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EFFECTS OF ENVIRONMENTAL FACTORS ON NUTRIENTS AND ANTINUTRIENT CONTENTS OF SELECTED LEAFY VEGETABLES

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAII IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN AGRONOMY AND SOIL SCIENCE

MAY 1986

By
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ABSTRACT

The purpose of this research was to test the hypothesis that plant composition in general, and plant antinutrient content in particular, are affected by environmental factors. To test this hypothesis three crops were grown in four benchmark locations which had been characterized for soils and provided with weather stations to monitor air and soil temperatures, relative humidity, rainfall, solar radiation and wind speed. The four experimental sites represented four soil series and two soil families. The Wahiawa and Lahaina soil series identified on sites on the Islands of Oahu and Molokai were members of the clayey, kaolinitic, isohyperthermic family of Tropeptic Eutrustox, whereas the Niulii and Kukaiau soil series identified on sites in the Kohala and Hamakua districts of the Big Island of Hawaii were members of the thixotropic isothermic family of Hydric Dystrandepts

Three test crops were used to test the hypothesis amaranth (Amaranthus gangeticus L), a crop cultivated for its tender leaves or grain, cassava (Manihot esculenta L), a crop normally cultivated for its starchy tubers, and taro (Colocasia esculenta L (Schott) a crop normally grown for its underground corms. The leaves of all three crops are consumed by people in the warm tropics. For this reason the leaves of all three crops were sampled and analyzed to measure the effects of soil and climate variables on oxalate intrate, and ionic contents of leaves

Amaranth experiments were installed at three sites. At each site irrigated and non-irrigated experiments were conducted. Within each

irrigation experiment, three fertilizer treatments consisting of (1) a basal treatment of lime, N, P, K, bases, and trace nutrients, (2) a N treatment superimposed on the basal treatment, and (3) a P treatment superimposed on the basal treatment, were arranged in a randomized complete block design with three replications. Plant tops were harvested at maturity for chemical analyses

Cassava leaves were sampled from ongoing experiments at the four experimental sites. Taro leaves were also sampled from ongoing experiments but from only three sites.

Soil and climatic factors significantly influenced the chemical compositions of crops. These effects differed for each crop. Nitrogen plays an important role in controlling the synthesis of oxalate and the accumulation of nitrate in amaranth. The highly variable oxalate and nitrate contents in plants grown in environmentally different sites were, to a large extent, due to different soil N contents.

Virtually all oxalate in cassava was in the form of calcium oxalate, so that tissue Ca content was an important factor in oxalate formation

In taro, K appeared to be the key factor accounting for the difference in oxalate content among sites

It was concluded from the results of this study that plant compositions can be controlled by management of the environment and crop
selection. It follows from this conclusion that the nutritional quality
of food crops can be measurably improved if more research is directed
towards achieving this goal

TABLE OF CONTENTS

ACKNOWLEDGME	NTS		111
ABSTRACT			11
LIST OF TABL	ES		x
LIST OF FIGU	RES		XVII
LIST OF ABBR	EVIATI	ONS	XX1
CHAPTER I	INTR	RODUCTION	1
CHAPTER II	REVI	EW OF LITERATURE	į
	2 1	Antinutrient oxalate and toxic agent nitrate in foods	Ę
	2 2	Oxalate accumulation in vegetables and other plants and its effects on the health of humans and animals	7
	2 3	Factors affecting oxalate contents in plants	12
		2 3 1 The concept of cation-anion balance	12
		2 3 2 Soil ionic environments	13
		2 3 3 Environmental factors	16
		2 3 4 State of growth or maturity	16
		2 3 5 Plant genetics	17
	2 4	Nitrate accumulation in vegetables and its effects on human and animal health	18
	2 5	Factors affecting nitrate accumulation in vegetables	22
		2 5 1 Nutrient supply	23
		2 5 2 Genetic factor	24
		2 5 3 Environmental factors	25
	2 6	Relationship between total N and nitrate-N concentration in plants	27
	2 7	Chemistry of oxalate with an emphasis on its biosynthesis in plants	28

	2 8	nitra	non between oxalate synthesis te accumulation and nitrogen olism in plants	32
	2 9	The u	se of amaranth cassava and taro	
		•	getables	37
		2 9 1	Distributions of crop and their uses	37
		2 9 2	Nutrition values and some limitations	39
	2 10	Estima	ation of oxalate contents	45
CHAPTER III	MATE	RIALS A	AND METHODS	47
	3 1	The ex	operimental site	47
	3 2	Crops		50
	3 3	Experi	ments	57
		3 3 1	Amaranth field experiments	57
		3 3 2	Cassava	63
		3 3 3	Taro	64
	3 4	Labora	tory Analyses	66
		3 4 1	Plant	67
		3 4 2	Soils	69
	3 5	Data a	nalysis	70
	3 6	Develo	pment of methods for oxalate tion, fractionation and	
			nation	72
		3 6 1	Extraction of total oxalate from plant tissue	72
		3 6 2	Extraction of oxalate fractions	73
		3 6 3	Preparation of the samples from the different oxalate fraction for	7.4
		3 6 4	HPLC analysis Description of oxalate determination	74
		3 6 5	by HPLC technique	75
		202	Comparison of the classical and HPLC methods of oxalate determination	77
		3 6 6	Oxalate recovery from the extraction and fractionation of plant	. .
			materials	84

enter enter enter enter enter en part enter enterente en

	3 7	specif enviro	nd weather characteristics measured ically for the study of agro-inmental effects on nutritional y of food crops	89
CHAPTER IV	RESU	LTS AND	DISCUSSION	97
	4 1	Growth	and development of amaranth	98
		4 1 1	Energy utilized by amaranth for emergence and growth	98
		4 1 2	Dry weight	100
		4 1 3	Moisture content of plant tissue	105
	4 2	Oxalat	e compositions of amaranth tissue	107
		4 2 1	Forms and amount of oxalate in amaranth	107
		4 2 2	Oxalate and calcium concentrations in amaranth tissue	116
		4 2 3	Comparison of chemical composition in plants grown at different sites	116
		4 2 4	Effect of irrigation on plant chemical composition	133
		4 2 5	Effects of N and P fertilizers on plant chemical composition	139
		4 2 6	Relationships between oxalate nitrate and agroenvironmental factors	155
	4 3	Respon factor	se of cassava to environmental s	186
		4 3 1	Forms and content of oxalate in cassava leaves	193
		4 3 2	Chemical compositions of plants grown in different sites	198
		4 3 3	Effects of irrigation on chemical compositions of plants grown in Molokai and Waipio sites	203
		4 3 4	Influence of plant soil and climatic factors on oxalate and nitrate	
			concentrations	204
	4 4	Respon	se of taro to environmental factors	209
		4 4 1	Forms of ovalate in tare	209

and Constitution of the same of

				1X
		4 4 2	Comparison of chemical composition of plants grown in different sites	215
		4 4 3	Relationships between oxalates in taro leaves and soil and climatic variables	223
CHAPTER V	SUM	MARY AND	CONCLUSIONS	226
APPENDICES				231
	Α	dependen	of variance of various it variables using combined amaranth experiments from tes	231
	В	variable	of variance of some dependent susing data of amaranth experiment plo site	246
	С	N concen	non matrix of oxalates and nitrate- etrations and various soil and evariables using data from non- ed plots of amaranth experiments at tes	253
	D	concentr variable	non matrix of oxalates nitrate-Neations and various soil and climatices using data from irrigated plots of experiments at three sites	264
	E		content of cassava leaves from rown in irrigated plots	279
	F	composit	non matrix of various plant nons and soil and climatic factors leaves from three experimental	
		sites	TEAVES ITOM CHIEF EXPERIMENTAL	280
REFERENCES				289

er en de la la description de la company de

THE THE PERSON OF THE PERSON O

LIST OF TABLES

Table		Page
2 1	Range of nitrate content of field-grown vegetables purchased in Columbia, Missouri	19
2 2	Nitrate-N contents of the leaves of some Amaranthus species	20
2 3	Uses and areas of origin of Amaranthus species	38
2 4	Composition of vegetable amaranth	41
2 5	Nutrient composition of taro leaves	44
3 1	Description of geographical positions and history of soil use of the experimental sites	51
3 2	Soil family properties as they relate to the taxa in Soil Taxonomy	53
3 3	Some physical and chemical properties of the soils at four experimental sites, Kukaiau, Iole, Molokai Waipio	54
3 4	Forms and rates of basal fertilizers and lime used in the amaranth experiments	59
3 5	Rates and timing of application of N and P fertilizers in addition to basal in amaranth experiments	60
3 6	Growing durations of amaranth grown in six experiments at three sites	62
3 7	Number of amaranth plants harvested from Iole, irrigated experiments	63
3 8	Planting date, sampling date and age for cassava crops at sampling	66
3 9	Planting date, sampling date and age of taro crop at sampling	67
3 10	Standard solutions of oxalic acid and oxalates	79
3 11	Relationships between oxalate ion (C ₂ O ₄ ²⁻)	81

Table		Page
3 12	Comparison of oxalate content determined by the classical and HPLC methods	83
3 13	Concentrations of internal standards for three forms of oxalate used in the recovery experiment	87
3 14	Solubility of magnesium oxalate in hot water	88
3 15	Recovery of water soluble water insoluble and total oxalate (Recovery I experiment)	88
3 16	Recovery of water soluble water insoluble and total oxalate (Recovery II experiment)	89
3 17	Chemical analysis of soils at three different experimental sites of amaranth experiments	91
3 18	Concentrations of some nutrients in soils at each experimental site of cassava and taro experiments	92
3 19	Soil chemical analysis results of irrigated and non-irrigated plots of three experimental sites	93
3 20	Weather characteristics during the growing period of amaranth grown at three sites	94
3 21	Weather characteristics during the period from planting to sampling of cassava grown at four sites	95
3 22	Weather characteristics during the period from planting to sampling of taro grown at three sites	96
4 1	Number of days and heat units (degree days) from date of planting to harvest in the amaranth experiments	100
4 2	Effect of irrigation treatment on dry weight of amaranth	102
4 3	Effects of fertilizers N and P on dry weights of amaranth in irrigated and non-irrigated plots at three sites	103
4 4	Tissue moisture contents of amaranth grown at different sites	105
4 5	Moisture content of amaranth grown at three different sites as affected by irrigation treatments	106

Table		Page
4 6	Correlation between soluble and insoluble fractions of oxalate with mineral composition of the plant tissue and of fractionation extracts of amaranth	108
4 7	Mineral concentrations in the (a) soluble and (b) insoluble fraction of extracts of oxalate in amaranth from all plots in three sites	114
4 8	Relative concentrations of different fractions of oxalate produced in amaranth grown at three sites	115
4 9	Comparison of the two means of total oxalate of amaranth by two different methods	115
4 10	Oxalate concentrations in amaranth grown at three sites	116
4 11	Oxalate production in amaranth grown at three different sites	118
4 12	Ionic concentrations and cation excess of amaranth grown at three sites	119
4 13	Ionic uptake by amaranth grown at three sites	120
4 14	Soluble oxalate concentration of amaranth grown in irrigated experiments of Iole and Waipio	122
4 15	Concentration of the sum of soluble and insoluble oxalate of amaranth grown in non-irrigated experiments of Kukaiau and Iole	122
4 16	Correlation between concentrations of different oxalate forms and cation excess	125
4 17	Proportion of total oxalate and the sum of soluble and insoluble oxalate relative to cation excess	130
4 18	Total N, nitrate-N concentration and crude protein content of amaranth grown in three sites	130
4 19	Total N and nitrate-N uptake by amaranth grown at three sites	131
4 20	Effect of irrigation on oxalate concentration of amaranth grown at three sites	134
4 21	Effects of irrigation on the production of oxalates in amaranth grown at three sites	136

		7111
Table		Page
4 22	Effect of irrigation on ionic concentration of amaranth grown at three sites	137
4 23	Effect of irrigation on ion uptake by amaranth grown at three sites	138
4 24	Effect of irrigation on total N and nitrate-N concentration of amaranth grown at three sites	139
4 25	Effect of irrigation on total N and nitrate-N uptake by amaranth grown at three sites	140
4 26	Effects of fertilizers on various forms of oxalate in amaranth grown at three sites	141
4 27	Correlation between N and P fertilizers with oxalates in irrigated and non-irrigated plots of amaranth	143
4 28	Effects of fertilizers on the production of various forms of oxalate in amaranth grown at different sites	144
4 29	Effects of N and P fertilizers applications on cation concentration in amaranth grown at three sites	148
4 30	Effects of fertilizers on anion concentrations in amaranth grown at three sites	150
4 31	Effects of fertilizers on cation uptake by amaranth grown at three sites	151
4 32	Effects of fertilizers on anion uptake by amaranth grown at three sites	152
4 33	Effects of N and P fertilizers on cation excess of amaranth grown at three sites	153
4 34	Effects of fertilizers N and P on total N concentrations and uptake in amaranth grown at three sites	155
4 35	Effects of N and P fertilizers on nitrate-N concentrations and uptake grown at three sites	156
4 36	Correlation between amaranth oxalates or nitrate content with air or soil temperatures at three experimental sites	157

		XVI
Table		Page
4 67	Correlation between taro oxalates and soil variables from three experimental sites	224
4 68	Correlation between taro oxalates and climatic variables from three experimental sites	225
5 1	Soil, climatic factors and cation excess correlated with the concentrations of oxalates and nitrate-N in amaranth, cassava and taro	228
5 2	Variables identified through multiple regression analysis to contribute significantly to the oxalate and nitrate contents of amaranth	229

LIST OF FIGURES

Figures					
The four proposed pathways to oxalate (1) from phosphoglycolate generated in RuBPo reaction, (2) from glyoxylate produced in the isocitrate lyase reaction, (3) from oxaloacetate via oxaloacetate lyase (4) from ascorbate by oxidation	30				
Cropping patterns for Tropeptic Eutrustox sites (a) and Hydric Dystrandepts sites (b) showing locations of cassava () and taro () rows and sampling locations for cassava (x) and taro (•)	65				
HPLC chromatograms illustrating the resolution of nitrate (upper) and oxalate peaks and the difference between spiked (right) and non-spiked samples * RT = Retention time in minutes	78				
Relationship between oxalate concentration and peak height	82				
4 l Effect of irrigation x fertilizer interaction on dry weight of amaranth grown at Waipio site	104				
Relationship between soluble oxalate and K concentration in the extract of soluble oxalate fraction (hot water extract) of amaranth tissue	109				
Relationship between soluble oxalate and the sum of potassium and magnesium concentration in the extract of soluble oxalate fraction (hot water extract) of amaranth tissue	110				
Relationship between insoluble oxalate and Ca concentration in the extract of insoluble oxalate fraction of amaranth tissue	111				
Relationship between insoluble oxalate and the sum of Ca and Mg concentration in the extract of insoluble oxalate fraction of amaranth tissue	112				
4 6 Comparative concentration of insoluble oxalate and tissue Ca in amaranth grown at three sites	117				
4 7 Relationship between total oxalate and cation excess in amaranth from all plots of the experiments at three sites	126				

AN TON THE PARTY OF THE PERSONAL PROPERTY OF THE PARTY OF

F	igure		Page
4	8	Relationship between the sum of soluble and insoluble oxalate and cation excess in amaranth from all plots of the experiments at three sites	127
4	9	Relationship between total oxalate and cation excess in amaranth from Waipio site	128
4	10	Relationship between the sum of soluble and insoluble oxalate and cation excess in amaranth from Waipio site	129
4	11	Effect of irrigation x fertilizer interaction on total oxalate concentration of amaranth grown at Waipio site	145
4	12	Effect of irrigation x fertilizer interaction on total N uptake of amaranth grown at Waipio site	146
4	13	Relationship between tissue total oxalate of amaranth and soil nitrogen from experiments at three sites	161
4	14	Relationship between tissue total oxalate of amaranth and soil Ca from experiments at three sites	162
4	15	Relationship between tissue total oxalate of amaranth and soil Mg from experiments at three sites	163
4	16	Relationship between tissue total oxalate of amaranth and soil K from experiments at three sites	164
4	17	Relationship between tissue total oxalate of amaranth and solar radiation from non-irrigated plots of experiments at three sites	165
4	18	Relationship between tissue total oxalate of amaranth and fertilizer P rate from non-irrigated plots of experiments at three sites	168
4	19	Relationship between tissue total oxalate of amaranth and solar radiation from irrigated plots of experiments at three sites	171
4	20	Relationship between tissue total oxalate of amaranth and fertilizer N rate from irrigated plot of experiments at three sites	172
4	21	Relationship between tissue soluble oxalate of amaranth and fertilizer P rate from non-irrigated plots of experiments at three sites	174

Figure		Page
4 22	Relationship between tissue soluble oxalate of amaranth and soil Ca from non-irrigated plots of experiments at three sites	175
4 23	Relationship between tissue soluble oxalate of amaranth and minimum air temperature from irrigated plots of experiments at three sites	177
4 24	Relationship between tissue soluble oxalate of amaranth and fertilizer P rate from irrigated plots of experiments at three sites	178
4 25	Relationship between tissue insoluble oxalate of amaranth and fertilizer P rate from non-irrigated plots of experiments at three sites	180
4 26	Relationship between tissue insoluble oxalate of amaranth and average air temperature from irrigated plots of experiments at three sites	182
4 27	Relationship between tissue nitrate-N concentration of amaranth and fertilizer N rate from non-irrigated plots of experiments at three sites	184
4 28	Relationship between tissue nitrate-N concentration of amaranth and wind speed from non-irrigated plots of experiments at three sites	185
4 29	Relationship between tissue nitrate-N concentration of amaranth and soil Mg from non-irrigated plots of experiments at three sites	187
4 30	Relationship between tissue nitrate-N concentration of amaranth and soil K from non-irrigated plots of experiments at three sites	188
4 31	Relationship between tissue nitrate-N concentration of amaranth and fertilizer N rate from irrigated plots of experiments at three sites	190
4 32	Relationship between tissue nitrate-N concentration of amaranth and soil N from irrigated plots of experiments at three sites	191
4 33	Relationship between tissue nitrate-N concentration of amaranth and minimum air temperature from irrigated plots of experiments at three sites	192
4 34	Relationship between total oxalate and Ca in the extract of insoluble oxalate fraction of lear tissue of cassava grown at four sites	195

Figure	Page
4 35 Relationship between insoluble oxalate and Ca in the extract of insoluble oxalate fraction of leaf tissue of cassava grown at four sites	196
4 36 Relationship between total oxalate concentration and cation excess in leaf tissue of cassava grown at four sites	201
4 37 Relationship between Ca and total N concentration in leaf tissue of cassava grown at four experimental sites	206
4 38 Relationship between insoluble oxalate and calcium in the extract of insoluble oxalate fraction of leaf tissue of taro grown at three sites	211
4 39 Relationship between insoluble oxalate and the sum of calcium and magnesium concentration in the extract of insoluble oxalate fraction of leaf tissue of taro grown at three sites	212
4 40 Relationship between soluble oxalate and potassium concentration in the extract of soluble oxalate fraction (hot water extract) of leaf tissue of taro grown at three sites	213
4 41 Relationship between soluble oxalate and the sum of potassium and magnesium concentration in the extract of soluble oxalate fraction (hot water extract) of leaf tissue of taro grown at three sites	214

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LIST OF ABBREVIATIONS

(for Appendices)

