SOME PHYSIOLOGICAL EFFECTS OF THE HERBICIDE BROMACIL (5-BROMO-3-SEC-BUTYL-6-METHYLURACIL) ON *ASPARAGUS OFFICINALIS* L.

A thesis presented in partial fulfilment
of the requirements for the degree of
Master of Science
at
Massey University,
Palmerston North.

Godwin Balasingam
February, 1985

ABSTRACT

The root-absorbed, photosynthesis-inhibiting herbicide bromacil (5-bromo-3-sec-butyl-6-methyluracil) was applied in sand culture to tissue-cultured 18-month-old Mary Washington 500W clone of Asparagus officinalis L. grown under controlled environmental conditions.

Dose-response characteristics were determined and ED_{20} and ED_{50} values computed by regression analysis for several parameters for asparagus plants exposed to a single application of 0, 2, 4, 8, 16, 32, 64, 128, 256, and 512 p.p.m. bromacil in non-draining pots. The results of this initial broad spectrum studies revealed a drastic decline in visually assessed foliage damage score, shoot growth and root fresh weight, and an increase in shoot death at relatively low concentrations. Good dose-response characteristics were obtained, and time-course data showed that the rate and severity of effects increased with increasing dose. The ED_{50} values 18 days after treatment were: visually assessed damage score, 2.7 p.p.m.; shoot growth, 25 p.p.m.; shoot death, 4.6 p.p.m.; and root fresh weight, 2.1 p.p.m.

A catalogue of colour plates showing visual phytotoxic effects was compiled. The injury symptoms observed were: yellowing of cladophyll tips followed by bleaching with the effects extending towards the base, cladophyll tipping and progressive cladophyll death leading to shoot death.

Equal increment dose-response experiments were conducted at 0, 2, 4, 6, and 8 p.p.m. bromacil, using a portable fluorometer (Model SF-10) to obtain fluorescence emission measurements. The results showed a dramatic decline in the initial rise in fluorescence yield from the cladophyll tips 156 hours after treatment. The ED_{50} value was computed to be 2.3 p.p.m. Fluorescence emission measurements from cladophyll tips from excised shoots placed in bromacil solution at the same concentrations showed a dramatic decline in fluorescence yield within 17 hours indicating that uptake and translocation was more rapid without the roots.

No significant changes in chlorophyll a, chlorophyll b and total chlorophyll concentrations, as determined by 80% acetone extraction technique, were evident in the samples in which a dramatic decline in fluorescence yield

occurred.

The results of this study, conducted under controlled environmental conditions, showed that the asparagus clone tested readily absorbed bromacil through its roots and translocated it to the foliage causing severe initial damage to the photosynthetic apparatus followed by detrimental effects on other parameters such as shoot growth, root fresh weight and shoot death. Even at a bromacil concentration of 2 p.p.m. the asparagus plants were found to susceptible to herbicide damage.

ACKNOWLEDGEMENTS

I wish to express my gratitude to the following people who made this project possible:

Mr A. Robertson and Dr G. Ivens, my supervisors, for their guidance during the course of this study and in the preparation of the thesis.

Dr D. Woolley and Mr D. Anderson for assistance with the use of the spectrophotometer.

Dr D. Cohen for discussions and provision of tissue culture facilities at the Plant Physiology Division, D.S.I.R.

Mr I. Warrington and his staff for assistance with the Climate Room facilities.

Mr A. Hardacre for assistance with the fluorometer.

Ph.D students; Kevin Kelliher, Robert Lai and Stuart Davis for advice on the SPSS, SPSS Graphics and Junior Word Processing Computer Programmes.

I am greatly indebted to my parents for the encouragement and support they have provided me to continue with my studies. To my wife Suzanne, my deepest appreciation for her understanding and support during the course of this project.

Godwin Balasingam

TABLE OF CONTENTS

Chapter			Page
		Abstract	
		Acknowledgements	
		Introduction	1
1		Crop: Asparagus officinalis L.	3
	1.1	Introduction	3
	1.2	Relevant Botanical Background	4
2		Herbicide: Bromacil	12
	2.1	History	12
	2.2	Chemical Formula	12
	2.3	Properties	12
	2.4	Persistence and Degradation in Soils	13
	2.5	Uptake, Translocation and Metabolism of Bromacil by Plants	16
	2.6	Mode of Action and Phytotoxic Responses to Bromacil	18
3		Materials and Methods	23
	3.1	Part I	23
	3.1.1	Objective	23
	3.1.2	Procedure for the Establishment of Aseptic Stock Plants	23
	3.1.3	Clonal Multiplication of MW500W Levin II clone	24
	3.1.3.1	The Basal Medium	24
	3.1.3.2	Clone Increase	24
	3.2	Part II	25
	3.2.1	Primary Objective	25
	3.2.2	Specific Objectives	25
	3.2.3	Materials and Methods	26
	3.3	Part III	27

	3.3.1	Objectives	27
	3.3.2	Materials and Methods	27
	3.3.3	Experiment 1	27
	3.3.4	Experiment 2	28
	3.3.5	Experiment 3	29
	3.4	Statistical Analysis	29
4		Results	30
	4.1	Part II: Broad Spectrum Dose-Response Experiments	30
	4.1.1	Phytotoxicity Observations	30
	4.1.2	Dose-Response Relationships	31
	4.1.2.1	Visually Assessed Foliage Damage Score	31
	4.1.2.2	Shoot Growth	33
	4.1.2.3	Shoot Death	34
	4.1.2.4	Root Fresh Weight	35
	4.2	Part III: Equal Increment Dose-Response Experiments	38
	4.2.1	Fluorometric Measurements	38
	4.2.1.1	Cladophyll tips from intact plants	38
	4.2.1.2	Cladophyll tips from excised shoots	39
	4.3	Chlorophyll Analysis	40
5		Discussion and Conclusion	43
	5.1	Discussion	43
	5.2	Conclusion	49
		Appendix A	50
		Appendix B	51
		Appendix C	52
		Appendix D	53
		Bibliography	54

