2.4	23
2.2.5	25
2.2.6	26
2.3	28
METABOLISM	28
2.3.1	28
2.3.2	29

		<u>PAGE</u>
CHAPTER 3	MATERIALS AND METHODS	31
3.1	PLANT PROPAGATION	31
3.2	THE HERBICIDES	32
3.3	ABSORPTION	33
3.4	SCANNING ELECTRON AND OPTICAL MICROSCOPY	34
3.5	TRANSLOCATION	34
3.6	METABOLISM	35
3.7	STATISTICAL ANALYSIS	36
CHAPTER 4	RESULTS	37
4.1	TECHNIQUES	37
4.2	ABSORPTION	41
4.3	TRANSLOCATION	52
4.4	METABOLISM	54
CHAPTER 5	DISCUSSION	55
5.1	TECHNIQUES	55
5.2	ABSORPTION	56
5.3	TRANSLOCATION	61
5.4	METABOLISM	63
CHAPTER 6	CONCLUSION	65
APPENDICES		67
BIBLIOGRAPHY		77

LIST OF TABLES

<u>TABLE</u>		<u>PAGE</u>
4.1	Detected ^{14}C levels of digested samples counted for 20 minutes at three gain settings.	37
4.2	<u>In vitro</u> absorption of the K-salt of ^{14}C -picloram at two time periods using two methods.	38
4.3	Comparison between newly produced stem tips from plants of two different age groups to absorb ^{14}C -2,4-D dimethylamine.	39
4.4	Estimates of the volatilization loss of ^{14}C -picloram (K-salt).	40
4.5	Loss of ^{14}C -picloram (K-salt) due to oxidation during perchloric acid/hydrogen peroxide digestion.	40
4.6	Temperature effect on the <u>in vitro</u> absorption of ^{14}C -2,4-D dimethylamine.	41
4.7	Temperature effect on the <u>in vitro</u> absorption of ^{14}C -picloram (K-salt) from a picloram:2,4,5-T (1:4) mixture.	42
4.8	The activation energies for uptake of ^{14}C -2,4-D dimethylamine and the K-salt of ^{14}C -picloram.	42
4.9	<u>In vitro</u> absorption of ^{14}C -2,4-D dimethylamine in darkness (tubes wrapped in aluminium foil), and in the light, with and without the respiratory inhibitor, KCN.	43
4.10	<u>In vitro</u> absorption of the K-salt of the ^{14}C -picloram in darkness and in the light.	44
4.11	<u>In vivo</u> absorption of the K-salt of ^{14}C -picloram formulated in the picloram:2,4,5-T mixture (1:4), diluted with water 1:250, under dry atmospheric conditions.	45

TABLEPAGE

4.12	<u>In vitro</u> absorption of picloram acid at different pH values. (Buffered with NaHPO_4 /citric acid).	46
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.	47
4.14	<u>In vitro</u> absorption of ^{14}C -2,4-D dimethylamine from solutions of different concentrations.	47
4.15	The effect of surfactant on <u>in vitro</u> absorption of ^{14}C -2,4-D dimethylamine, 764 ppm and picloram acid, 440 ppm.	48
4.16	The <u>in vitro</u> absorption of the K-salt of ^{14}C -picloram from an alkane based solvent.	49
4.17	The effect of inorganic and organic additives on the <u>in vitro</u> absorption of 2,4-D dimethylamine, 764 ppm.	50
4.18	Lipid recoveries from glasshouse grown gorse following a one minute dip in chloroform.	51
4.19	The <u>in vitro</u> absorption of ^{14}C -2,4-D dimethylamine by a gorse genotype grown outdoors and in a glasshouse, and by a leaf of blackberry grown outside.	51
4.20	Estimation of the velocity of ^{14}C -picloram translocation.	54
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.	54
5.1	Example of the insensitivity of the difference method for determining absorption when the ^{14}C -herbicide concentrations are measured with a 2 percent error.	56