AIR1, AIRMAX Maximum air temperature

AIR1SQ Square of maximum air temperature

AIR2, AIRMIN Minimum air temperature

AIR2SQ Square of minimum air temperature

AIR3, AIRAVE Average air temperature

AIR3SQ Square of average air temperature
AIR4 Difference between maximum and minimum

air temperature

AIR4SQ Square of difference between maximum and

minimum air temperature

CA Calcium concentration

CANUPT Anion uptake
CATUPT Cation uptake
CAUPT Calcium uptake
CC-A, C-A Cation excess

CL Tissue chloride concentration

CLUPT Chloride uptake

CNO3 Tissue nitrate concentration

CNO3UPT Nitrate uptake DW Dry weight

K Tissue potassium concentration

KUPT Potassium uptake

MG Tissue magnesium concentration

MGUPT Magnes ium uptake

NA Tissue sodium concentration

NAUPT Sodium uptake

NO3N Tissue nitrate-N concentration

P Tissue phosphate PUPT Phosphate uptake

OX Total oxalate concentration
OXPRO Total oxalate production
OX2 Soluble oxalate concentration
OX2PRO Soluble oxalate production

OX2-3 Sum of soluble and insoluble oxalate concentration OX2-3PRO Sum of soluble and insoluble oxalate production

OX3 Insoluble oxalate concentration
OX3PRO Insoluble oxalate production

RF Cumulative rainfall Square of rainfall

RHAVE RH3 Average relative humidity RHMAX, RH1 Maximum relative humidity RHMIN RH2 Minimum relative humidity

RH4 Difference between maximum and minimum

relative humidity

RH1SQ RH2SQ RH3SQ RH4SQ	Square of maximum relative humidity Square of minimum relative humidity Square of average relative humidity Square of the difference between maximum and minimum relative humidity
SOLCA	Soil calcium content
SOLK	Soil potassium content
SOLMG	Soil magnesium content
SOLNA	Soil sodium content
SOLP	Soil phosphate content
SOLTN	Soil total nitrogen content
SOL1	Maximum topsoil temperature
SOLISQ	Square of maximum topsoil temperature
SOL2	Minimum topsoil temperature
SOL2SQ	Square of minimum topsoil temperature
SOL3	Average topsoil temperature
SOL3SQ	Square of average topsoil temperature
SOL4	Difference between maximum and minimum topsoil temperature
SOL4SQ	Square of the difference between maximum
	and minimum topsoil temperature
SOL5	Maximum subsoil temperature
SOL5SQ	Square of maximum subsoil temperature
SOL6	Minimum subsoil temperature
SOL6SQ	Square of minimum subsoil temperature
SOL7	Maximum subsoil temperature
SOL7SQ	Square of maximum subsoil temperature
SOL8	Difference between maximum and minimum
	subsoil temperature
SOL8SQ	Square of the difference between maximum
•	and minimum subsoil temperature
SR	Solar radiation
SRSQ	Square of solar radiation
SSAVE	Average subsoil temperature
SSMAX	Maximum subsoil temperature
SSMIN	Minimum subsoil temperature
SUPT	Sulfate uptake
TN, TOTALN	Tissue total nitrogen
TNUPT	Total nitrogen uptake
TSAVE	Average topsoil temperature
TSMAX	Maximum topsoil temperature
TSMIN	Minimum topsoil temperature
WIND, WSP	Wind speed
WSPSQ	Square of wind speed

CHAPTER I

INTRODUCTION

Agricultural practices and research have been geared toward high production to cope with the high rate of increase in world population. The genetic characteristics that have led to yield improvements were mainly dwarfism and leaf erectness in cereals, such as wheat and rice. These key genetic characteristics gave birth to the green revolution in the 1960s. A third characteristic photperiod insensitivity made it easy to fit the short, erect high yielding varieties into a wide range of environments. Other genetic traits including resistance to diseases and insects, and tolerance to environmental stress continue to increase yields.

Besides genetic factors, cultural factors are also another means employed by agronomists to increase yields. These include fertilizer application and irrigation

Low consumer acceptance of several promising high yielding varieties compelled plant breeders to include factors that affect consumer preference into their breeding strategy. In addition, some cultural practice has been implemented with the dual purposes of increasing yield and improving quality with regard to consumer preference for example heavy applications of nitrogen fertilizer in order to increase yields and to produce green and succulent produce

Quality is therefore often measured in terms of consumer

Preference However, preference alone does not ensure food quality

The nutritional value of an agricultural product is not always apparent

to the consumer More elusive quality factors are the antinutrient and toxic agent contents of food crops. Their effects on the consumer are often slow and insidious

Food compositions tables are used indiscriminantly to identify and quantify the nutrients present in various foodstuffs, in spite of their knowledge of the high variability in nutrient contents among cultivar of food crops and among similar crops grown in different places. The source of variability could be genetic and/or environmental and cultural. With regard to environmental factors, growers need to know the environmental factors that affect food quality.

This study was based on the hypothesis that variance in nutritional quality of food crops is strongly influenced by cultural and environmental factors

The type of food crops selected for this study were leafy vegetables because they are important sources of oxalate (an antinutrient) and nitrate (a toxic agent) which are nutritionally significant

Vegetables are important sources of vitamins and minerals essential for humans and animals. Some of them are also an unconventional source of protein recommended for the tropics where meat is relatively scarce. However some vegetables, such as amaranth (Amaranthus species), cassava leaves (Manihot species), taro leaves (Colocasia species) purslane (Portulaca oleracea L.) and ungchoi (Ipomoea aquatica) of the tropics, and spinach (Spinacea oleracea L.), rhubarb (Rheum rhaponicum) beet (Beta vulgaris) of the temperate regions contain high levels of antinutrient oxalate. Oxalate is considered an antinutrient because it can render some mineral nutrients unavailable by binding them to form

insoluble salts which are not absorbed by the intestine. Many vegetables also contain high content of nitrate which is a toxic agent to humans and animals

The main interest in oxalate is its complex formation with calcium (Ca) to form insoluble Ca-oxalate both within plants and the human body. This precludes the utilization of Ca. Furthermore, high level of soluble oxalate and free oxalic acid can combine with Ca from other foods, further reducing Ca availability in diets.

There exist relationships between oxalate and nitrate contents in plants and factors of the agroenvironment. These include soil nutrient status, temperature, soil moisture, and intensity and duration of radiation. Such knowledge has prompted the idea of reducing the content of these two compounds in plants by manipulating agroenvironmental factors. The most widely researched factor is perhaps plant nutrient supply. For example, different forms of nitrogen fertilizers affect the oxalate and nitrate contents in plants differently. While nitrate fertilizer tends to increase oxalate and nitrate contents in plants, ammonium fertilizer tends to decrease them

Cation-anion balance is a sound concept to explain the presence of oxalate in plants. It was proposed that organic acids (including oxalate) were produced to balance excess cations. In this way, any external factors which can exert influence on the content of cation excess in plants should in turn affect oxalate content.

Amaranth, taro and cassava chosen for this study contain high levels of oxalate and are widely consumed as vegetables in the tropics Amaranth is also gaining popularity in the continental U.S. as a leafy vegetable

This study was undertaken to investigate the effects of agroenvironmental factors on the production of oxalate and nitrate in these tropical
vegetables in the hope that the additional knowledge and understanding
gained would lead to ideas as to how these factors can be managed to
produce high quality vegetables with regard to their contents of oxalate
and nitrate. The objectives of this study are, therefore, as follows

- l To assess the contribution of soil and climate variables to the variance in oxalate and nitrate contents of food crops
- 2 To ascertain the relationship between cation-amon balance and the biosynthesis of oxalate
- 3 To identify practical measures to control oxalate and nitrate contents of food crops

CHAPTER II

REVIEW OF LITERATURE

This study draws upon two disciplines—agronomy and human nutrition—Agronomy deals with understanding and manipulating genotype by environment interactions to optimize food production whereas human nutrition deals with understanding and optimizing human health through nutrition—The relationship being considered is primarily between agroenvironment and the nutritional quality of food crops—Inferences are made on the relationship between plant, and man to some extent

Oxalate and nitrate content, as well as the content of other nutrients of leafy vegetables were chosen as plant parameters that may be affected by soil and climatic factors. Oxalate and nitrate were selected for study because they are an antinutrient and a toxic agent respectively to humans. They are also present in many leafy vegetables consumed in the tropics. Moreover, earlier studies indicate that there is a definite relationship between plant tissue nitrate content and agroenvironment. Comparable studies on oxalate, on the other hand are scarce.

The background information forming the basis for this study is presented and includes the relationships among agroenvironmental factors and oxalate and nitrate contents in plants, the mechanism of oxalate synthesis, and the accumulation of oxalate and nitrate in vegetables

2 l Antinutirient oxalate and toxic agent nitrate in foods

Antinutrients are chemical substances (natural or synthetic) that can inhibit nutrients from performing their normal functions

Antinutrients can be divided into 3 groups according to the mechanisms by which they inhibit nutrient function

- 1 Those that bind nutrients chemically and make them unavailable for absorption by the digestive system. Examples of antinutrients are oxalate which binds Ca and phytate which binds trace elements, such as Fe. Zn and Cu. These antinutrients lower the nutritional quality of food with respect to minerals, because the nutritional quality is determined by the proportion of absorbable and utilizable essential elements in a meal (Mertz, 1980, Quarterman, 1973 as cited by Welch and House, 1984)
- 2 Those that compete with nutrients for intestinal absorption sites
- 3 Those that compete with nutrients for biological (enzymic) reaction sites. For example, antivitamins such as isoniazid (a tuberculosis drug), which competes with pyridoxine (vitamin B_6)

Antinutrients that bind nutrients (group 1) do so in the food before it is ingested and/or in the body. Oxalic acid belongs to this group of antinutrients

Oxalic acid $(H_2C_2O_4)$ is a relatively strong dicarboxylic acid. Like other acids oxalic acid can be converted into salts. This property is important for this study because oxalic acid becomes an antinutrient when it forms insoluble salts with nutrients such as Ca and Mg. Hodgkinson (1977) has described the properties of various oxalate salts in detail. Of particular interest are the oxalate salts of Ca. Mg. K. Na and ammonium (NH₄) that are frequently encountered in plants.

Oxalic acid forms neutral and acid salts with monovalent cations including ammonium. With most divalent cations it forms only one salt Potassium forms three salts, a neutral and acid salt and a tetraoxalate (Hodgkinson, 1977)

Except for the salts of the alkalı metals (L1, Na, K), ammonium, and Fe III, most oxalates are sparingly soluble in water. The most soluble oxalate salt associated with a bivalent metal is magnesium oxalate (MgC $_2$ O $_4$) and the least soluble among the common oxalates are calcium oxalate (CaC $_2$ O $_4$) and lead oxalate (PbC $_2$ O $_4$). All are soluble in strong acids (Hodgkinson, 1977). Knowledge of the solubility of oxalates is important for developing techniques to fractionate oxalates in plants

Oxalic acid becomes an antinutrient when it forms insoluble salts with Ca and Mg. However, the potential of it becoming an antinutrient is high when it is in the form of free acids or soluble salts. Therefore, assessing food quality with respect oits oxalate contents involves fractionating and identifying the kind of salts and/or free oxalic acid a food contains.

Nitrate (NO_3^-) is considered a toxic agent because even though nitrate itself presents no health hazard to human and animal, its derivatives, namely nitrite (NO_2^-) and nitrosamine—are health hazards. The effects of nitrate on health are discussed in section 2.4

2 2 Oxalate accumulation in vegetables and other plants and its effects on the health of humans and animals

Oxalate exists in plants mainly as water soluble acid soluble (water insoluble) and free acid forms. The water soluble forms include

sodium- potassium- and ammonium oxalate and the water insoluble forms include calcium- and magnesium oxalate Hodgkinson (1977, p. 130) has reviewed the various forms of oxalate in plants. For example, the oxalate in Begonia semperflorens exists almost entirely as free acid, and sodium salts predominate in Halogeton glomeratus Potassium salts predominate in Oxalis, Rumex, and several Nigerian vegetables, such as Talmium triangulare (Oke 1969) Ammonium oxalate appears to be the principal form in the tropical grass, Setaria sphacelata and calcium oxalate is found in plants such as beet, spinach, and buckwheat Marderosian et al (1980) discovered that almost half of the oxalate in amaranth is in the soluble form. Singh and Saxena (1972) found both soluble and insoluble forms of oxalate in six leafy vegetables consumed in India (i e , amaranth [Amaranthus gangeticus L], bathua [Chenopodium album L] kharbathua [C murale L], purslane [Portulaca oleracea L] spinach [Spinacea olearacea L] and beet [Beta vulgaris L]) correlations were found between the concentration of soluble oxalate and K and Na, and between insoluble oxalate and the sum of Ca and Mg Sap pH has been used to identify high oxalate-producing plants, as sap pH correlates well with the form of oxalate (James 1972 p 232) Two categories of plants are recognized

- Plants such as Oxalis pes-caprae and some species of Rumex which have a sap pH of about 2 in which case the oxalate anion is present chiefly as the acid oxalate ($HC_2O_4^{-1}$)
- 2 Plants of some Chenopodiacae, which have a sap pH of about 6 indicating that the oxalate is present chiefly as the oxalate anion $({\rm C_2O_4}^{2-})$

According to James (1972), the oxalate in the first category of plants is present chiefly as potassium acid oxalate, and the oxalate in the second category is present mainly as soluble sodium oxalate and the insoluble calcium and magnesium oxalates

One of the plants with the highest known oxalate content is Halogeton glomeratus (Chenopodiaceae) This plant contains as much as 30% oxalate on a dry weight basis. Another high oxalate plant is Oxalis cernua (Oxalidiaceae) (Fassett, 1973) In spinach the total oxalate content ranges on a dry weight basis from 5 42° in savoyed-leaf variety to 9 81% in smooth leaf variety (Kitchen et al., 1964a) Fassett (1973) summarizes oxalate contents in various plants, expressed as percent on a fresh weight basis. The contents range from 0 3-1 2% in spinach, 0 2-1 3% in rhubarb, 0 3-0 9% in beet leaves, 0 3-2 0% in tea Suvachittanont et al (1973) determined the and 0 5-0 9% in cocoa oxalic acid content of some vegetables from Thailand and reported oxalic acid contents of 3 3% on a dry weight basis (DW) or 0 4% on a fresh weight basis (FW) for Amaranthus gangeticus Linn and 0 6% DW or 0 1% FW for cassava leaves According to Standal (1982), the oxalate content of taro leaf (Colocasia esculenta var Bun-long) was 0 5% FW

The oxalate content of plants is generally higher in the leaves than the stalk (Fassett 1973) as has been demonstrated in amaranth (Der Marderosian et al. 1980) taro (Standal, 1983) and spinach (Kitchen and Burns 1965). Kitchen and Burns (1965) found only trace amounts of oxalic acid in spinach seed. They stated that oxalate concentration decreased with increasing distance from the leaves, which is assumed to be the site of oxalate production.

The probable detrimental effects of oxalic acid and oxalate on human health are as follows

- l According to Suvachittanont et al (1973) ingestion of dilute oxalic acid solution produces little gastrointestinal distress but may cause weakness, muscular twitchings, sometimes convulsions, coma or death
- 2 It has also been suggested that oxalic acid might play a role in cardiovascular disease (Anderson et al., 1971)
- 3 A harmful effect, particularly in relation to calcium utilization, has been demonstrated for dietary oxalate. For example, Pingle and Ramasastri (1978) showed that in humans, the availability of Ca from a diet of amaranth leaves was very low relative to that from milk In addition, the intake of amaranth leaves together with milk adversely affected the absorption of milk Ca
- 4 Excessive intake of oxalates in the diet is an ethological factor for the formation of renal and urinary calculi. Renal and urinary calculi are known to contain oxalate, especially Ca oxalate. In an area in Thailand where urinary bladder stone disease is endemic, local vegetables and forest plants commonly consumed by villagers of all ages contained very high levels of oxalic acid (Valyasevi and Dhanamitta, 1974). Oxalate consumption in Rajasthan, India has been suggested as a cause of the incidence of renal calculi (Singh et al., 1972).

The different forms of oxalate have different implications to human nutrition. Insoluble calcium oxalate precludes the utilization of calcium in the intestine. Soluble oxalate and free oxalic acid can combine with calcium from other foods and thus reduce calcium availability. Hodgkinson has cited work which shows that oxalic acid and

soluble oxalates interfere with the intestinal absorption of calcium and that calcium oxalate is poorly utilized (Hodgkinson 1977 p. 207)

Oxalate poisoning in animals, especially ruminant animals is welldocumented There is ample evidence that many pasture plants and weeds containing oxalate, are poisonous to animals James (1972) gave a thorough and comprehensive review of incidences of oxalate poisoning in animals with an emphasis on oxalate toxicosis. According to James (1972), oxalate poisoning in livestock results primarily from the ingestion of plants. She gave examples of plants that contain dangerously high levels of oxalate (10% or more anhydrous oxalic acid on a dry weight basis) including Halogeton glomeeratus, greasewood (Sarcobatus vermiculatus) and sugar beet (Beta vulgaris) of the Chenopodiaceae family, soursobs (Oxalis pes-caprae) and the sorrels of the Oxalidaceae family, and certain Rumex species, and docks of the Polygonaceae family Grasses also produce oxalate, but they do so at a lower level than the previously listed forbs and shrubs In the tropical grass Setaria (Setaria sphacelata), in which oxalate poisoning of grazing animals has been reported the oxalate contents of some varieties reach values as high as 4-5% on a dry weight basis (Jones Pigweed (Amaranthus retroflexus) leaf contains oxalate et al . 1970) content as high as 12-30% on a dry weight basis (Marshall et al., 1967) Poisoning and sometimes death have been reported in swine (Osweiler et al., 1969 as cited by James 1972) and cattle (Brown 1974 Stuart et al 1975) ingesting this plant

According to James (1972) most oxalate-producing plants are palatable to livestock, and therefore the husbandry of ruminants with access to these plants becomes critical. The extent of damage to

livestock can be extensive A case where 1200 sheep were poisoned of which 100-800 died, has been reported Deaths in numbers less than a hundred are common (James, 1972)

James (1972) has described in detail the symptoms of oxalate poisoning and its accompanying pathological changes. There appears little doubt that if intake of oxalate from forage exceeds the capacity of rumen decomposition, sheep, cattle, swine and horses may develop serious (acute and chronic) symptoms accompanied by deposition of calcium oxalate crystals in various tissues. Furthermore, she pointed out that the response of animals to oxalate intoxication varied with animal species as well as with species of plant consumed. This is an indication that the kind and amount of oxalate found in plants affect the health of consumers.