LIST OF ILLUSTRATIONS

Table		Page
4.1	Time-course data for visually assessed damage score	33
4.2	Time-course data for dead shoots	35
4.3	Time-course data for fluorescence emission measurements of cladophylls from intact shoots	39
4.4	Time-course data for fluorescence emission measurements of cladophylls from excised shoots	40

FIGURES

		Page
1.1	Asparagus production in New Zealand	Facing 4
1.2	Rhizome growth, root and shoot development from bud clusters	5
1.3	Main events in the life cycle of *Asparagus officinalis* L. in New Zealand	8
2.1	Structural formula of bromacil	12
2.2	Degradation mechanisms for bromacil inactivation in the soil	15
4.1	Dose-response curve, bromacil effects on visually assessed damage score	Facing 31
4.2	Dose-response curve, bromacil effects on shoot growth	Facing 33
4.3	Dose-response curve, bromacil effects on shoot death	Facing 34
4.4	Dose-response curve, bromacil effects on root fresh weight	Facing 35
4.5	Dose-response curve, bromacil effects on fluorescence induction of cladophyll tips from intact shoots	Facing 38
4.6	Dose-response curve, bromacil effects on fluorescence induction of cladophyll tips from excised shoots	Facing 39
5.1	Schematic presentation of photoinduced electron transport system showing postulated site of action of bromacil	Facing 46

Plates		Between Page
3.1	Jar of stock plants	23-24
3.2	1-bud nodal segments	24-25
3.3	Asparagus plantlets after 10 weeks in culture	25-26
3.4	Asparagus plants established in soil	25-26
3.5	Asparagus plants in Climate Room	26-27
3.6	Kautsky apparatus (Model SF-10)	28-29
3.7	Aluminium plate with cladophyll tips	28-29
3.8	Excised shoots in flasks containing various concentrations of bromacil	29-30
4.1	Yellowing of foliage after treatment with bromacil	30-31
4.2	Bleached cladophyll tips after treatment with bromacil	30-31
4.3	Curling and twisting of new shoots	30-31
4.4	Representative samples of plants exposed to 0, 2, 4, 8 and 16 p.p.m. bromacil	30-31
4.5	Representative sample of plants exposed to 32, 64, 128, 256 and 512 p.p.m. bromacil	30-31
4.6	Root systems of asparagus plants treated with 0, 4 and 8 p.p.m. bromacil	30-31
4.7	Fluorescence induction curve on storage oscilloscope screen of untreated control sample	38-39
4.8	Fluorescence induction curve showing a decrease in induced rise in chlorophyll fluorescence due to bromacil damage	38-39

INTRODUCTION

During the past two or three decades we have seen a widespread acceptance of herbicides in agriculture, horticulture and forestry, and a rapid introduction of new chemicals and application techniques. In New Zealand some 123 different formulations and mixtures are now commercially available (O'Connor,1984) and the estimated expenditure on herbicides for 1984 was $8.4 million (Popay,1984).

In horticulture, a number of soil-applied herbicides have been widely used in most parts of New Zealand. One of these is bromacil. Bromacil (5-bromo-3-sec-butyl-6-methyluracil) is used for the long-term selective weed control of many annual and perennial weeds on asparagus plots in New Zealand. The herbicide is marketed under the trade name "Hyvar X" which contains 800g/kg (80%) of the active ingredient, bromacil. The manufacturer recommends that rates up to 3kg/ha of "Hyvar X" be applied on asparagus which has been established for at least 12 months, as a pre-emergence broadcast treatment before the harvesting season commences.

Since bromacil is a persistent, root absorbed broad spectrum herbicide and asparagaus is a perennial crop, the possibility exists for crop damage due to accumulatory effects. It is known that herbicides act differently under differing conditions. The soil texture (sand,silt,clay), amount of organic matter,climate (precipitation, temperature), all have a bearing on the effectiveness, residual life and safety of the chemical. In New Zealand many people have expressed doubts concerning the safety of bromacil on asparagus (Franklin, 1983).

Recommendations for soil-applied herbicides like bromacil are usually based on field tests carried out over a period of 2-3 years on a range of soil types, chosen to ensure that the conditions experienced will include the extremes encountered in commercial usage. In practice, reliability of the results, especially in respect to crop safety, is very dependent on environmental factors, especially rainfall. More recently, pot tests with plants grown in sand have been developed by researchers at the Weed Research Organisation at Oxford in England to provide more reliable information on crop tolerance to herbicides (Clay

& Davidson, 1978). Pot test tolerance studies on the effects of bromacil on asparagus, conducted under controlled environmental conditions, have not been reported.

The objectives of this research project are to study, under rigorously controlled conditions and using sand culture techniques developed at the Weed Research Organisation, the tolerance of asparagus to bromacil in terms of dose-response relationships. This study will be in three parts:

Part I: In vitro clonal propagation of asparagus plants for use in Part II and Part III of the project.

Part II: A broad spectrum experiment to investigate a wide range of herbicide concentrations to obtain a full range of responses and to determine the ED_{20} and ED_{50} phytotoxic limits. (ED_{20} or ED_{50}= "Equivalent Dose 20 or 50 ": herbicide concentration that causes 20% or 50% plant growth response compared to the untreated control. ED_{20} or ED_{50} values can be derived from dose-response curves).

Part III: Equal increment dose-response experiments around the ED_{20} and ED_{50} limits to determine the speed of action, degree of response and tolerance to increase in herbicide concentration.