LIST OF FIGURES

	<u>AFTER PAGE</u>
FIGURE 4.1: Calibration curve derived for counting ^{14}C in aqueous samples (gain 9.0%).	37
FIGURE 4.2: Calibration curve derived for counting ^{14}C in digested samples (gain 25.0%).	37
FIGURE 4.3: Correlation between fresh weight and surface area of gorse stem tips.	38
FIGURE 4.4: The influence of precooling on the recorded counts.	39
FIGURE 4.5: <u>In vitro</u> 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).	44
FIGURE 4.6: <u>In vivo</u> 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.	44
FIGURE 4.7: The effect of misting on the <u>in vivo</u> absorption of the K-salt of ^{14}C -picloram from a picloram: 2,4,5-T mixture (1:4). Plants harvested after five days.	44
FIGURE 4.8: The effect of pH on the <u>in vitro</u> absorption of ^{14}C -2,4,-D.	45
FIGURE 4.9: The effect of pH on the <u>in vitro</u> absorption of the K-salt of ^{14}C -picloram from a picloram: 2,4,5-T mixture (1:4).	45
FIGURE 4.10: The effect of surfactants on the <u>in vitro</u> absorption of the K-salt of ^{14}C -picloram from a picloram: 2,4,5-T mixture (1:4).	48

- 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. 51
- 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. 53
- FIGURE 4.13: The distribution of the ^{14}C -picloram formulated as the K-salt along a 30cm length of stem following application of the herbicide 6cm below the stem tip. 53
- FIGURE 4.14: Radiochromatogram scan for ^{14}C -picloram and metabolic products, following 24 hours incubation. 54
- APPENDIX VI: Arrhenius plot of data on the absorption of ^{14}C -2,4-D dimethylamine and the potassium salt of ^{14}C -picloram. 71

LIST OF PLATES

xi.

PLATE

AFTER PAGE

1.1	Gorse bush 12 months after an aerial application of a picloram/2,4,5-T (1:4) spray mixture.	9
3.1	Gorse cutting at six months. This plant is typical of the cuttings used in the study.	31
3.2	Stem of gorse showing the terminal six cm excised in preparation for an <u>in vitro</u> absorption experiment.	31
4.1	Scanning electron micrograph of a gorse spine from glasshouse propagated material. Magnification x 100.	52
4.2	Scanning electron micrograph of an unhardened gorse stem from glasshouse propagated material. Magnification x 95.	52
4.3	Scanning electron micrograph of a gorse spine from material grown out of doors. Magnification x 90.	52
4.4	Scanning electron micrograph of hairs on a gorse spine from glasshouse propagated material. Magnification x 150.	52
4.5	Scanning electron micrograph of wax formation on a gorse leaf from glasshouse propagated material. Magnification x 1900.	52
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.	52

- 4.7 Optical micrograph of a 1 μ l water droplet
 on a gorse spine. Contact angle measured
 at 78°. Magnification x 150. 52
- 4.8 Autoradiograph of gorse plant shoots 24 hours
 after the application of the K-salt of
 ^{14}C -picloram as a picloram:2,4,5-T mixture,
 (1:4). 53
- 4.9 Autoradiograph of the roots of the gorse plant
 shown in Plate 4.8. 53
- 4.10 Autoradiograph of 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). 53
- 4.11 Autoradiograph of the upper shoots of a gorse
 plant four days after the application of the
 K-salt of the ^{14}C -picloram as a picloram:
 2,4,5-T mixture, (1:4). 53
- 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. 53
- 4.13 Autoradiograph of the remaining roots not shown
 in Plate 4.12. 53
- 4.14 Autoradiograph of the shoots of a gorse plant
 seven days after the application of the K-salt
 of ^{14}C -picloram as a picloram:2,4,5-T mixture,
 (1:4). 53
- 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
 the seed pods. 53

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

53

LIST OF APPENDICES

<u>APPENDIX</u>	<u>PAGE</u>
I Nutrient solution.	67
II Liquid scintillation counting.	68
III Digestion of plant material.	69
IV Decontamination procedure.	69
V The effect of pre-cooling vials on the counts recorded by scintillation spectrometry.	70
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.	71
VII Absorption of ^{14}C -picloram during different time intervals.	72
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.	73
IX The effect of pH on <u>in vitro</u> absorption.	74
X The effect of surfactants on the <u>in vitro</u> absorption of the K-salt of ^{14}C -picloram.	75
XI Conversion of wax recovery data from mg/gDW to mg/cm^2 .	76

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-dipyridylum dibromide
DMSO	dimethyl sulfoxide
DNP	2,4-dinitrophenol
dpm	disintegrations 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
picloram	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