2 3 Factors affecting oxalate contents in plants

Factors such as soil fertility, light intensity season, temperature, plant age, and genotype influence the oxalate content of plants. These factors can be grouped into broader categories--soil, agroclimate, genetic make up of plants, and stage of growth

2 3 1 The concept of cation-anion balance

The concept of cation-anion balance is the most widely used explanation for the variation in the organic acid concentration of plants (Kirkby and Mengel, 1967 Kirkby 1969 Ismandji and Dijkshoorn 1971, Breteler 1973, Dijkshoorn 1973 Nelson and Selby, 1974 Kirkby and Knight 1977 Peck et al. 1980 Israel and Jackson 1982). The concept is based on the assumption that in higher plants the sum of cation including Ca. Mg, K and Na (denoted by C) and expressed as cmol.

 kg^{-1} (me 100 g^{-1}) of dry weight minus the sum of inorganic anion concentrations including NO_3^- , $H_2PO_4^ SO_4^{-2}$ and Cl^- (denoted by A), represents the cations excess (C-A) that should occur as the salts of organic acids (Dijkshoorn, 1962)

The following is an example to illustrate this concept
The results of plant analyses show that

- Sum of cations $(Ca^{2+}+Mq^{2+}+K^{+}+Na^{+}) = 180 \text{ cmol kg}^{-1}$
- Sum of unassimilated anion concentrations $(H_2PO_4^{-1}+SO_4^{-2}+NO_3^{-1}+CI^{-1}) = 70 \text{ cmol kg}^{-1}$
- Excess cations = $180 70 = 110 \text{ cmol kg}^{-1}$
- Sum of organic anion concentrations

 (e g malate citrate fumarate,
 succinate, malonate, quinate
 oxalate and polyuronate = 110 cmol kg⁻¹

In some plants, such as those in the family Chenopodiaceae and some tropical grasses, oxalic acid predominates (Dijkshoorn, 1973, Zindler-Frank, 1976), but in most other plant species malic acid dominates (Dijkshoorn 1973) About 10-20% of (C-A) is polyuronic acid in cell walls and the remainder being other organic acids (Dijkshoorn 1973)

2 3 2 Soil ionic environments

Soil fertility exerts some effects on oxalate levels of some plants High soil fertility associated with high soil carbon nitrogen available phosphorus and potassium is associated with high total oxalate in Amaranthus and Basella (Schmidt et al. 1971). However differences among species were much greater than differences due to soil fertility.

Other studies have shown the existence of a relationship between calcium and oxalate contents in plants Rasmussen and Smith (1961) demonstrated a positive correlation between calcium and oxalic acid in Valencia orange leaves Olsen (1939) suggested that calcium absorption from the soil is related to oxalic acid production in plants Brumagen and Hiatt (1966) found that oxalate interfered with calcium translocation and utilization in tobacco plants. They measured more oxalic acid in tobacco varieties that were susceptible to calcium deficiency than in non-susceptible varieties. De Kock et al. (1973) studied the effect of oxalate on the absorption of calcium in Lemna gibba L and demonstrated that Ca uptake and oxalate production were stimulated in each other's presence. They suggested that oxalate acts as a carrier for calcium in the absorption process and is subsequently reduced and condensed with coenzyme A to form malic acid However. some contradictory evidence has been presented by Osmond (1967) who found that in Atriplex (Australian salt bush) calcium absorption and oxalate synthesis varied independently, and that oxalate content was generally correlated with a high cation content as a whole rather than with the calcium alone

Since liming is a common practice in crop production, further studies on the effects of liming on oxalate content in plants should indicate if certain modification in this practice needs to be made to obtain a desirable crop quality with regard to oxalate content

Smith (1972) showed that a positive correlation exists between potassium applied and oxalate level in the grass <u>Setaria sphacelata</u> Moreover, significant relationships between oxalic acid and cation excess values were shown suggesting that accumulation of oxalic acid

was the result of high application rates of potassium which in turn affected the cation excess

Soil pH affects the level of oxalic acid. Wadleigh and Shive (1939) found that the total organic acid content, including oxalic acid, increased in corn plants as substrate pH was increased.

Nitrogen in various forms exert profound effects on oxalate contents in plants. The forms of nitrogen fertilizers investigated were mainly ammonium and nitrate (e.g., Kirkby, 1968, Kirkby and Knight, 1977), and in some investigations, urea (e.g., Kirkby and Mengel 1967). Vityakon 1979). Generally nitrate resulted in a higher oxalate content than ammonium. This observation has been explained in terms of cationanion balance. In general, nitrate fertilizer results in higher cation excess than ammonium. Therefore, one recommended method of reducing the oxalate content in plants is to stabilize ammoniacal fertilizers with a nitrification inhibitor.

The effects of phosphorus and zinc fertilizers on oxalate contents of plants have been investigated by Peck et al (1980). In table beets, which is a well-known oxalate accumulator, there was a clear relationship between increasing rates of phosphorus application and reduced oxalate levels in blades, petioles, and roots. These workers suggested that phosphorus application lowered oxalic acid by reducing cation concentrations and also by lowering cation excess. Zinc fertilizer did not appear to have any significant effects on oxalic acid in table beets. They discussed the implication of these results from a human nutrition point of view and speculated that phosphorus fertilization of table beets might result in more available calcium.

2 3 3 Environmental factors

Research on environmental factors affecting plant chemical composition is sparse and sometimes contradictory. Singh and Saxena (1972) stated that environmental factors, such as light intensity season and temperature influenced the synthesis of oxalic acid in plants. Eheart and Massey (1962) conducted a greenhouse experiment to study the effects of environmental factors on the oxalate content of spinach and concluded that plant variety was the only factor that had any significant effect on oxalic acid content. Light intensity, soil moisture, and all interactions did not affect the oxalate content of spinach.

Kitchen et al (1964b) found that high temperatures decreased oxalates in spinach leaves and postulated that at high temperatures more oxalic acid was used as a respiratory substrate. Gnedkov (1963 as cited by Singh and Saxena, 1972) observed an increase in oxalic acid concentration in some succulent plants maintained in the dark at a low temperature.

The effect of soil moisture on organic acid content in plants was clearly demonstrated in tomato fruits by Lee and Sayre (1946). Citric acid contents were lower in tomatoes grown on wet soils than on dry soils.

2 3 4 Stage of growth or maturity

The literature presents contradictory conclusions on the effect of stage of growth or maturity on the level of oxalate in plants

Kitchen and Burns (1965) observed that both total and soluble oxalates

In spinach decreased with age. However, some other scientists reported

increases in oxalic acid contents in spinach with the development of the plant (e.g., De Vilmorin and Bilques, 1957. Doesburg and Sweede 1948 as cited by Kitchen and Burns, 1965). Der Marderosian et al (1980) demonstrated that oxalate levels in chard, spinach and amaranth tended to increase with plant age. The same trend was also found by Singh and Saxena (1972) in the leaves of six leafy vegetables including amaranth (Amaranthus gangeticus L.), bathua (Chenopodium album L.) kharbathua (C. murale L.), purslane (Portulaca oleracea L.) spinach (Spinacea olearacea L.) and beet (Beta vulgaris L.). Furthermore the fractions of soluble and insoluble oxalate increased in leaves and decreased in stems with amaranth age. However, Kitchen and Burns (1965) reasoned that the discrepancy was due to a combination of growth stage and season.

2 3 5 Plant genetics

Eheart and Massey (1962) reported significant varietal differences in the oxalate content of spinach even though the range in oxalate content of 12 varieties was only 10 30-11 38% on a dry weight basis. Kitchen et al. (1964a) related the oxalate contents of different spinach varieties according to leaf morphology. Total oxalate content ranged from 5 42% on a dry weight basis in savoyed-leaf varieties to 9 81% on a dry weight basis in smooth-leaf varieties.

Different varieties of the tropical grass, <u>Setaria sphacelata</u> also contain different levels of oxalate. For example, the Kazungula and Bua River cultivars have 5 0% and 4 1/ total oxalate respectively on a dry weight basis (Jones et al. 1970). Amaranth varieties exhibit

differences in oxalate content as shown by Der Marderosian et al. (1980)
These varietal and species differences offer a basis for selection and
breeding work to lower oxalate contents in food crops

2 4 Nitrate accumulation in vegetables and its effects on human and animal health

Many plant species accumulate nitrate if nitrate is supplied in excess of N requirement. Nitrate levels in plants cannot be measurably reduced without reducing yields (Lorenz, 1978). Nitrate accumulates in plants when uptake rate exceeds reduction and assimilation rates. Such plant nitrate represents a major source of nitrate-N in human diets (Mills et al., 1976). Vegetables such as beets (Beta vulgaris L.), spinach (Spinacea oleracea L.), broccoli, celery (Apium graveolens), lettuce (Lactuca sativa L.), radishes (Raphanus sativus L.), kale (Brassica oleracea) mustard greens (Brassica juncea) collard (Brassica oleracea var acephala) (Table 2.1) and Amaranthus species (Table 2.2) may accumulate large quantities of nitrate

Nitrate distribution is not uniform in the plant. In general nitrate levels are highest in petioles and stems moderate in leaves and roots and very low in fruit and flowers (Maynard and Barker 1972 Lorenz, 1978)

Levels of nitrate and nitrate derivatives in foods should be considered in evaluating potential health hazards in foods and feeds

Nitrate itself is non-toxic to man and animals being readily absorbed and excreted but its derivatives (i.e. nitrite and nitrosamine) are toxic

Table 2 l

Range of nitrate content of field-grown vegetables purchased in Columbia, Missouri

Crop	Nitrate-N (% dry weight)
Beans	0 04 - 0 25
Beets	0 09 - 0 84
Broccoli	0 01 - 0 09
Brussel sprouts	0 01 - 0 06
Cabbage	0 01 - 0 09
Carrots	0 00 - 0 30
Cauliflower	0 00 - 0 31
Celery	0 11 - 1 12
Corn	0 00 - 1 01
ucumber	0 00 - 0 16
indive	0 06 - 0 67
.ettuce	0 02 - 1 06
Parsnip	0 00 - 0 04
Peas	0 00 - 0 02
Radish	0 41 - 1 54
Spinach	0 07 - 0 66
iquash yellow	0 09 - 0 43
Tomato	0 00 - 0 11

Source Brown and Smith 1967 as cited by Lorenz 1978

Table 2 2
Nitrate-N contents of the leaves of some Amaranthus species

ec1es	Nitrate-N (% dry weight)
A blitum	0 21
cruentus	0 74
dubius	0 27
gangeticus	0 36 - 0 90
hypochondriacus	0 65

(after Der Marderosian et al , 1981)

Nitrate is reduced to nitrite in humans and animals in their gastrointestinal tract. Vegetables represent a major source of nitrate in human diets. Nitrate in some vegetables (e.g., lettuce and spinach) has been reported to be converted to nitrite during storage (Gersons 1974)

Nitrite reacts with hemoglobin to form methemoglobin and causes impairment to oxygen transport (Methemoglobinemia). In humans when the methemoglobin concentration exceeds 70%, asphyxia occurs. At lower levels the reaction is reversible. Apparently the reaction of nitrite with hemoglobin is inconsequential in adults, but it can be critical in infants (Committee on Nitrate Accumulation, 1972, Wolff and Wasserman, 1972). In cattle, abortion was associated with a methemoglobin level of 40% (Wolff and Wasserman, 1972).

Nitrosamines are toxic derivatives of nitrate and nitrite as well as of secondary and tertiary amines. Nitrosamines may have carcinogenic teratogenic (impaired development of embryo foetus) and mutagenic (cause of chromosomal aberation and gene mutation) properties. Nitrosamines occur in foodstuffs such as cooked bacon polluted air water, and tobacco plants (Committee on Nitrate Accumulation 1972). Nitrosamines precursors may be converted to nitrosamines in the stomachs of mammals (Deeb and Sloan, 1975).

Lorenz (1978) has reviewed various works concerning the potential toxic level of nitrate for humans. Sollman (1957) has given 0.70 g of nitrate-N as a possible single lethal dose for humans. This result agrees with that proposed by Brown and Smith (1967), who stated that an adult weighing 70 kg would need to ingest approximately 0.7 to 1.0 g of nitrate-N for a toxic dose. Deeb and Sloan (1975) set the lethal dose at 18 to 68 mg nitrate-N kg⁻¹ of body weight for adults. The lethal dose for nitrite was 22 to 33 mg nitrite-N kg⁻¹. The U.S. Public Health Service (1962) suggested limits and ranges of nitrate-N in food and vegetables are as follows. (1) zero for squash and tomatoes for strained baby foods and 833 ppm nitrate for spinach. (2) 50 ppm (0.005%) for asparagus (dry weight) and 3600 ppm (0.36%) for spinach (dry weight)

Toxic levels of nitrate for animals vary greatly and depending upon other factors. Lorenz (1978) has reviewed this subject and it appears that a level of 0 20% nitrate-N on a dry weight basis is potentially dangerous in livestock. The University of Missouri (1958) has suggested that hay with less than 0 08% nitrate-N is safe for cattle

while levels above 0 21% may result in death. They suggest that the nitrate-N intake for a cow should not exceed 12 g per day. The minimum lethal dose of nitrate is about 15 g per 45 4 kg of animal weight. Deeb and Sloan (1975) list levels of 75 to 140 mg nitrate-N kg $^{-1}$ of body weight as the toxic level for cattle and slightly lower values for sheep

Sources of nitrate for animals include a wide range of pasture plants and green feeds (e.g., barley, wheat, corn, sugar beet, sunflower, turnips, pigweed, thistle, lamb siquarter, bindweed, nightshade ragweed, certain algae, and sometimes alfalfa). Animal processed food, such as soybean meal and molasses from cane and sugar beets also contain nitrate. Nitrate-accumulating plants become the source of nitrite for animals when the plant materials are bruised and acted upon by plant enzymes or bacteria. Moreover, any nitrate-containing food or feed processed by bacterial fermentation may, at some stage, contain appreciable amounts of nitrite. Thus, silage prepared with water high in nitrate, commonly contains some nitrite, especially in the early stages of fermentation (Committee on Nitrate Accumulation 1972)

2 5 Factors affecting nitrate accumulation in vegetables

Two opposing processes regulate nitrate accumulation by plants nitrate uptake and nitrate assimilation. Any factors that exert influence on these processes will, in turn, affect nitrate accumulation. Before nitrate can be assimilated it must first be reduced to nitrite then to ammonium. The first enzyme which catalyzes this process is nitrate reductase.

2 5 1 Nutrient supply

Available soil nitrogen is the most important factor in nitrate accumulation in plants (Maynard and Barker 1972). Nitrate in plants is derived primarily from soil organic matter, plant residues, manures and fertilizer nitrogen. The amount, source, time method of application, and numerous environmental conditions govern the effects of N fertilizers on nitrate accumulation in vegetables (Maynard et al., 1976).

Increasing rates of N application increase the nitrate concentration of vegetables (e.g., spinach [Barker et al., 1971], table beets [Peck et al., 1971] and many other vegetables [Lorenz, 1978])

Due to mineralization and nitrification, nitrate is the primary N form absorbed by most plants regardless of the type of N applied Therefore, within limits, as much nitrate may be accumulated from organic or ammoniacal fertilizers as from nitrate fertilizers if sufficient time is allowed for mineralization and nitrification to occur (Peck et al., 1971). Usually, however, nitrate accumulates less from ammoniacal and urea fertilizers than from nitrate sources (Barker et al., 1971, Peck et al., 1971, Lorenz and Weir, 1974).

Nitrate concentration in plants is also affected by the timing of N application. For example, spinach and table beets (Barker et al. 1971, Peck et al., 1971) accumulated more nitrate when they were exposed to the same quantity of nitrate fertilizer earlier and for longer periods (i.e., broadcast application as opposed to side-dressing one week before harvest)

The accompanying cation influences nitrate absorption and hence
its accumulation by plants. Ammonium ion suppressed nitrate absorption

more than Ca, K. Na, or Mg ions (Minotti et al., 1969). There is much evidence to show that increasing K supply increases nitrate accumulation (Barker 1962, Minotti et al., 1968). Solution culture studies have shown that K generally stimulates nitrate absorption with respect to the effects of other cations (Minotti et al., 1968, Tottingham et al., 1934). Potassium has also been found to be the most regular counterion for transport of nitrate ion from root to shoot (Locher and Brouwer 1964, 1965, Louwerse and Alberda, 1965, Minotti et al., 1968).

Phosphorus supply does not have a strong effect on nitrate accumulation by vegetables. Cantliffe (1973) did not find any influence of fertilizer P on nitrate concentration in spinach and table beets. Brown and Smith (1967) in their field study on 30 species of vegetables showed that low P levels had little effect on nitrate levels in plants. On the other hand. Baker and Tucker (1971) reported that the addition of P reduced the amount of nitrate-N in wheat

2 5 2 Genetic factor

Nitrate accumulation is a function of variety in spinach (Barker et al , 1974), and lettuce (Maynard et al , 1976). Savoyed-leaf spinach varieties tend to accumulate more nitrate than smooth-leaf varieties, and semi-savoyed leaf types fall between them (Barker et al 1974). Differences in nitrate accumulation may be related to differences in uptake, assimilation or translocation. Olday et al. (1976) observed that nitrate reductase activity in smooth-leaf spinach was higher than in savoyed-leaf types. It appears that differential assimilation is the primary cause for the observed differences in spinach varieties.

The lettuce variety that accumulated least took up N most (Maynard et al., 1976)

Maynard et al (1976) suggested that the ratio of nitrate-N to total N could be taken as an indirect measure of assimilatory capacity. This ratio varied greatly among varieties

2 5 3 Environmental factors

Environmental factors which affect nitrate accumulation in plants include light intensity, photoperiod and light duration water relation, and temperature

Cantliffe (1972b and 1972c) observed that increased nitrate reductase activity associated with high light intensity and long light duration decreased nitrate accumulation in spinach and other vegetables. The need for light to activate nitrate reductase results in a diurnal fluctuation in nitrate concentration. More recent work on spinach by Steingrover et al. (1982) on spinach gave similar results. A low-light' treatment, where plants were exposed to low light intensity throughout the night as opposed to normal darkness, seemed to affect nitrate reduction rather than nitrate uptake and transport. Nitrate reductase activity in the leaf blades of the low light treated plants was about twice that in the dark-treated' plants. It was suggested that a low-light' treatment one night before the harvest may provide a way to lower the nitrate content of commercially grown vegetables.

Shading increased nitrate-N content in tall fescue (Festuca arundinacea Schreb) a nitrate accumulating forage crop (Stritzke et al 1976) Death of cattle grazing on tall fescue was attributed to nitrate poisoning. This study showed that 30% shade and high N

fertility increased the nitrate-N content of tall fescue significantly over those that received less shade and lower N

Moisture stress leads to excessive nitrate accumulation in forage (Wright and Davison, 1964) Water stress affects general assimilatory processes in plants and reduces nitrate reductase activity (Huffaker et al. 1970), so that nitrate assimilation is depressed. A more recent study by Leclerc and Robin (1983) showed that if soil water content of his test sample was near or below 2% marram grass (Ammophila arenaria L.) diminished its assimilation activity and stored nitrate. Above 2% soil water content, the nitrate reductase activity increased and the endogenous nitrate was consumed. Several other processes in soils and plants that are moisture-dependent such as microbial activity movement of N to absorbing roots, and transport in the xylem appear to increase nitrate levels in plants (Wright and Davison, 1964).

Maynard et al (1976) speculated on the possible effects of humidity and, thus transpiration, on nitrate accumulation. They postulated that reduced humidity and increased transpiration rates were involved in maintaining a continuous movement of nitrate to reduction sites, thereby helping to maintain nitrate reductase and keeping accumulated nitrate low. The authors postulated that high humidity might enhance nitrate accumulation and suggested that the higher nitrate in glasshouse plants relative to plastic-house plants (10% lower light intensity in plastic house) might not be due to the light intensity effect but rather to the higher humidity in glasshouses

There is considerable evidence to show that high temperatures increase nitrate content in various plants such as barley (Hordeum vulgare L) (Onwueme et al. 1971) lettuce (Frota and Tucker, 1972)

corn (Zea mays L) (Mattas and Pauli, 1965), pasture plants (Bathurst and Mitchell, 1958), and spinach (Cantliffe, 1972a). Cantliffe (1972a) attributed this effect to deactivation of nitrate reductase and also to higher soil availability of nitrate resulting from increased microbial activity at high temperatures.

According to Maynard et al (1976) a general statement about the effects of temperature on nitrate accumulation should not be made. because N absorption, translocation and assimilation are all affected by temperature. The relative degree to which each process was affected depended on other factors Factors such as light, moisture, and N availability are important which could in turn be markedly affected by temperature They cited examples of the effects of the interactions of two factors on N accumulation Cantliffe (1972a) noted that nitrate accumulation in spinach was affected by the interaction of temperature and amount of N applied At zero fertilizer N, nitrate accumulation did not begin until temperature was greater than 15° C, but at 50° and 200 mgN kg⁻¹ soil, nitrate accumulation began at 10° C and 5° C respectively Hoff and Wilcox (1970) reported effects of temperature and light interaction on nitrate accumulation in tomatoes (Lycopersicon esculentum Mill) They found that temperature exerted its greatest effect (highest nitrate accumulation) at high N and low light and that light exerted its greatest effect at high temperature and high N

2 6 Relationship between total N and nitrate-N concentrations in plants

Terman et al (1976) have shown that total N and nitrate-N concentrations in plants are related. In pot and nutrient solution experiments, nitrate-N accumulation by corn commenced when total N

exceeded 2 5% in the leaves and 1 5% in the stems. In spinach and mustard nitrate-N concentration increased rapidly when total N exceeded 4 0% but approached zero below 4 0%. Nitrate-N in fescue grass was less than 0 1% up to 3% of total N and beyond that increased linearly with total N. The workers concluded from these studies that

- l The occurrence of high levels of total and nitrate-N during early growth depended on the abundance of available soil and fertilizer N $\,$
- 2 As plant growth continued, total and nitrate-N decreased as a result of dilution and assimilation. Such decreases occurred with age and in response to P, K, and other factors
 - 3 As total N decreased all forms of unassimilated N were depleted
- 4 Except during the early growth stage high nitrate-N concentrations in plants are usually the result of diminished growth due to unfavorable conditions such as drought, low or high air temperatures, poor light together with continued uptake of nitrate-N
- 5 If the rate of plant growth was higher than that of N absorption much of the nitrate would be incorporated into proteins. However, low concentrations of nitrate-N (< 0.05%) can remain in conductive tissues

2 7 Chemistry of oxalate with an emphasis on its biosynthesis in plants

Franceschi and Horner (1980) have reviewed studies on the synthesis of oxalic acid in high oxalate plants such as rhubarb buckwheat

Begonia Oxalis, Atriplex Setaria spinach and beetroot According to Raven et al (1982) there are four major pathways of oxalate

biosynthesis Interestingly, all four pathways have been suggested for spinach. The four pathways are shown in Figure 2 1 and include

- l Formation of glyoxylate from glycollate by the action of glycollate oxidase, followed by oxidation of the glyoxylate to oxalate by action of the same enzyme
- 2 Formation of glyoxylate from isocitrate by the action of isocitrate lyase, followed by oxidation of glyoxylate to oxalate
- 3 Formation of oxalate from oxaloacetate by the action of oxaloacetate lyase
 - 4 Formation of oxalate from C_1 and C_2 of L-ascorbate

The first 2 mechanisms have glyoxylate as the immediate precursor of oxalate Glyoxylate is oxidized to produced oxalate (reaction)



Mechanism 1 appears to have some connection with photosynthesis or more precisely, photorespiration. The conversion of glyoxylate to oxalate occurs in green tissue and is facilitated by light (Sengupta and Sen 1976). Both photorespiration and oxalate synthesis via mechanism 1 use the same substrate glycollate with subsequent formation of glyoxylate.

Mechanism 2 is contrastingly different from mechanism 1 because oxalate is synthesized in the dark 3-carboxylation of phosphoenol pyruvate (PEP) provides the basis of a non-crassulacean acid metabolism common in plant tissue. This process is also called dark CO₂ fixation

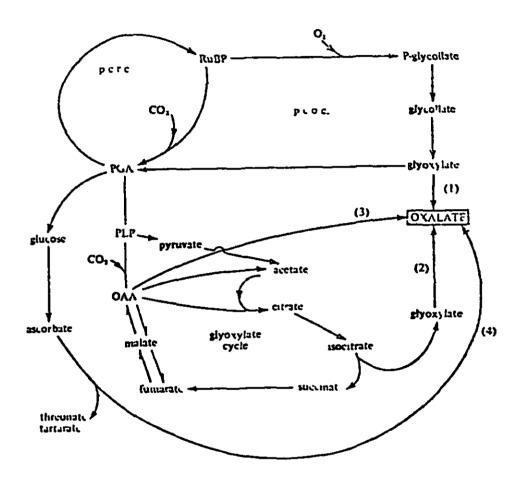


Figure 2 1 The four proposed pathways to oxalate (1) from phosphoglycolate generated in the RuBPo reaction (2) from glyoxylate produced in the isocitrate lyase reaction, (3) from oxaloacetate via oxaloacetate lyase (4) from ascorbate by oxidation OAA = Oxaloacetic acid, p c o c = photosynthetic carbon oxidation cycle, p c r c = photosynthetic carbon reduction cycle PEP = phosphoenol pyruvate PGA = 3-phosphoglycerate P-glycollate = phosphoglycollate (Source Raven et al 1982)

because it does not require light. The products of this CO_2 fixation are C_4 compounds which can enter the tricarboxylic acid (TCA) cycle to be metabolized with subsequent energy generation. According to Osmond and Avadhani (1968) oxalate is not an early product of carboxylation reactions but is synthesized from intermediates of subsequent TCA cycle metabolism. The TCA cycle intermediate that is a likely oxalate precursor is isocitrate, which gives rise to glyoxylate by the action of isocitrate lyase.

Mechanisms 3 and 4 do not have glyoxylate as the substrate for oxalate. Chang and Beevers (1968), who discovered mechanism 3 indicated that oxalate was produced as a by-product of acetate utilization in the TCA cycle. Oxalate synthesis involves the cleavage of C_4 oxaloacetate to yield acetate and oxalate. The enzyme which catalyzes this reaction is oxaloacetate lyase.

Mechanism 4 uses L-ascorbic acid as a precursor of oxalic acid. This mechanism has been found in many oxalate accumulating plants, such as Rumex crispus L (curly dock), Amaranthus retroflexus L (red root pigweed), Chenopodium album L (Lamb s quarters). Beta vulgaris L (sugar beet), Halogeton glomeratus M Bieb (halogeton) and Rheum rhabarbarum L (rhubarb) (Nuss and Loewus, 1978), and also in species with low oxalate content. such as barley (Wagner. 1981). It was found by labelling studies that C_1 and C_2 fragments of ascorbic acid give rise to the carbons in oxalic acid (refer to the structure of the acid below) (Nuss and Loewus, 1978). More biochemical work needs to be done to establish the enzymic basis of this conversion and to determine the site of the activity.

L-ascorbic acid

Recent work on the subject of oxalate biosynthesis by Raven et al (1982) uses non-radioactive isotopes, $^{18}O_2$, ^{12}C , and ^{13}C , to investigate the relative significance of the four mechanisms of oxalate biosynthesis. Their results indicate that the role of mechanism 1 is not important for oxalate synthesis in spinach. This mechanism is the only one that requires light or in other words, is the only one that occurs in green tissue. Earlier work has shown that organic acid or carboxylate synthesis is connected to dark $^{CO}O_2$ fixation (e.g. Osmond and Avadhani 1968. Chang and Beevers. 1968)

2 8 Relation between oxalate synthesis nitrate accumulation and nitrogen metabolism in plants

There are connections between oxalate synthesis instrate accumulation, and nitrogen metabolism in plants. As mentioned in section 2.5 instrate accumulation depends upon the rate of nitrate uptake and the rate of nitrate assimilation. Nitrates accumulate in plant tissue when the rate of nitrate assimilation ceases or is slow. Nitrate enters

the protein metabolism pathway after it is reduced to ammonia. The connection between protein metabolism and carboxylate (organic acid) synthesis will be described

Studies on nitrate and oxalate accumulations on different spinach varieties have shown that there is a relationship between leaf type and nitrate and oxalate accumulations. Savoyed-leaf spinach cultivars tend to accumulate the most nitrate while smooth-leaf spinach are able to maintain low nitrate concentrations. The semi-savoyed leaf type behave in an intermediate manner (Barker et al., 1974, Maynard and Barker, 1974). On the other hand, Kitchen et al. (1964a) found the savoyed-leaf types to have the lowest oxalate content and smooth and semi-savoyed leaf types to have the highest content. While this can be taken as evidence of the connections between nitrate accumulation, N metabolism and oxalate accumulation, it is not a direct one.

Studies on the role of carboxylic acids in nitrogen metabolism up to the mid 1960s were reviewed by Beevers et al (1966). The effects of ammonium and nitrate ions on organic acid contents have been observed in various plants (Beevers et al., 1966, p. 231). The acid content increases in plants treated with ammonium or nitrate nitrogen relative to the untreated ones, and the increase is greater in nitrate-fed plants than ammonium-fed plants (Beevers et al., 1966, Kirkby and Mengel, 1967 Kirkby, 1969. Kirkby and Knight. 1977, Vityakon. 1979). Similar response to ammonium and nitrate has been found in oxalate accumulating plants such as spinach and beetroot (Vityakon. 1979).

According to Beevers et al. (1966) ammonium and nitrate utilization impinged directly upon the metabolism of carboxylic acids. Work in the 1970s and 1980s has used isotopically labelled ${\rm CO_2}$ to examine

the effects of ammonium and nitrate nutrition on dark ${\rm CO}_2$ fixation. Ikeda and Yamada (1981) investigated the effect of ammonium and nitrate nitrogen on dark ${\rm CO}_2$ fixation in tomato leaves. Dark ${\rm CO}_2$ fixation involves the enzyme phosphoenol pyruvate carboxylase which catalyses the following reaction.

PEP + ${\rm CO_2}$ \longrightarrow OAA + ${\rm P_1}$ (2) phosphoenol pyruvate oxaloacetate inorganic P Ammonium-fed plants were remarkably less able to fix ${}^{14}{\rm CO_2}$ in the dark compared with nitrate-fed plants. Furthermore, the phosphoenol pyruvate carboxylase activity of ammonium-fed plants was lower than that of nitrate-fed plants. Fixed ${}^{14}{\rm CO_2}$ was more rapidly utilized for amino acid synthesis in ammonium-fed plants than in nitrate-fed plants.

More than 50% of total organic acids in tomato consist of malic acid. Not only is malic acid synthesized through the TCA cycle in mitochondria but it is also produced by the carboxylation of C_3 compounds catalyzed by soluble enzymes in the cytoplasm (i.e. oxaloacetate produced in reaction 2 can be metabolized to malate by the enzyme malate dehydrogenase). Inference can also be made concerning the synthesis of oxalate from the oxaloacetate precursor produced in reaction 2 along the pathway of mechanism 2 or 3

Hammel et al (1979) investigated the effect of ammonium on carbon metabolism in isolated mesophyll cells of <u>Papaver somniferum</u> L.

Ammonium chloride solution was used as the nitrogen source. Addition of ammonium ion to isolated mesophyll cells resulted in an increase in the rate of dark. ¹⁴C fixation relative to that in untreated cells. The most rapid increase in labelling the metabolite occurred in

aspartate and was accompanied by a decrease in the level of labelled phosphoenol pyruvate. This indicates that ammonium stimulates phosphoenol pyruvate carboxylation in this system.

Possible effects of ammonium on glycolysis and respiratory metabolism were investigated by Hammel et al (1979). The level of labelled pyruvate did not change nor were there effects of ammonium on the pyruvate kinase reaction in the dark (reaction 3). Such results indicate that ammonium is not important to the reaction.

PEP pyruvate kinase enol pyruvate ———> pyruvate (3)

However Ikeda et al. (1974) showed that there was an enhancement of glucose degradation (glycolysis) and respiratory metabolism (TCA cycle) in the leaves of tomato plants supplied with high levels of ammonium nitrogen for seven consecutive days. They suggested that the enhanced respiration in ammonium-fed plants is due to assimilation/detoxification of $NH_4^{-1}-N$. This reaction keeps the levels of $NH_4^{-1}-N$ and organic acids low. The organic acids are replenished mainly by intermediates in glycolysis and the TCA cycle.

Paul et al (1978) reported the enhancement of both pyruvate kinase and phosphoenol pyruvate carboxylase in isolated mesophyll cells of Papaver somniferum L supplied with ammonium and light. They concluded that the net effect of an addition of ammonia to mesophyll cells was a redistribution of newly fixed carbon away from carbohydrates and into amino acids.

In a study of effect of ammonium on dark CO_2 fixation products of Euglena Peak and Peak (1980) measured an overall increase in

glutamine and the amount of $^{14}\text{CO}_2$ fixed. Both were heavily labelled Furthermore, they postulated with some supporting evidence that the labelled CO_2 entered the amino acids via the TCA cycle by way of the carboxylation of phosphoenol pyruvate

Peak and Peak (1980) reported that ammonium stimulation caused increased incorporation of labelled CO_2 into some amino acids not derived from TCA cycle intermediates including for example glycine serine, and alanine, which are all derived from the glycolytic pathway Since it is known that <u>Euglena</u> possesses the glyoxylate shunt enzymes isocitrate lyase and malate synthase, these researchers suggested that it should be feasible that CO_2 could enter glycine and serine via the TCA cycle intermediates, oxaloacetate and isocitrate, which are glyoxylate precursors

Although the studies presented so far do not directly relate nitrogen to oxalate synthesis, the connections of these nitrogen-related blochemical processes to oxalate metabolism can be seen. The process of dark CO₂ fixation, which involves the enzyme phosphoenol pyruvate carboxylase and the product oxaloacetate is a direct precursor of oxalate formation, also oxaloacetate can lead to the synthesis of isocitrate, which can lead to the synthesis of glyoxylate by the catalysis of isocitrate lyase. Furthermore, these studies have thrown some light on the long-observed phenomenon of a relatively higher organic acid content (including oxalate) in nitrate-fed plants compared with ammonium-fed plants.

2 9 The use of amaranth, cassava, and taro as vegetables

Amaranth, cassava, and taro have been consumed in the tropics since Amaranth was an important grain crop of the Aztecs and the Incas (National Research Council 1984) Taro is grown throughout the humid tropics for edible corms and leaves as well as for traditional ceremonial uses (Wang, 1983) Although widely used, the term 'underexploited crop has been applied to amaranth and taro (National Academy of Science, 1975) because they are relatively unknown to the industrial-In contrast, in the industrialized world key food crops have been bred to suit large scale monoculture farming systems with consequent high yields for economic purposes. From this point of view, cassava is different from amaranth and taro Cassava is a well-known root crop, now grown on a large scale and is an important commodity in international trade Much research work has been done on cassava, for example at the Centro Internacional de Agricultura Tropical (CIAT) ın Columbia The use of cassava leaves is relatively unknown although they are rich in protein

2 9 1 Distributions of the crops and their uses

Amaranth seeds and leaves are used for human consumption. Principal uses and areas of origin of many Amaranthus species are summarized in Table 2.3. Amaranth grows in many regions of the world. As far as the edible vegetative parts (i.e., leaves and young shoots) are concerned. Amaranthus species such as A. tricolor. A. dubius. and A. cruentus. are grown for their value as soup vegetables or for boiled salad greens particularly in the hot, humid regions of Africa. Southeast Asia (especially Malaysia and Indonesia) southern China.

Table 2 3
Uses and areas of origin of Amaranthus species (after Teutonico and Knorr, 1985)

			• • •				
Species	How found	Use ———————	Area of origin				
A blitum (A	Cultivated	Vegetable,	Asıa				
lividus, A		ornamental					
oleraceus)							
A caudatus	Cultivated	Grain	South America				
A edulis		vegetable,	(Andes)				
(<u>A mategazzianus</u>)		ornamental					
A cruentus (A	Cultivated	Grain, vegetable	South America				
paniculatus)			(Guatemala)				
A dubius	Weed,	Vegetable	South America				
	cultivated						
A hybridus	Weed	Vegetable	South America				
A hypochondriacus	Cultivated	Grain, vegetable	North America				
(A leucocarpus,			(Mexico)				
A leucosperma,							
A flavus)							
A retroflexus	Weed	Vegetable	North America				
A spinosus	Weed	Vegetable	Asıa				
A tricolor (A	Cultivated	Vegetable	Asıa				
gangeticus, A							
mangostanus)							
A viridis (A	Weed	Vegetable	Africa				
ascendens, A							
gracilis)							

India, and the Caribbean A palmeri is a major wild green for the Indians in the North American deserts, and A blitum leaves have been a favorite salad in Greece since ancient times (National Research Council, 1984) Amaranthus species along with other wild plants are used by the Tanzanians as relishes that are an integral and essential part of the diet in all seasons of the year (Fleuret, 1979)

Taro (<u>Colocasia esculenta</u> (L) Schott) is a member of the Araceae family Primarily it is a root crop but leaves are also consumed extensively in Africa, Asia, the West Indies, and South America However, taro consumption is greatest in the Caribbean, Hawaii the Solomon Islands, American Samoa, Western Samoa, the Philippines, Fiji, Sri Lanka, India, Nigeria, Indonesia, New Hebrides, Tonga, Niue, Papua New Guinea, and Egypt Young taro leaves are an important vegetable throughout Melanesia and Polynesia (Wang, 1983)

Cassava (Manihot esculenta L) is one of the most important root crops of the world. The root is the fourth most important energy staple of the tropics, providing food and income for 750 million people in three continents of Africa, Asia and America

The consumption of cassava leaves as a vegetable is widespread among the native peoples of many Asian countries such as Malaysia, Indonesia, and Thailand The cyanide content of cassava leaves precludes their use as a vegetable in Nigeria (Oke, 1968)

2 9 2 Nutritional values and some limitations

Considerable work has been done in India on evaluating the nutritional value of amaranth. Many species of amaranth are used as greens in India and their nutritional value to low income groups is well known According to Rao et al (1980), amaranth is considered the king of vegetables with regard to its nutritional value and its low price. Like many other green leafy vegetables in India, amaranth is 'within reach of the poor man'. Much of the research has been focused on the use of amaranth as a food supplement. For example, Devadas et al. (1964) investigated the use of amaranth and other green leafy vegetables as supplements for rice-based diets. They found that amaranth and vegetable supplements improved the calcium status of the body as indicated by a positive calcium balance and higher calcium levels in the bones of experimental albino rats. In addition, the overall growth of animals on a diet that included amaranth was improved.

Amaranth, like other green leafy vegetables, is an important source of vitamins A and C, which are of considerable nutritional significance (Table 2 4) (National Research Council, 1984, Teutonico and Knorr, 1985) In places like Kenya amaranth also provides a cheap source of vitamin C relative to more expensive sources like citrus (Abe and Imbamba, 1977)

With regard to minerals, amaranth has high iron and calcium contents relative to other greens such as spinach, Malabar spinach (Basella) and chard (National Research Council, 1984). Guttikar et al. (1966) also reported that amaranth greens (A. tricolor) was a very rich source of magnesium when compared with other Indian foodstuffs including pulses, leafy vegetables, other vegetables and fruits. Data reported by Teutonico and Knorr (1985) confirm that amaranth contains potassium, iron, magnesium and calcium in significant concentrations (Table 2.4)

Protein is another important nutrient of amaranth greens. Its leaf can be used in the wet tropics to ameliorate protein shortages (Pirie

Table 2 4 Composition of vegetable amaranth

										Co	ntent												
Analysis	A cruent	u s	edu	A lís	hypoch	A ondriacus	retro	A if 1exi	us 1	A tric		cal	A idat	u \$	spio	A nost	ıs	viri	A idis	graeci	l Zans	hybr	A idus
Moisture										85	7	70	19	0 9	79	0 1	33 0	94	0	84	0		
Crude protein (%N x 6 25 dm)	20 9 3	3 0	18	0	21	6	21	1 21	2	32	7	17	4 2	9 7	28	4 :	31 0	3 (3	23	2	22	1 33
Total lipidș ^a	1 58	67	1	60	1	96	1	58		3	5 10 6	1	0 2	8	1	8	5	1	7	1	7 3 2	1	3 6 !
Crude fiber a	8 6 1	3 1	12	4	11	8	13	1 13	5	7	0	5	4 9	2	9	4		13	3 3	14	5	10	5 24
Crude ash ^a	16 1 2	16	22	0	21	2	20	4 22	2			19	3 2	1 0	22	1				21	7	7	6 19
Na Na Ka												0	01	0 04	. 0	01				0	01		
K ^a	296	t	3	7	5	6	5	4 5	6			0	3 5	0	0	3				0	2		
Ca ^a	192	6	6	2	2	6	3	0		3	5	2	3 2	6	1	1	1 8	7	2 3	ι	8	3	3
Mq	1 1 2		2	2	ł	2	1	4 1	7	1	7	1	1 1	3	2	2				1	5		
Mg Fe ^a	0 02		0	02		02	0	04 0	05			0	02		0	01			008	3		0	00
Zn ^a	0 05		0	002	0	004	0	005	0 00	8													
Zn P	0.4		0	3		5		3 0		0	7	0	3 (4	0	4							
Riboflavin b										2													
Niacin										8	4(0 8C	4	7		7	7				6	2		
Ascorbic acid											3(55°)				25			1	47	•	-	30	48 ^C
Thiamin ^C											02		-			-		•	• -			30	••
carotene											000	25	400)	54	11	0	41	3 000)			
(1U/100 g d	ωì										710 ^d }				- •	- •		•					

a % dry matter b mg/ 100 g dry matter c mg/ 100 g fresh weight d g/ 100 fresh weight

1966) Leaves of this and other specific tropical plants contain protein of high quality (Akeson and Stahmann, 1965. Gerloff et al., 1965 as cited by Madamba, 1972). Attempts to promote the use of leaf protein have been underway for some time in the tropics. In Nigeria the traditional corn grinding mill was used to extract leaf protein from vegetables such as Amaranthus caudatus (Olatunbosun, 1976). Adding approximately 10 g of leaf protein a day to the low protein diet of children suffering from protein-calorie malnutrition resulted in significant clinical improvement.

Research on the production of protein concentrate from amaranth leaves (e.g., Carlsson, 1980 in Sweden, Cheeke et al., 1981) has been designed to make proteins in green plants available to non-ruminants because leaf protein concentrate no longer has the cellulose and fiber that is indigestable to non-ruminants. Amaranthus species contain large amounts of extractable protein (Lexander et al., 1970), but protein concentrates from amaranth have not improved growth of experimental rats. Cheeke et al. (1981) postulated that poor growth may be associated with a high ash content and organic substances such as saponins phenolics, and oxalates. These factors need to be overcome before amaranth or its protein concentrate can be utilized successfully

The lysine content of amaranth is high. This amino acid is frequently deficient in cereal-based diets (Oliveira and de Carvalko 1975, Kamath and Sahonie, 1959). Oliveira and de Carvalko (1975) reported that in Mozambique, \underline{A} spinosus leaves had lysine contents as high as 5.2°. The benefit of supplementing a maize meal with \underline{A} spinosus was evidenced by the fact that the chemical score of the

mixture was raised to 43, compared to 28 for maize meal alone and 32 for amaranth leaves alone

The National Research Council (1984) has reported leaf-protein levels (on a dry weight basis) as follows 27% for A blitum, 28% for A hybridus, 30% for A caudatus, 32% for A gracilis, 23% for A gracilis, 23% for A gracilis and 28% for A spinosus The range of protein content in various species of amaranth is presented in Table 2.4

Taro leaves

Young leaves and petioles of taro are used as vegetables. They are useful sources of vitamin A and vitamin C (Table 2.5). Taro leaves are used extensively for cooking in Pacific islands such as Hawaii Fiji, Samoa and Tonga. Taro leaves are also rich in mineral calcium and potassium (Table 2.5) (Standal, 1983). Crude protein contents are comparable to those of amaranth and cassava as shown in Table 2.5.

Cassava leaves

Cassava leaves are rich in protein, carotenes, vitamins B_1 , B_2 and C, and minerals (Oke, 1968). Rogers and Milner (1963) reported that the protein content of cassava leaves ranged from 17.8 to 34.8% on a dry weight basis for Brazlilian varieties and from 18.5 to 32.4 for Jamaican varieties. Concentrations of essential amino acids are adequate except for methionine (Eggum, 1970. Rogers and Milner. 1963). Adding methionine to a diet of cassava leaves raised the biological value from 49 for the leaves alone to 80 for the mixture (Eggum. 1970). Rogers and Milner. (1963) considered the composition of essential amino acid of cassava leaf protein to be similar to that of soybean. which is one of the best in nutritive value among the readily available.

Table 2 5
Nutrient composition of taro leaves

Composition per 100 g of fresh wt	Ind	1 a	Phili	ppines	Hawa11		
Edible portion (%)	-		55	0	-		
Moisture (g)	78	8	79	6	88	0	
Protein (g 100g ⁻¹ dry wt)	32	1	21	6	22	7	
Fat (g)	2	0	1	8	0	7	
Carbohydrates (g)	8	1	12	1	4	8	
Fiber (g)	1	8	3	4	2	0	
Food energy (kcal)	77	0	69	0	36	0	
Ash (g)	-		2	0	2	0	
Calcium (mg)	460	0	268	0	107	0	
Phosphorus (mg)	125	0	78	0	60	0	
Iron (mg)	38	7	4	3	2	3	
Sodium (mg)	-		11	0	2	0	
Potassium (mg)	-		1237	0	437	0	
Vit A value (IU)*	2000		20385	0	5028	0	
Thiamine (mg)	0	1	0	1	0	14	
Riboflavin (mg)	0	5	0	33	0	31	
Niacin (mg)	1	9	2	0	1	0	
Ascorbic acid (mg)	63	0	142	0	37	0	

^{* 0 6} ug = 1 IU

Source Standal, 1983

vegetable proteins The use of cassava leaf for producing protein concentrates is being investigated by several food scientists (e.g. Luiza et al., 1979)

The vitamin A content of cassava leaves is as high as 0.5 unit g^{-1} Various sources report vitamin C content ranging from 29 to 180 cmol kg^{-1} in cassava leaves (Oke, 1968)

Limitations

Amaranth, taro, and cassava leaves contain oxalate and accumulate a certain amount of nitrate. The presence of an antinutrient and toxic agent lowers the nutritive value of these vegetables, as discussed in section 2.2

2 10 Estimation of oxalate contents

Two techniques of oxalate quantification are to be discussed

The first, termed the 'classical method' was developed through a series

of improvements of existing techniques before the 1950s. Papers by

Baker (1952) and Moir (1953) are frequently cited by researchers who

use the 'classical method in their work. The Association of Official

Analytical Chemists (AOAC) has certified this technique for total

oxalate determination (Horwitz, 1975)

The classical method of oxalate determination has three steps

- 1 Separation of oxalate by means of calcium oxalate precipitation at pH 4-5,
- 2 Determination of calcium by titrimetric or spectrophotometric methods.
- 3 Calculation of oxalate equivalents from calcium analysis (Baker, 1952 Moir, 1953 Horwitz 1975)

One difference between the methods employed by Baker (1952) and Moir (1953) is that Baker used fresh plant material to avoid loss of oxalate from drying whereas Moir used dried (100° C), plant material

Baker (1952) proved the effectiveness of this technique by demonstrating 99-100% recovery of added oxalate

The second technique is based on the use of high performance liquid chromatography (HPLC) developed in the 1970s. In HPLC a mobile phase is allowed to percolate through the stationary phase packed in a column continuously and the sample in the same solvent as the mobile phase can be injected into the mobile phase as desired.

A paper describing the effectiveness of the HPLC technique for determining oxalic acid and comparing this technique with the classical method has been published (Libert, 1981). Libert used a reversed-phase column called the Lichrosorb RP-8 (C_8) as the stationary phase and a solution containing 0.5% KH $_2$ PO $_4$ and 0.05 M tetrabutyl ammonium hydrogen sulphate (TBA), buffered at pH 2 with orthophosphoric acid, as the mobile phase. One advantage of HPLC is the simultaneous determination of organic acids other than oxalic acid. The determination of oxalic acid levels in rhubarb by the HPLC technique has been shown to give comparable results to the classical method

CHAPTER III

MATERIALS AND METHODS

In order to test the hypothesis that the variance in the nutritional quality of food crops is strongly influenced by cultural and environmental factors, the following experimental sites crops, experimental design and data collection and analysis procedures were selected

3 1 The experimental sites

Four experimental sites were selected to represent a range of agroenvironments. These sites were developed by the Benchmark Soils Project (BSP) of the University of Hawaii to test the hypothesis that agroproduction technology can be successfully transferred from one location to other distant sites around the world provided the biophysical environment of the site where the technology was developed was similar to the transfer site. The project defined similar environments according to the criteria set forth to define the soil family in Soil Taxonomy (Soil Survey Staff, 1975). Soil Taxonomy is a comprehensive system of soil classification for making and interpreting soil surveys issued by the Soil Conservation Service of the U.S. Department of Agriculture

By 1980, the project had established experimental sites in the Cameroons in Africa, Brazil Puerto Rico the Philippines Indonesia and Hawaii Three soil families were selected to test the hypothesis that similar agroproduction technologies and management practice would result in similar crop performances and yields among soils belonging to the same soil family regardless of where they occurred. The three

soil families selected were the clayey, kaolinitic isohyperthermic family of the Tropeptic Eutrustox, the thixotropic isothermic family of Hydric Dystrandepts and the clayey, kaolinitic, isohyperthermic family of Typic Paleudults

The Tropeptic Eutrustox are found in the warm semi-arid tropics and are nutrient-rich Oxisols Experimental sites with this soil were established in Puerto Rico, Brazil and Hawaii In Hawaii the experimental sites were established in Waipio on the Island of Oahu and on the west end of the Island of Molokai

The Hydric Dystrandepts are impoverished soils that have formed from volcanic ash and are well known for their low phosphorus levels. The isothermic members of Hydric Dystrandepts occur in the cool tropics and the mean annual temperature is between 15 and 22°C. Experimental sites with soils of this family were established in the Philippines, Indonesia and Hawaii. In Hawaii, the sites were established on the Big Island of Hawaii at Iole and Niulii on the upper slopes of the Kohala Mountains and at Kukaiau on the slope of Mauna Kea

Experimental sites for the Typic Paleudults were established in Cameroon, Indonesia and the Philippines. These soils are highly acid nutrient-poor and occur in the warm humid tropics. No experimental site of this soil was established in Hawaii.

Each experimental site was carefully characterized for soil and provided with a weather station that recorded wind speed and direction relative humidity, solar radiation maximum and minimum air temperature topsoil and subsoil temperatures and rainfall. The results of this project are summarized in a book entitled. Soil-Based Agrotechnology Transfer' (Silva 1985)

In 1982 a workshop on A Multidisciplinary Approach to Agrotechnology Transfer (1984) was held at the University of Hawaii. At this workshop, the nutritionists reminded the agronomists that the objective of agriculture research was not simply to increase yield but to provide adequate nutrients for people

In a paper on the 'Relationship of Soil Composition to the Nutrient and Antinutrient Contents of Plants" presented at that workshop, Van Reen (1984) suggested that the data in food composition tables were not very reliable because there is evidence that the composition of the same edible plant grown in different parts of the world is very different (Food and Agriculture Organization 1954)

In the same paper Van Reen listed factors that may contribute to variations in food compositions. These are

- 1 Genetics of the variety of the plant
- 2 Light intensity and duration (season)
- 3 Temperature condition
- 4 Water conditions
- 5 Soil types
- 6 Type and amount of pesticide/herbicide
- 7 Type and amount of fertilizers
- 8 Age of plant material
- 9 Part of the sample analyzed

It was evident from Van Reen's paper that the Benchmark research sites established by the Benchmark Soils Project were ideally suited to assess a number of the factors which could influence variation in nutrient contents

It was decided to select four Benchmark research sites to test the hypothesis that nutrient composition in general and antinutrient content in particular vary with genotype and environment. These sites were the Waipio site on the Island of Oahu the Molokai site on the west end of the Island of Molokai the Iole site in the Kohala district of the Island of Hawaii and the Kukaiau site in the Hamakua District also on the Island of Hawaii. Tables 3 1, 3 2 and 3 3 summarize the characteristics of each site.

3 2 Crops

Three crops were selected on the basis of their availability for growing and/or obtaining samples and their relevance to the nutritional quality of interest, namely the contents of oxalates and some mineral nutrients. These crops were amaranth (Amaranthus gangeticus L.), cassava (Manihot esculenta L.) and taro (Colocasia esculenta (L.) Schott.) These crops, with the exception of amaranth were part of the existing cropping systems experiments of the Benchmark Soils Project. They were known to have high oxalate contents.

Amaranth belongs to the family Amaranthaceae It is commercially known in the United States as tampala. In Hawaii, it is also known as Hinn Choi or Chinese spinach. It is a spinach-like vegetable with dark green leaves which are elongated and smooth (Cole 1979). The plants are succulent, low growing and compact with growth habit much like spinach (National Research Council 1984). Under optimum conditions, the plants reach their harvestable size in 45 days after seed sowing. The leaves and young tender stem are eaten as greens. A gangeticus L has been proved to be the same species as A tricolor L

Table 3 1

Description of geographical positions and history of soil use of the experimental sites

Sites

Geographical position and history of soil use

Kukanau

This site is situated on the Island of Hawaii, approximately 2.5 km southeast of the town of Honokaa and approximately 0.6 km from a plantation road southeast of the junction of highways 19 and 24 and 0.3 km southeast of an abandoned church. The elevation is approximately 395 m and the slope is moderate to strong (6 percent slope, north aspect)

The soil was formerly grown to sugarcane In 1976, it was planted to soybean and in 1977 to corn. It was then fallowed until 1982 when cropping systems experiments were begun in which the fertilizers (lime epsom salt, borax and zinc sulfate), in quantities as shown in Table 3.4 were applied yearly. The soil on this site is a member of the thixotropic, isothermic family of Hydric Dystrandepts.

Iole

This site is situated in North Kohala, Island of Hawaii, approximately 5 6 km south southeast of the town of Hawi, approximately 7 2 km southwest of Kapaau village. The elevation is approximately 545 m and the site occurs on moderately sloping to strongly sloping (6 percent slope north aspect) land

The soil was formerly planted to sugarcane Maize experiments in which different rates of N and P were applied, were conducted in 1977. It was left fallow after the maize experiments until 1982 when cropping systems experiments were started, and fertilizers (lime epsom salt borax and zinc sulfate) in rates shown in Table 3.4 were applied yearly. The soil on this site is a member of the thixotropic, isothermic family of Hydric Dystrandepts.

^aSource Ikawa 1979

^bPatrick Ching personal communication

Table 3 1 (continued) Description of geographical positions and history of soil use of the experimental sites

Sites	Geographical position and history of soil use
Waipio	This site is situated on the Island of Oahu, Hawaii, approximately 8 km north of Waipahu and approximately 225 m east of the road leading to Mililani Cemetery from Kamehameha Highway The elevation was 150 m. It is on nearly level upland (2 percent slope)
	The land was an abandoned pineapple field and was used to conduct maize transfer experiments in 1978 after which it was left fallow until 1982 when cropping systems experiments were started and the fertilizers (lime epsom salt, borax and zinc sulfate) of rates shown in Table 3.4 were applied yearly. The soil on this site is a member of the clayey, kaolinitic isohyperthermic family of Tropeptic Eutrustox.
Molokai	This site is situated in Maunaloa, Island of Molokai, Hawaii approximately 1 5 km north-northwest of the Maunaloa Village and approximately 900 m northwest of highway 46. It is at approximately 257 m elevation on gently sloping upland (5 percent)
	The soil was formerly grown to sugarcane Maize transfer experiments were conducted in 1980 after which it was left fallow until 1982 when the cropping systems experiments were started and the fertilizers (lime, epsom salt, borax and zinc sulfate) in rates shown in Table 3.4 were applied yearly. The soil on this site is a member of the clayey kaolinitic isohyperthermic family of Tropeptic Eutrustox.

Taxonomic name	Inherent soil family characteristics
Thixotropic, iso	thermic Hydric Dystrandepts
thixotropic	High surface activity of colloids
ısothermic	Cool soil temperatures (mean annual temperature 15-22 ^o C)
Hydric	Moist humid soils
Dystr-	Low base saturation (< 35%)
-andept	Low bulk density ($< 0.85 \text{ g cc}^{-1}$), amorphous colloids
Clayey kaolinit	ic, isohyperthermic Tropeptic Eutrustox
clayey	More than 35% clay in the subsoil
kaolinitic	Dominated by low activity clay
1 sohypertherm1c	Warm soil temperatures throughout the year (mean > 22 ⁰ C), small difference between summer and winter temperatures (< 5 ⁰ C)
Tropeptic	Moderate structure or less than 125 cm deep, or both
Eutr-	Moderately enriched with nutrients medium to high base saturation (>50%)
-ust-	Pronounced dry season (dry for more than 90 cumulative days per year)
-ox	Presence of oxides of iron and aluminum low cation-exchange capacity

Source Benchmark Soils Project 1979

Table 3 3

Some physical and chemical properties of the soils at four experimental sites (a) Kukaiau, (b) Iole, (c) Molokai and (d) Waipio

(a)

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(b)

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Table 3 3 (continued) Some physical and chemical properties of the soils at four experimental sites (a) Kukaiau (b) Iole, (c) Molokai, (d) Waipio

(c)

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The variety used in the study was White Leaf' The seeds were obtained from Zsang and Ma, Belmont, California

Cassava belongs to the family Euphorbiaceae which also include para rubber. It is a short-lived shrub, 1-5 m in height, with latex in all its parts (Purseglove 1974a). It is known for its edible roots but young leaves are also consumed in different parts of the world. Leaves are variable in size, color of stipules, petioles, midribs and lamina, in number of lobes, depth of lobing and in shape and width of lobes. The cassava used in this study had leaves with 5-7 lobes, midribs, and veins were green in color. The plants were approximately 2 m tall when the leaves were sampled.

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Taro belongs to the Araceae family Like cassava, it is well known for its starchy underground part and the leaves are also consumed in many parts of the world. The plants can grow to 1-2 m tall, with underground starchy corm, producing at its apex a whorl of large leaves with long erect petioles. The leaves are peltate 20-50 cm long, oblongovate glabrous, with rounded basal lobes. The petioles are stout 1 m or more long and clasping at their bases (Purseglove, 1974b)

The variety used in this study was Miyako which is probably native of Japan (Whitney et al. 1939). It is a short variety, shorter than 1 m, with erect and moderately stocky structure. The leaf blades are 35-50 cm long, 25-35 cm wide. 30-40 cm from tip to base of sinus ovate, firm-chartaceous dark green with a bluish cast, the lobes are wide and obtuse with shallow wide sinus.

3 3 Experiments

3 3 1 Amaranth field experiments

The field experiments were installed in Waipio Iole and Kukaiau to represent a range of agroenvironments

In addition to the effect of natural environmental factors on plant chemical composition, imposed management factors can also affect the nutrient and antinutrient contents of farm crops. Two factors which farmers frequently control are water supply and nutrient supply. To assess the effect of water supply on plant chemical composition, irrigated and non-irrigated experiments were conducted on each site. Water was supplied to the irrigated experiments with drip irrigation. The duration and frequency of irrigation were left to the discretion of project staff. In general, soil conditions were used as the main criterion to decide whether irrigation was required or not. Within each irrigation experiment, treatments to test the effect of nitrogen (N) or phosphorus (P) application on plant compositions were included. The fertilizer treatments consisted of

- a basal treatment of lime, N P K bases and trace nutrients
 (Table 3 4)
- 2) a N treatment superimposed on the basal treatment designated basal+N (Table 3 5), and
- 3) a P treatment superimposed on the basal treatment designated basal+P (Table 3 5)

These fertilizer treatments were arranged in randomized complete block design with 3 replications within each irrigation treatment. Therefore

there were 9 plots of irrigated experiments and 9 plots of non-irrigated experiments. The plot size was 1.5 \times 1 m²

Lime, epsom salt borax and zinc sulfate (Table 3.4) were applied on an annual basis as a part of the routine operation of cropping These materials were applied before the amaranth systems experiments Urea, triple superphosphate and muriate experiments were installed of potash (Table 3 4) were applied 1-2 days prior to the planting of The lime rates were adjusted so that the resulting soil the amaranth pH would be near pH 6 Higher lime rates were applied on Hydric Dystrandepts than on the Tropeptic Eutrustox (Table 3 4) because the former has a higher buffering capacity. The rates of various nutrients in the basal fertilizers applied (Table 3.4) were adjusted to provide The rates of N and P fertilizer adequate nutrients for good growth applied at planting and as a sidedressing (Table 3.5) were calculated in such a way that the response of plants to these fertilizer treatments would be reflected in their yields and chemical compositions P rate was applied to the Hydric Dystrandepts soils than the Tropeptic Eutrustox soil because the former is known to fix P more than the latter Response to P fertilizer was expected from plants grown on the Hydric Dystrandepts soil, while responses to N or both N and P were expected from plants grown on the Tropeptic Eutrustox soil

At all locations the land was ploughed to 15 cm depth before the fertilizer was applied. All fertilizers used were of commercial grade. All basal fertilizers and lime (Table 3.4) and N and P fertilizers applied at planting (Table 3.5) were broadcast by hand before being worked into the soil. The plants were sidedressed with N fertilizer.

(Table 3 5) after the plants had emerged and had been thinned to required spacing. The fertilizer was applied by hand, close to the plants and covered with soil

Table 3 4

Forms and rates of basal fertilizers and lime used in the amaranth experiments

Element	Chemical formula (Common name)	Rate (kg elem	ent ha')
		Hydric Dystrandepts	Tropeptic Eutrustox
Ca	CaCO ₃ (lime)	1000	750
Mg	MgSO ₄ 7H ₂ O (epsom salt)	100	100
В	$Na_2B_4O_7$ 10 H_2O (borax)	5	2
Zn	ZnS0 ₄ H ₂ 0	15	15
N	CO(NH ₂) ₂ (urea)	50	50
Р	CaH ₄ (PO ₄) ₂ H ₂ O	25	25
	(triple superphosphate)		
Κ	KCL (muriate of potash)	170	100

After the application of lime basal and preplant fertilizers seeds were dropped by hand into furrows at 2.5 cm deep. No attempt was made to space the seeds as they were to be thinned to the required

Table 3 5

Rates and timing of application of N and P fertilizers in addition to basal in amaranth experiments

Site	Treatment		Rate (kg ele	ment ha ⁻¹)	
		Preplant (Basal)	At planting	Sidedress	Total
Iole and	N	50	100	100	250
Kukarau	P	25	200	-	225
Waipio	N	50	100	100	250
	Р	25	120	-	145

spacing later There were 5 rows spaced 16 6 cm apart in each plot.

The edge rows were used as guard rows

In most cases the seeds emerged to form a thick stand. At the two- to four-leaf stage they were thinned to a linear density of one plant per 15 cm resulting in 11 plants per row leaving 55 plants per plot

In Waipio non-irrigated plots the plants did not emerge until approximately 2 months after sowing. Emergence followed 15 mm of rains which fell during March 8-15 1983 and the growth was sustained by 54 1 mm of rains which fell from March 16 to April 27 1983. The insecticide Sevin was applied to all Waipio plants when they were approximately 1 month old to minimize insect damage. In valpio irrigated plots the plants emerged seven to ten days after seed sowing.

In Iole, plants in both the irrigated and non-irrigated treatments grew poorly. The seedlings emerged ununiformly and several weeks late and the plant growth was not uniform within the same plot. The emergence and growth were poorer in the irrigated plots than in the non-irrigated ones. However, the plants in both experiments did respond to P fertilizer treatment (basal+P) where the plants were larger than those receiving the other fertilizer treatments.

In Kukaiau the plants in both irrigated and non-irrigated plots emerged uniformly and grew vigorously. Responses to fertilizer treatments, especially P, were clearly evident

The experiments at each location were monitored by the project staff who also determined water requirements for the irrigated treatment. They also recorded the general conditions of the experiments.

The plants were harvested when the flower buds were apparent

An attempt was made to harvest the plants in all experiments at the same physiological stage. Due to the uneven emergence and growth in several treatments, all treatments were not harvested at the same time as shown in Table 3.6. The uneven emergence and growth rate resulted in exposures to different sets of environmental conditions for experiments conducted at the same site.

With the exception of the irrigated treatment at Iole which produced less than 10 plants (Table 3.7). 10 representative plants from each plot (judged on their appearance i.e., height leaf and stem size physiological stage such as appearance of flower buds) were sampled for analysis.

Table 3 6 Growing durations of amaranth grown in six experiments at three sites

	Kukatau		<u>lole</u>		Waipio	
	NI*	I*	NI*	I*	NI*	[*
Planting date	1/29/83	1/29/83	1/29/83	1/29/83	1/13/83	1/13/83
Harvesting date	4/10/83	4/10/83	5/4/83	6/28/83	6/23/83	3/24/83
Growing duration (days)	71	71	95	150	161	70
Number of plants harvested/plot	10	10	10	< 10	10	10
Physiological stage and appearance at harvest	flower buds	flower buds	flower buds	flower buds some at seeding stage	flower buds small leaves	flower buds

Table 3 7

Number of amaranth plants harvested from Iole irrigated experiment

Plot #	Fertilizer treatment	Replication	No of plants	Fresh weight (g)	Physiological stage
1	Basal	1	1	35	Seeding
2		2	1	232	Flowering
3		3	6	46	Flowering+seeding
4	Basal+N	1	6	256	Flowering
5		2	3	501	Flowering
6		3	0	0	-
7	Basal+P	1	9	833	Flowering
8		2	3	64	Flowering
9		3	4	134	Flowering

The plants were cut at the soil level and placed in polyethylene bags for transportation to the laboratory. At the laboratory, they were washed free of dust and soil with tap water and again with distilled water and blot-dried with paper towels. After washing and toweldrying, they were weighed to obtain fresh weights

The edible parts consisting of leaves and young succulent shoots, were separated from the non-edible hard and fibrous stems and branches. The edible parts were stored in sealed polyethylene bags and stored in the freezers for later analysis.

3 3 2 Cassava

Cassava leaves were sampled from the Tropeptic Eutrustox soils at Waipio and Molokai and from the Hydric Dystrandepts soils at Kukaiau

and Iole At all sites cassava was grown in hedge rows as wind breaks for other crops in the cropping systems experiments as illustrated in Figure 3 l

In the Waipio and Molokai sites casssava was grown in irrigated and non-irrigated plots, whereas in Kukaiau and Iole all plants were irrigated. Cassava is a drought resistant crop, therefore, it was expected to be able to thrive without irrigation in areas with low rainfall such as in Waipio and Molokai.

The youngest fully expanded leaves (1 e , the third or fourth leaf from the top) including petioles were harvested from each branch for a total of 50 leaves from each sampling location of the cropping pattern described in Figure 3 l. The 50 leaves from each location constituted a composite sample. Two composite samples were collected from each of two rows in Waipio and Molokai with Tropeptic Eutrustox soils, whereas three composite samples were collected from each of two rows in Kukaiau and Iole with Hydric Dystrandepts soils. Table 3 8 shows the age and sampling date of the cassava experiments

The leaf samples were placed in plastic bags and transported to the laboratory where they were washed with tap and then distilled water to remove dust and soil, blotted-dried weighed to determine fresh weights and frozen

3 3 3 Taro

Taro leaves were sampled from the irrigated and non-irrigated plots at both the Kukaiau and Iole sites. No taro was grown at the Molokai site and all taro plants received irrigation water at Waipio owing to the low rainfall there

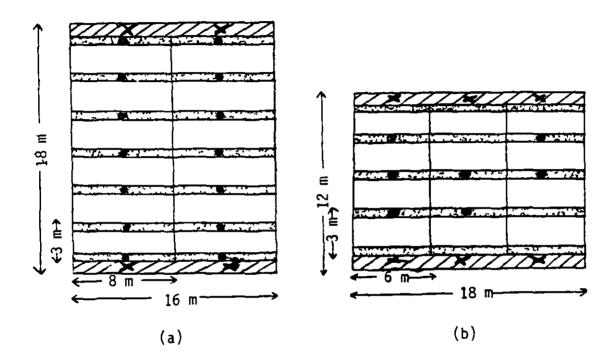


Figure 3 1 Cropping patterns for Tropeptic Eutrustox sites (a) and Hydric Dystrandepts sites (b) showing locations of cassava ([777]) and taro ([1]) rows and sampling locations for cassava (X) and taro (*)

Table 3 8

Planting date, sampling date and age of cassava crops at sampling

Site	Planting date	Sampling date	Plant age (weeks)
Kukanau	1/19/82	11/ 4/82	42
Iole	1/22/82	11/ 4/82	41
Waipio	2/17/82	11/ 1/82	37
Molokai	2/22/82	10/19/82	35

For analysis the youngest fully developed leaf was cut close to the blade to exclude the petiole. Each composite sample of eight leaves was collected from each row as of the cropping pattern described in Figure 3.1

Fourteen samples were collected from the Waipio site and seven samples per experiment were collected from the Kukaiau and Iole sites for a total of 14 samples per site

Table 3 9 gives the age and sampling date of the taro experiments Each taro sample was cleaned and stored the same way as cassava leaves

3 4 Laboratory analyses

This study involved many laboratory techniques, which were standardized and described in other publications and are mentioned briefly in this section. However, the fractionation of oxalates from plant tissue and their measurements by the high performance liquid chromatography (HPLC) technique was newly developed in this study in

Table 3 9
Planting date sampling date and age of taro crops at sampling

Site	Planting date	Sampling date	Age at sampling date (weeks)
Kukaiau	4/13/82	11/4/82	29
Iole	4/14/82	11/4/82	29
Waipio	5/3/82	11/1/82	26

order to obtain data for objectives 1 and 3 of this study. The techniques are described in section 3.6

3 4 1 Plant

Determination of dry weight and moisture content of plant tissue

To obtain moisture content of plant tissue (edible part) and at the same time to obtain dry plant material for chemical analyses—the frozen plant materials were freeze-dried at 40°C. Freeze-drying is an effective technique to obtain dry plant material for analysis of organic compounds. Through this technique plant material can be dried at low temperatures thus avoiding changes in organic compounds.

The moisture contents of edible parts of amaranth were obtained by subtracting the freeze-dried weights from the frozen weights. Dry weights of harvested plants (10 plants) were then calculated from their fresh weights and the corresponding moisture contents. In the case of the Iole irrigated plants, after the dry weight per plot was

obtained, the value was adjusted to represent the 10 plants, because less than 10 plants were harvested from these plots (Table 3.7)

Moisture contents of cassava and taro were obtained by subtracting the freeze-dried weight from the fresh weight

The freeze-dried plant materials were ground to fine powder for further chemical analyses

Determination of sap pH

Approximately 1 g of freeze-dried ground plant material was mixed with 10 g of deionized water for pH measurement

Determination of total nitrogen in plant tissue

Total-N in plant tissue was digested in a micro-Kjeldahl apparatus and measured by a colorimetric procedure using a Technicon autoanalyzer (Clements, 1980)

Determination of ionic and trace element contents in plant tissue

Concentrations of Cations (Ca, Mg, K, and Na) and anions (P ($H_3PO_4^{-1}$), S (SO_4^{-2-}) and C1), and some trace elements including A1, Mn, Zn, Cu, Fe and S1 were determined by a x-ray fluorescence spectrometer, model 72 000 manufactured by Applied Research Laboratories

Nitrate-N was determined by the ${\rm H_2SO_4}$ -salicylic acid method (Cataldo et al , 1975). Boron was determined by automated carminic acid-phenol method (Clements 1980). The technicon autoanalyzer was used as the main analytical apparatus for both nitrate-N and B determinations

The concentrations of all major cations and anions were expressed in the units of cmol kg^{-1} on a dry weight basis which is identical to

the unit of me/100 g on a dry weight basis, for comparative (ionic balance study) purposes and as percent of dry weight for general quantitative purposes

Determination of oxalate in plant tissue

This consisted of the extraction of the total as well as various fractions of oxalate and the subsequent measurement of oxalic acid concentrations by the HPLC technique. The detailed procedures are described in section 3.6

The concentrations of oxalates were expressed in cmol kg⁻¹ on a dry weight basis and percent of dry weight for the purposes stated earlier

Determination of bases in the oxalate fractionation extracts of plant tissue

The water soluble fraction and water insoluble but acid soluble fraction were analyzed for Ca, Mg, K and Na concentrations. Ca and Mg were determined in an atomic absorption spectrophotometer (Perkin and Elmer Model 303) and K and Na on a flame photometer (Coleman Junior II, Model 6/20). The concentrations were expressed as cmol kg⁻¹ on a dry weight basis

3 4 2 Soils

To characterize the agroenvironment further soil chemical analyses for their total N exchangable bases and P contents were conducted Soil samples from only the amaranth plots were chemically analyzed Soil data for cassava and taro were obtained from the soil analysis of 1983 of the Benchmark Soils Project

Determination of total-nitrogen in soils

The total-N in soils was determined using the micro-Kjeldahl method (Soil Conservation Service USDA, 1972). Soil samples weighing 0.5-1 g and a catalyst mixture of K_2SO_4 FeSO $_4$ CuSO $_4$ Se=7.9 l.l. 0.1 by weight were used. Ammonia was collected in boric acid and titrated with 0.0371 or 0.0519 NH $_2SO_4$ depending on the N concentrations. This method of total-N determination did not include NO $_3$ -N

Determination of exchangeable bases in soils

Exchangeable bases (i.e., Ca, Mg, K and Na) were extracted from 10 g of air-dried soils with 15 ml of $\frac{1}{N}$ NH₄OAc. After the extract was filtered, the filtrate volume was made up to 100 ml (Soil Conservation Service USDA, 1972). Calcium and Mg were determined by atomic absorption spectrophotometry and K and Na by flame photometry

Determination of extractable phosphate in soils

One gram of oven-dried soil was extracted with 100 ml of modified Truog extractant (0 025 \underline{N} H₂SO₄+0 38% (NH₄)₂SO₄) Phosphorus concentration was determined colorimetrically using ammonium paramolybdate and ascorbic acid as color developer (Ayres and Hagihara 1952, Olsen and Sommers, 1982)

3 5 Data analysis

The Statistical Analysis System (SAS) computer package was used for the analysis of variance, means comparisons and correlation and regression analyses

In amaranth the fertilizer treatments within each irrigation experiment were arranged in a randomized complete block design and

analyzed accordingly The effects of irrigation fertilizer and their interactions at each site were analyzed as if the experiment was a split plot design with irrigation as the main plots and fertilizer treatments as the subplots. The difficulty in randomizing the irrigation treatment necessitated this approach and therefore the interpretation of the statistical analysis requires some caution.

The effects of sites and various interactions were analyzed using the split split plot design with site as the main plots, irrigation as the subplots and fertilizer as the sub-subplots

For the cassava study, all sites had irrigated experiments but only the Molokai and Waipio sites had both irrigated and non-irrigated trials. In the taro study, the Kukaiau and Iole sites had both irrigated and non-irrigated experiments wheras the Waipio site had only irrigated experiments. No taro experiment was conducted at the Molokai site. Since the irrigated experiments were conducted at all sites the effect of site on plant chemical composition was tested on data collected from the irrigated experiments.

The effect of irrigation was analyzed for each site containing both types of experiments, and the effect of site by irrigation interaction was obtained by combining the irrigated and non-irrigated cassava experiments from the Waipio and Molokai sites and by combining the irrigated and non-irrigated taro experiments from Kukaiau and Iole

The Duncan-Waller's multiple range test was used for comparing the effects of site and fertilizer on amaranth and the effect of site on cassava and taro. This test is appropriate for cases with unequal subclass numbers as was the case with the cassava and taro experiments.

In order to evaluate results affected by treatment interactions the least significant difference (LSD) analysis was employed because the Duncan-Waller's multiple range tests only compare main effects

The relationships between various plant, soil and weather variables were investigated through correlation and regression techniques

Multiple regression models were used to assess the effects of climatic and soil variables on oxalate and nitrate contents of amaranth cassava and taro

3 6 Development of methods for oxalate extraction fractionation and determination

The development of these methods was designed to meet the conditions for organic acid determination by high performance liquid chromatography (HPLC)

3 6 l Extraction of total oxalate from plant tissue

This was essentially the standard technique described in AOAC (Horwitz, 1975) with minor modifications

Freeze-dried, finely ground plant material weighing 2.5 g was boiled for 30 minutes in 300 ml of 1.1 \underline{N} HCl (55 ml 6 \underline{N} HCl and 245 ml distilled water). Two to three drops of caprylic alcohol were added to prevent foaming during boiling

The boiled mixture was allowed to cool to room temperature and its volume subsequently adjusted to 500 ml with distilled water. It was then filtered through Whatman #541 filter paper. The first 100 ml of the filtrate was discarded and the remaining filtrate was stored in a refrigerator at #40C for subsequent oxalate determination by the HPLC technique

3 6 2 Extraction of oxalate fractions

Previous findings—such as those of Baker (1952), Moir (1953),
Singh and Saxena (1972) and Hodgkinson (1977), suggest that oxalates
should be fractionated into 3 parts, namely—free oxalic acid, hot water
soluble oxalate and hot acid soluble (hot water insoluble) oxalate

Free oxalic acid is known to be highly soluble in ethanol at room temperature, while the other forms of oxalate, such as K-, Ca- Mg-, Na- and NH₄-oxalates are insoluble (Weast 1973) Thus, ethanol was used to extract free oxalic acid

Potassium- Na-, NH₄-oxalates and to a lesser extent Mg oxalate are known to be water soluble and their solubilities increase with increasing temperature (Weast 1973, Hodgkinson, 1977). Boiling water was, therefore used to extract this fraction

Calcium oxalate and to a lesser extent Mg oxalate are known to be relatively insoluble in water (Weast 1973, Hodgkinson 1977)

Therefore hot acid, similar to that used to extract total oxalate was used to extract this fraction

Procedure for extraction of oxalate fractions

Freeze-dried, finely ground plant material weighing one gram was shaken for one hour in 30 ml of 95% ethanol. The mixture was filtered through ashless filter paper and subsequently washed with ethanol. The filtrate was made to 100 ml volume with ethanol. This extract was used for free oxalic acid determination.

The plant residue on the filter paper was dried to room temperature and was transferred into a 600 ml beaker. One hundred ml of distilled water were added to the beaker along with two to three drops of caprylic

alcohol to prevent foaming. The mixture was boiled for 30 minutes. After cooling to room temperature it was filtered through fast, ashless filter paper into a 200-ml volumetric flask. The volume was made up with distilled water. This extract was used for the determination of water soluble oxalate, Ca, Mg, K, and Na contents.

The wet plant residue on the filter paper was transferred to a 1000-ml beaker along with 150 ml of distilled water 27 5 ml 6 \underline{N} HCl and a few drops of caprylic alcohol. The mixture was boiled for 30 minutes. After cooling to room temperature, the content was poured into a 250-ml volumetric flask and the volume was made up with distilled water. The content in 250-ml volumetric flask was filtered through fast, ashless filter paper (Whatman #541). The first 100 ml of the filtrate was discarded and the remainder was kept for determination of insoluble oxalate. Ca. Mg. K., and Na contents

3 6 3 Preparation of the samples from the different oxalate fraction for HPLC analysis

An aliquot of 1-2 ml from each extract was pipetted into a test tube. With the exception of the alcohol extract, all aliquots were evaporated to dryness in a vacuum oven at $40\text{-}42^{\circ}\text{C}$. This process took approximately 5-8 hours. The aliquots from the alcohol extract were evaporated in an air convection oven at 40°C . The dry residues were dissolved in 10 ml of 0 013 NH₂SO₄ since this acid was also used as the standard eluent (mobile phase) for the ion exchange column of the HPLC

After each residue was dissolved in 0 013 \underline{N} H_2SO_4 the solution was filtered through Whatman #1 filter paper and refiltered through

a special filter 'Sep Pak , manufactured specifically for HPLC use

The solution prepared from ethanol extract required several refilterings with Sep Pak' (Water Assoc , Mass) to remove color. The final solution prior to analysis by HPLC was clear and colorless. The samples were frozen if an immediate HPLC run was not possible.

3 6 4 Description of oxalate determination by HPLC technique

The instrument which is called collectively HPLC consisted of an automatic injector, a high pressure pump—a chromatographic column a detector and an integrator

The automatic injector (Micromeritics Model 725, Norcross Georgia 30093) enables the samples to be fed to the system automatically Approximately 0.8 ml was required from each sample. The samples were placed in special glass vials with a tightly fitting plastic stopper. The stoppered vials containing the samples were arranged in a self-revolving tray attached to the injector. A needle which automatically penetrated the plastic stopper of the vial fed sample solutions to the column.

The column pressure was maintained between 1500-1850 psi with a Beckman solvent metering pump. Model 110A. The pressure is determined by the flow rate and the chemical nature of the mobile phase. In most cases the flow rate was maintained at 0 6-0 8 ml/minute.

The chromatographic column is known as an ion exchange column. It detects and measures organic acids by ion exclusion and partition chromatography by means of a strong cation exchange resin Aminex ion exclusion HPX-87, manufactured by BIO-RAD. In general organic acids

emerge from the column in the order of increasing pKa when dilute sulfuric acid is used as the solvent

The detector used was a combination of the Model 100-10 Hitachi spectrophotometer and Beckman's Altex spectrophotometer flow cells. The combination results in a high performance variable wavelength absorbance detector for use in liquid chromatography. For organic acid analysis, the UV wavelength of 210 nm was used

A small, single-channel plotting/reporting integrator Model 3390A Hewlett and Packard was also a part of the assembly

Minor modification of the procedures

High nitrate content in the leaf tissue interfered with oxalate determination by the HPLC column. Nitrate and oxalate have the same absorbance wavelength (210 nm) and their retention times are very close, especially when 0 013 \underline{N} H_2SO_4 is used as the eluent. This eluent did not permit the complete separation of oxalate and NO_3 . Better separation was achieved when more concentrated sulfuric acid (i.e. 0.05 \underline{N}) was used. However, when the column was replaced with a new one of the same specification, it was found that 0.05 \underline{N} H_2SO_4 by itself was not sufficient to separate NO_3 and oxalate. The use of 5% acetonitrile with 0.05 \underline{N} H_2SO_4 resulted in better separation of oxalate and NO_3 . However, acetonitrile shortened the life span of the column

Use of an internal standard to study oxalate recovery from HPLC determination

During each HPLC run, recovery study was run simultaneously, by using an internal standard. For this purpose 0 1 ml of 1 000 ppm

standard oxalic acid was added to a two ml aliquot of plant extract. This spiked' sample (Figure 3.2) was run in duplicate. The rest of the procedure remained the same as described in section 3.6.3. The 10 ml spiked sample (section 3.6.3) contains oxalic acid from the plant extract and internal standard. As shown in Figure 3.2, the addition of 0.1 ml of 1,000-ppm oxalic acid should result in a 10 ppm higher concentration for 100% recovery.

The percent recovery was calculated by taking the difference in oxalate concentration between the spiked and non-spiked sample. This difference divided by the concentration of the added internal standard is the fraction recovered. This value was used to adjust all HPLC reading to 100% oxalate recovery.

3 6 5 Comparison of the classical and HPLC methods of oxalate determination

In order to measure the several forms of oxalate in plant tissue, it is necessary to use sensitive methods of analyses. In this experiment the recoveries of various forms of oxalate using the classical and HPLC methods of oxalate determination were compared. Analytical grades of oxalic acid and three forms of oxalate were used to prepare a range of solution concentrations as shown in Table 3.10. Aliquots of these working standards were analyzed by both the HPLC and classical methods.

The classical procedure

The classical technique for oxalate determination (Horwitz 1975)

18 based on the precipitation of oxalate in the plant extract as Ca

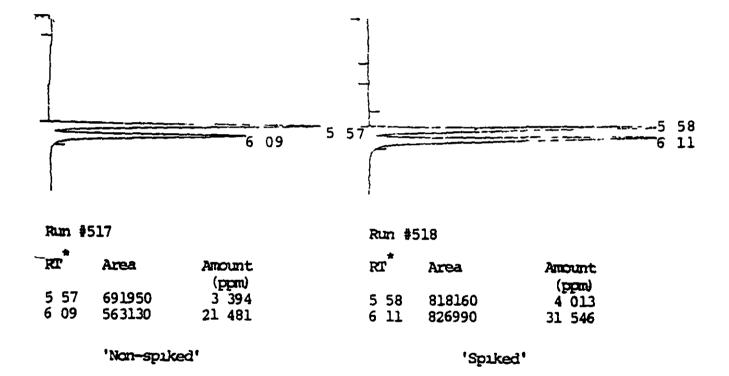


Figure 3 2 HPLC chromatograms illustrating the resolution of nitrate (upper) and oxalate peaks and the difference between spiked (right) and non-spiked samples

*RT = Retention time in minutes

Table 3 10 Standard solutions of oxalic acid and oxalates

Form	Formula and formula weight	Concen	tration	of oxa (ppm	late 10	on (C ₂ 0,	2-)
Oxalıc acıd	нососоон 2н ₂ о (126 00)	1000	500	250	125	62 5	6 3
Sodium oxalate	Na ₂ C ₂ O ₄ (134 00)	656 7	328 4	164 2	82 1	41 0	4 1
Ammonlum oxalate	(HH ₄) ₂ C ₂ O ₄ H ₂ O (142 12)	709 0	345 5	177 2	88 6	44 3	4 4
Potassium oxalate	K ₂ C ₂ O ₄ H ₂ O (184 24)	529 4	264 7	132 3	66 2	33 1	3 3

oxalate at pH 4 to 5, and the subsequent titrimetric or spectrophotometric determination of Ca

An aliquot of 20 ml of each standard oxalate solution shown in Table 3 10 was pipetted into a beaker. The pH of the aliquot was adjusted to 4-4 5 with concentrated NH $_4$ OH. Then 5 ml of a calcium oxalate solution in acetate buffer (pH 4 5) were added. The mixture was allowed to stand overnight at room temperature to provide enough time for Ca oxalate to precipitate. This was centrifuged at 3 000 rpm for 15 minutes. After removal of the supernatant liquid, the precipitate was washed twice with a fine jet stream of 20 ml filtered, cold, wash liquid. Five ml of 10% $\rm H_2SO_4$ were added to dissolve the Ca oxalte.

and the contents quantitatively transferred and made to volume in a 25-ml volumetric flask with $1 \, \underline{N}$ HCl. The Ca in this solution was measured by atomic absorption spectrophotometer

The HPLC procedure

The standard oxalic acid and oxalate solution (Table 3 10) were analyzed directly without sample preparation because of their high purity. The resulting peak heights are shown in Table 3 11 and related to oxalate concentration as illustrated in Figure 3 3

As shown in Table 3 12 the classical method gave good results between the range of 325-700 ppm but overestimated oxalate content below 300 ppm and underestimated the true value above 700 ppm

The HPLC method resulted in a curvilinear relationship between peak height and oxalate ion concentrations (Figure 3 3). A linear relationship was found between 0-125 ppm (Figure 3 3). The particular chromatographic column used in this experiment could not detect oxalic acid below 6 3 ppm (Table 3 12), but throughout much of this study there was evidence that this technique could determine oxalic acid concentrations as low as 2 ppm. Different forms of oxalate salts had little effect on the accuracy of oxalate determinations for both techniques

This study shows that the HPLC is a good method for plant oxalates because the concentration range in plant extract samples (10-100 ppm) falls within the linear range

Table 3 11 Relationships between oxalate ion (${\rm C_2O_4}^{2^+}$) concentrations and HPLC peak height

O ₄ ²⁻ concentration (ppm)	Peak height (cm)
33 1	0 9
41 0	1 0
44 3	1 2
62 5	1 7
66 2	1 8
82 1	2 2
88 6	2 4
125 0	3 3
132 3	3 9
164 2	4 8
177 2	5 2
250 0	7 4
264 7	8 8
328 4	11 0
354 5	12 5
500 0	16 4

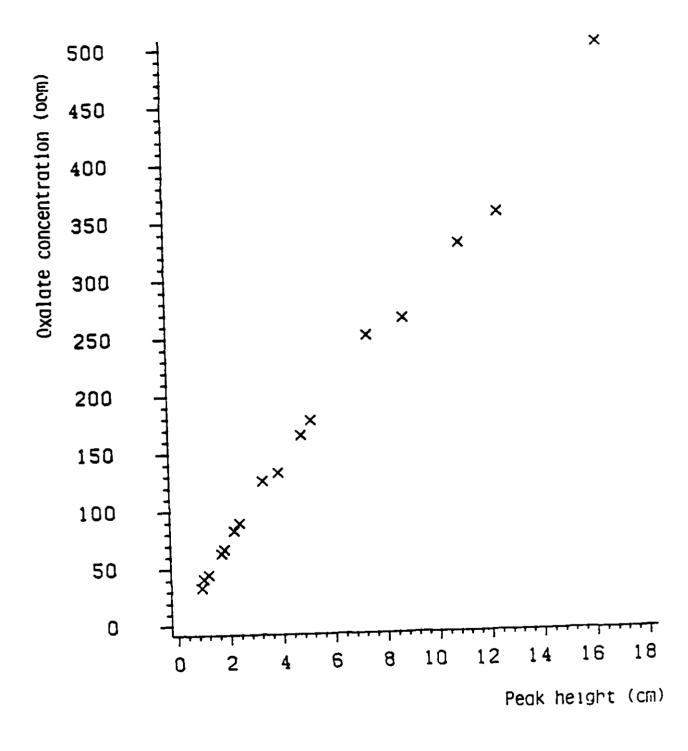


Figure 3 3 Relationship between oxalate concentration and peak height

Table 3 12

Comparison of oxalate content determined by the classical and HPLC methods

	Oxalate ion $(C_2O_4^{2-})$ concentration				
	Standard concentration	Measured c	Measured concentration		
		Classical	HPLC*		
Na oxalate	656 7 328 4 164 2 82 1 41 0 4 1	663 4 329 7 192 5 101 5	336 2 157 6 82 5 37 5 n d **		
NH ₄ oxalate	709 0 354 5 177 2 88 6 44 3 4 4	718 1 350 6 215 8 120 0	378 2 169 4 90 0 45 0 n d		
K oxalate	529 4 264 7 132 3 66 2 33 1 3 3	551 0 275 8 157 8 90 6	273 8 131 0 67 5 33 8 n d		
Oxalic acid	1000 500 250 125 62 5 6 3	840 0 476 2 265 7 140 5	485 3 233 5 123 8 63 8 n d		

^{*} The regression equation y = 0.1906 + 37.3293x in Figure 3.3 was used to calculate oxalate concentrations below 125 ppm but $y = 13.83 + 30.46 \times -0.10 \times 2000 \times 200000 \times 2000 \times 2000$

^{**} Non-detectable due to exceedingly low concentrations

3 6 6 Oxalate recovery from the extraction and fractionation of plant materials

This part of the study consisted of three parts which are called Recovery I, Recovery II and Mg oxalate solubility. The Recovery I experiment was conducted for the purpose of assessing the recovery of total, Ca- K- and Mg oxalates from plant material using the total oxalate extraction and oxalate fractionation techniques described in sections 3 6 l and 3 6 2. Since Mg oxalate is partially soluble in hot water, the Recovery II experiment was conducted for the purpose of assessing the recovery of only the total, Ca- and K oxalates from plant material using the technique described in sections 3 6 l and 3 6 2. Concentrations of the internal standards used for these recovery studies are given in Table 3 13

Based on published chemical data (Weast, 1973) three assumptions were made (1) that K oxalate was totally soluble in boiling water (2) that Ca oxalate was totally insoluble in hot water but was totally soluble in hot acid, and (3) that Mg oxalate was partially soluble in hot water and also soluble in hot acid

A magnesium oxalate solubility experiment was conducted to measure the proportion of Mg oxalate which is soluble in hot water and in hot acid. The results from this experiment were used to calculate recovery of Mg oxalate in the recovery I experiment. The two recovery experiments made use of added internal standards as described in section.

3 6 4 The experiment included a set of controls which did not receive oxalate internal standards. The difference in oxalate concentration between the treated and the control samples was the added.

concentration of internal standards to the treated' samples Recovery was calculated as follows

% recovery = ppm of 'treated sample - ppm of 'control sample X 100
Actual concentration (ppm) of oxalate internal standard added

Magnesium oxalate solubility experiment

Three replications of 0 0659 g of analytical grade Mg oxalate (MgC₂O₄ 2H₂O) were weighed and placed in 250-ml beakers. One hundred ml of distilled water a volume equal to the amount used to extract water soluble oxalate fractions were added to the beakers. The contents were boiled for 30 minutes, cooled to room temperature, filtered through #40 Whatman filter paper into a 200-ml volumetric flask and made up to volume with distilled water. This is the same treatment that was used for plant samples except that #40 filter paper was used here

A 1 ml aliquot was diluted 25 times with distilled water and magnesium was determined in this solution by atomic absorption spectrophotometry

The Mg concentration was converted to an equivalent amount of Mg oxalate. This portion of Mg oxalate was considered to be water soluble. By subtracting this soluble portion from the total Mg oxalate originally added the quantity of water insoluble portion was obtained

On an average 72 1% of the ${\rm MgC_2O_4}$ ${\rm 2H_2O}$ was soluble in boiling water while 27 9% was insoluble (Table 3 14). These percentages were taken to represent the solubility of plant Mg oxalate. However, it should be emphasized that this experiment did not simulate the true conditions of plant samples where other oxalates might interfere with the

Mg oxalate may overestimate the solubility of Mg oxalate in plant materials and underestimate the amount of insoluble Mg oxalate

Recovery I experiment

Dried, ground plant material weighing 1 g was transferred to a 250-ml beaker. Care was taken to keep sample weights between 1 0000± 0 0001 g to allow for direct computation of recovery from HPLC reading in ppm. For the 'treated samples internal standards of 0 0409, 0 0324 and 0 0658 grams of K-, Ca- and Mg oxalates, respectively were added. These quantities were pre-calculated to enable the final oxalte concentration to be read directly from the HPLC.

The results showed that recoveries of the water soluble fraction and total oxalate were low, while the insoluble fraction exceeded 100% (Table 3 15) These results appear to support the concern regarding the decreasing effect that plant oxalates could have on the recovery value of Mg oxalate determined on the pure reagent grade salt

Recovery II experiment

To eliminate the uncertainty regarding the solubility of Mg oxalate in boiling water—the addition of Mg oxalate crystals were excluded in the Recovery II experiment—The procedures—otherwise—were the same as those of the recovery I experiment

The recoveries of oxalate in the water soluble and water insoluble fractions were close to 100% (Table 3 16). These recoveries are considered satisfactory and indicate that all K oxalate and Ca oxalate are recoverable by the methods of fractionation and extraction employed in this study.

Table 3 13

Concentrations of internal standards for three forms of oxalate used in the recovery experiment

Oxalate fractions	K oxalate	Ca oxalate	Total for Recovery	Mg oxalate	Total for Recovery
	(a)	(b)	(a)+(b) (ppm)	(c)	(a)+(b)+(c)
Soluble oxalate	16 0	0 0	16 0	11 5*	27 5
Insoluble oxalate	0 0	8 0	8 0	4 5	12 5
Total oxalate	16 0	8 0	24 0	16 0	40 0

^{*}Using 72 1% solubility value

Table 3 14
Solubility of magnesium oxalate in hot water

Portion of Mg oxalate		Mg oxalate
	mg	% total
Total amount added	65 8	100 0
Boiling water soluble	46 5	72 1
Boiling water insoluble (acid soluble)	19 4	27 9*

^{*}A preliminary experiment showed that Mg oxalate was completely soluble in hot acid

Table 3 15

Recovery of water soluble water insoluble and total oxalate (Recovery I experiment)

Treatments	Oxalate conc	entrations	in c	infferent	fractions		
	Water soluble (1)	Water insoluble	(2)	Total (1)+(2)	Total analyzed		
Treated ^a (ppm)	22 9	50 9		73 8	70 3		
Control ^a (ppm)	13 5	26 6		40 4	41 2		
Difference ^a (ppm) 94	24 3		33 7	29 1		
Recovery (%) ^a	34 2 ^b	194 4 ^C		84 3 ^d	72 8		

a Means of 3 replicates

b Calculation (9 4/27 5) x 100

c Calculation (24 3/12 5) x 100

d Calculation $(33 7/40 0) \times 100$

Table 3 16

Recovery of water soluble, water insoluble and total oxalate (Recovery II experiment)

Treatments	Oxalate conc	entrations in c	infferent f	ractions
	Water soluble (1)	Water insoluble (2)	Total (1)+(2)	Total analyzed
Treated a (ppm)	36 2	48 8	85 0	87 2
Control ^a (ppm)	19 3	40 6	59 9	61 6
Difference ^a (ppm) 16 9	8 2	25 1	25 6
Recovery (%) ^a	105 6 ^b	102 5 ^C	104 6 ^d	106 7

- a Means of 3 replicates
- b Calculation (16 9/16 0) x 100
- c Calculation (82/80) x 100
- d Calculation, (25 1/24 0) x 100

3 7 Soil and weather characteristics measured specifically for the study of agroenvironmental effects on nutritional quality of food crops

In order to characterize the agroenvironment of the experimental sites, measurement of soil and climatic components of the agroenvironments were undertaken at each site. Soil data from amaranth experiments were obtained for each experimental plot, but those from cassava and taro experiments were already measured and made available by the Benchmark Soils Project.

The chemical analysis of the soil samples from amaranth plots were made after the plants were harvested. The results of the chemical analysis of the soil for the amaranth cassava and taro (Tables 3 17)

3 18) plots are comparable to the previous results of soil analysis from the same sites (Table 3 3) reported by Ikawa (1979). The Tropeptic Eutrustox soils in Molokai and Waipio had higher exchangeable bases than the Hydric Dystrandepts soils of Iole and Kukaiau as shown in Tables 3 17 and 3 18. On the other hand, the Tropeptic Eutrustox soil at Waipio had significantly lower total N content than the Hydric Dystrandepts soils of Iole and Kukaiau (Table 3 17). Higher P contents were found in the Kukaiau soil than the soils from the other sites as shown in Tables 3 17 and 3 18. This was likely residual P from previous heavy P applications on the Kukaiau soil

Since the irrigated and non-irrigated plots were not situated adjacent to each other in all three sites of amaranth experiments, the soil chemical properties of both irrigated and non-irrigated plots are presented in Table 3 19. In Kukaiau, higher total N and P were found in the irrigated than in the non-irrigated plots. In Iole, higher total N was found in the irrigated plots but higher Ca and Mg concentrations were found in the non-irrigated plots. In Waipio, higher concentrations of Na and P were found in the irrigated plots but Ca and K were higher in the non-irrigated plots. The difference in soil chemical properties between irrigated and non-irrigated plots can result in differences in plant chemical composition.

There are distinct differences regarding the climatic factors of the four experimental sites. Kukaiau and Iole sites are generally wetter than the Waipio site (Tables 3 20 3 21 3 22). However, the Molokai site, although considered to be a dry site due to low rainfall had a higher relative humidity than the other three sites (Table 3 21).

The Kukaiau and Iole sites received lower solar radiation and had lower air and soil temperatures than the Molokai and Waipio sites (Tables 3 20, 3 21, 3 22). Both Kukaiau and Iole sites were situated in higher elevations and because of the frequent cloud cover associated with frequent rains and cool temperatures, the energy supply was lower compared to the Waipio and Molokai sites

Comparing the two sites at the Island of Hawaii, [ole tended to be cooler as indicated by lower air and topsoil temperatures and solar radiation (Tables 3 20, 3 21, 3 22). Since the non-irrigated amaranth in Waipio were left in the field much longer than its irrigated counterpart, it received almost 5 times more rain than the irrigated amaranth (Table 3 20). A similar situation was observed in Iole since irrigated and non-irrigated plants were harvested at different times

Table 3 17

Chemical analysis of soils at three different experimental sites used for the amaranth experiments

Location	total N %	P ppm		Mg K _cmol kg ^{-l} soil	Na
Kuka1au	0 55 A	81 2 A	2 4 C	1 0 C 0 25 C	0 09 B
Iole	0 48 B	72 9 B	3 3 B	1 2 B 0 32 B	0 09 B
Waipio	0 18 C	78 3 AB	5 6 A	3 4 A 0 74 A	0 43 A

^{*}Means in the same column with the same letters are not significantly different at 0 05 probability level (Waller-Duncan's multiple range test)

Table 3 18

Concentrations of some nutrients in soils at each experimental site of cassava and taro experiments

Site		ppm			
	Ca	Mg	K	Na	Р
Molokai	4 6	2 0	1 3	0 20	20 2
Waipio	3 3	3 5	2 1	0 23	81 4
Iole	3 4	1 4	0 6	0 07	83 8
Kukalau	2 6	0 9	0 4	0 07	110 1

Table 3 19 Soil chemical analysis results of irrigated and non-irrigated plots of three experimental sites

Site	Treatment*	Total N %	P ppm	Ca	Mg _cmol kg l so	K 011	Na
Kukatau	NI I	0 54 0 56	74 3 88 0	2 4 2 4	0 9 1 0	0 23 0 27	0 08 0 11
Significance level (F-test)		0 0016	0 0270	0 7231	0 4641	0 5386	0 1637
Iole	NI I	0 45 0 50	75 6 70 2	4 0 2 5	1 4 1 0	0 32 0 31	0 11 0 06
Significance level (F-test)		0 0003	0 1459	0 0001	0 0004	0 7053	0 1864
Waipio	NI I	0 18 0 17	70 7 85 9	5 9 5 3	3 3 3 5	0 99 0 49	0 11 0 77
Significance level (F-test)		0 2920	0 0426	0 0120	0 1597	0 0001	0 0001

^{*}NI - non-irrigated I = irrigated

Table 3 20

Weather characteristics during the growing period of amaranth grown at three sites

Site Irri gation			ο .			Soil temperature ^a					Relative humidity				r	a i n	fa 1 1		Solar radiation ^a	'									
					<u></u>			Topsoil				Subsoil			(%) 				'	(mm)		(Langley)							
		Ма	×	Mi	n	Av	e 	Ma	ĸ	Min		Ave	: 	Ma	x	Hir) 	Ave		Max		Min		Av	e				
Waipio	NI	27	3	15	7	21	5	21	1	20	4	20	8	21	9	21	7	21	8	78 7	,	45	6	62	2	115	5 6	768 2	7 2
	1	27	0	14	6	20	8	20	3	19	9	20	1	21	7	21	4	21	6	79 5	i	44	4	62	0	35	7	696 2	7 1
lole	NI	22	9	13	2	18	1	18	8	18	1	18	5	20	4	20	1	20	3	90 ()	55	6	72	8	332	2 0	363 6	4 1
	1	23	0	13	4	18	2	19	1	18	5	18	8	20	6	20	3	20	5	90	l	57	9	74	0	440	4	371 9	5 9
kukaiai	u NI	25	9	16	5	21	2	20	3	19	3	19	8	19	0	19	0	19	0	80	ì	50	9	65	5	293	5 0	391 4	5 0
	& I																												

Average daily values

Cumulative values for the whole growing period

Table 3 21
Weather characteristics during the period from planting to sampling of cassava grown at four sites

Site	Air	temper	ature			So11 t (emperat C)	ure ^a			Relative numidity (%)	•	Cumulative rainfall (mm)	Solar radiation ^a (Langley)	Wind speed (km/hr
					Topso	11		Subso	i1		(-/		(mm·)	(congrey)	(C. 111)
	Max	Min	Ave	Max	Min	Ave	Max	Min	Ave	Max	Min A	ve			
Mo loka i	28 7	20 5	24 6	21 5	21 1	21 3	22 0	21 8	21 9	97 8	71 0 8	14 4	441 9	510 0	19 0
Waipio	29 6	19 3	24 5	22 9	22 3	22 6	23 6	23 3	23 5	85 0	53 6 6	9 3	843 8	481 4	7 6
Iole	24 4	16 3	20 4	20 7	19 7	19 9	21 0	20 4	20 7	90 9	63 8 7	7 4	2354 8	351 9	9 8
Kukaiau	25 0	17 6	21 3	22 2	21 0	21 6	20 5	20 5	20 5	85 5	64 9 7	5 2	3088 6	370 7	8 1

^aAverage daily values

^bCumulative values from planting to sampling

Table 3 22

Weather characteristics during the period from planting to sampling of taro grown at three sites

Site	Air	temper	ature		Soil	temper (°C)	ature a			huc	lative nidity ⁽ (%)	1	Cumulative rainfall (mm)	Solar radiation ^a (Langley)	Wind speed (km/hr)
				Tops	1011		Sub	soil					, mm)	(Langley)	(KW/N/)
	Max	Min	Ave	Max	Min	Ave	Max	Min	Ave	Max	Min	Ave			
Waipio	31 2	19 9	25 6	23 8	23 2	23 5	24 4	24 0	24 2	84 1	51 8	68 0	585 0	533 5	6 9
lole	24 8	16 6	20 7	20 9	20 4	20 7	21 7	21 5	21 6	90 7	64 9	77 8	1363 6	372 5	9 5
Kukaiau	25 9	18 6	22 2	23 3	22 0	22 7	21 3	21 3	21 3	85 3	65 8	75 5	1485 5	392 4	8 8

Average daily values

^bCumulative values for the period from planting to sampling

CHAPTER IV

RESULTS AND DISCUSSION

The hypothesis to be tested is that the nutrient and antinutrient content and therefore the overall composition of food crops, are affected by controlled and uncontrolled environmental factors that regulate crop growth, development and performance This was tested by growing several test crops in a number of environmentally different sites The test crops were amaranth, taro and cassava with amaranth receiving the greatest attention Experimental sites were located on Oahu, Molokai, and two locations on the Big Island Hawaii varied in temperature, moisture, and radiation regimes as well as in the chemistry and physics of the substrate. The imposed treatments were fertilizer rates and water supply. The aim of this study is to account for the expected variance in food composition and crop performance

The effect of both controlled and uncontrolled environmental factors on the number of days required for a crop of amaranth to mature differed among sites, and in some instances between treatments within sites. It will become increasingly evident in this study that environmental factors other than those considered in this study contributed to variances in the dependent variables. In the absence of insects weeds and pathogens water nutrients and energy supply are expected to affect crop performance. If water nutrients and pests are controlled, energy supply in heat units becomes the major contributor.

to differences in growth and development. Photoperiod can also affect crop performance, but in the amaranth experiment all plantings were completed within a two week period and the small latitudinal difference between Oahu and the Big Island of Hawaii was not expected to affect daylength

This chapter is written in two parts. The first deals with the effect of controlled and uncontrolled environmental factors on crop growth, development, and performance of crops with emphasis on amaranth, and the second with the effect of environmental factors on the nutrient and antinutrient content of the same crops

4 1 Growth and development of amaranth

In evaluating the effects of controlled and uncontrolled environmental factors on amaranth, three crop-related parameters are considered
which include (1) number of heat units required by amaranth to develop
from planting to harvesting (2) dry weight of amaranth, and (3) moisture
content of amaranth

4 1 1 Energy utilized by amaranth for emergence and growth

All things being equal the heat units required by plants of the same genotype to develop to a particular phenological stage should be the same. In order to obtain information about the temperature requirement of amaranth in this study, heat units in degree days were computed

Heat unit/day =
$$\frac{T_{max} + T_{min}}{2}$$
 - Tbase

Heat unit (degree days)

for the period between planting and harvest of the plants (Table 4 1) With the exception of the Iole, irrigated and Waipio non-irrigated, the number of heat units required by the plants to reach harvest age after planting ranged from 581 to 769 degree days (Table 4 1). The higher cumulative heat units exhibited by Iole, irrigated plants are likely due to the uneven emergence and the late harvest of these plants. The higher cumulative heat units exhibited by Waipio, non-irrigated plants are due to the delayed and uneven emergence resulting from lack of moisture.

Disregarding the Waipio, non-irrigated and the Iole, irrigated treatments for the reason stated above, Iole plants took the longest chronological time to reach harvest age. But in terms of heat units, or thermal time, the Iole plants required nearly identical heat units to reach harvest age as did plants from Waipio. This suggests that amaranth requires the same number of heat units to reach a particular phenological stage. Because Iole occurs in a cooler environment in takes longer to accumulate the same amount of heat units. According to a National Research Council report (1984), optimal germination temperatures of various accessions of amaranth varied between 16-35°C. The speed of seed emergence was increased at the upper end of this range Iole had the lowest minimum temperature of 13°C (Table 3-20).

where

 $T_{\text{max}} = \text{Maximum daily air temperature}$

min = Minimum daily air temperature

Thase = The lowest temperature whereby the metabolic functions and growth of plants ceases The temperature of 10°C was used for amaranth

Heat unit for a period of plant development is the sum of heat units/day over that period

Table 4 l

Number of days and heat unit (degree days) from date of planting to harvest in the amaranth experiments

Growth duration	Treatment*			Site		<u>. </u>	
			Iole	Kı	ukarau	Wa	סוקו
		Days	Degree days	Days ————	Degree days	Days	Degree days
Planting	NI	95	769	71	581	161	1942
to harvest	I	150	1255	71	581	70	757

^{*} NI = Non-irrigated treatment, I = Irrigated treatment

The lower degree days recorded for the Kukaiau site can be attributed to the early harvest date. Although every attempt was made to harvest each crop just before flowering, it was not possible to forecast flowering and to be on each island to await the proper harvest date.

The fact that it took approximately 25 additional days in Iole non-irrigated treatment to accumulate the same number of heat units as in Waipio clearly indicates that the agroenvironments are different This difference is essential to test the hypothesis that nutrient contents of cultivar depends on the agroenvironment in which it is grown

4 1 2 Dry weight

Based on the concept of equal energy utilized by plants of the same genotype to develop to the same phenological stage, the dry weight

comparisons are made on those that exhibited equal energy utilization (Table 4 1) These include the dry weight comparison between Iole, non-irrigated plants and Waipio, irrigated plants, and the comparison between Kukaiau, non-irrigated and irrigated plants

Table 4 2 shows that the plant dry weights from non-irrigated plots of Iole (90 5 g) and irrigated plots of Waipio (29 5 g) were significantly different. This difference may be attributed almost totally to the difference in agroenvironment. The moisture contents of these two groups of plants were similar as shown in Table 4 5 and, indicate that irrigation in Waipio compensated for natural rainfall in Iole. As shown in Tables 4 24 and 4 25, total N concentration and uptake in Iole non-irrigated plants were significantly higher than those from Waipio irrigated plants. This was likely because there was more N in the Iole soil than in that of Waipio soil (Table 3 17). This should be the main reason for higher dry weight in Iole.

Higher dry weight from the non-irrigated than irrigated plants
(Table 4 2) was also found by Varde (1984) for soybean and cabbage grown
in Kukaiau Tables 4 23 and 4 25 show that uptake of cations anions
and N was higher in plants from the non-irrigated plots than the
irrigated ones

Phosphate fertilizer increased yields significantly in both nonirrigated and irrigated plants in Kukaiau and apparently also increased
yields of non-irrigated plants at Iole (Table 4.3). These results confirm the notion that P is a limiting nutrient in Hydric Dystrandepts
Application of N fertilizer increased the dry weight of Waipio
irrigated plants significantly (Table 4.3). This shows that N was a
major limiting nutrient in the Tropeptic Eutrustox. The Benchmark Soils

Table 4 2
Effect of irrigation treatment on dry weight of amaranth

Treatment	Site								
	Kukanau	Iole	Waipio						
		(g/10 plants)							
Non-irrigated	65 3	90 5 ^a	31 0						
Irrigated	37 3	124 6	29 5 ^a						
Significance level (F-test)	0 0871	0 6864	0 8151						

^aThe two means are significantly different at 0 05 probability level according to the least squares comparison

Project (1979) also demonstrated that P deficiency was frequently encountered in Hydric Dystrandepts while N was deficient in most cultivated Eutrustox due to its susceptability to leaching

Dry weight was not affected significantly by irrigation (Table 4 2) but the irrigation x fertilizer interaction was significant in Waipio plants (Appendix A). Significantly higher dry weight was found in N-treated Waipio, irrigated plants than in the N-treated non-irrigated, Waipio plants (Table 4 3 Figure 4 1). N and uptake of other nutrients in Waipio plants also showed this significant irrigation x fertilizer effects (Appendix A, Table 4 12). This indicates that providing adequate water is essential for proper growth and nutrient uptake in a dry site like Waipio.

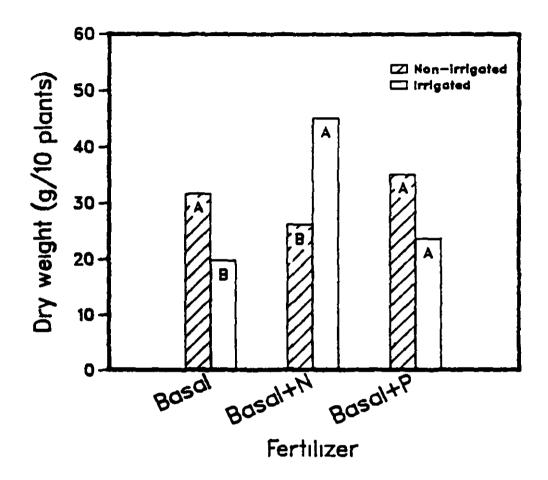
Table 4 3

Effects of fertilizer N and P on dry weights of amaranth in irrigated and non-irrigated plots at three sites

Fertilizer			Dry weigh	t (g/10 plants)			
	Kukanau		Iole		Waipio		
treatment	NI ^a	I ^a	NI ^a	I a	NI ^a	I a	
Basal	35 4 Ba ^b	20 6 Ba ^b	67 5 Aa ^b	151 2 Aa ^b	31 7 Ab ^b	19 8 Ba ^b	
Basal+N	46 1 Ba	23 1 ABa	59 9 Aa	165 3 Aa	26 2 Ab	45 1 Aa	
Basal+P	114 5 Aa	68 2 Aa	144 O Aa	70 9 Aa	35 1 Aa	23 6 Ba	

aNI = Non-irrigated, I = Irrigated

Means within the same location and irrigation treatment followed by the same capital letter are not significantly different at the 0.05 probability level. Means within the same location and fertilizer treatment followed by the same small letter are not significantly different at 0.05 probability level according to the least squares pair comparison



Means of the same fertilizer treatment with the same letter are not significantly different at 0 05 probability level (Waller-Duncan's multiple range test)

Figure 4 1 Effect of irrigation x fertilizer interaction on dry weight of amaranth grown at Waipio site

4 1 3 Moisture content of plant tissue

The higher rainfall in Kukaiau and Iole led to significantly higher tissue moisture contents than those from Waipio (Table 4.4). Table 4.5 shows that in the Kukaiau and Iole sites irrigation did not increase tissue moisture content. In Iole, however, the non-irrigated plants had significantly higher tissue moisture content than the irrigated plants. These observations are not unexpected as Iole and Kukaiau receive sufficient rainfall during much of the year, and irrigation may not be required or in some cases may depress plant growth through excess water. If irrigation is to have sufficient beneficial effects, it would do so in Waipio where rainfall is infrequent. The non-irrigated Waipio plants displayed their response to water stress by their small leaves and large proportion of stems (Table 3.3)

Table 4 4
Tissue moisture contents of amaranth grown at different sites

Site	Moisture content* (% fresh weight)
Kukarau	89 7 A
Iole	87 1 B
Waipio	85 7 C

^{*}Means followed by the same letter are not significantly different at 0 05 probability level (Waller-Duncan's multiple range test)

Table 4 5

Moisture content of amaranth grown at three different sites as affected by irrigation treatments

Site	Treatment ^a	Tissue moisture conter (% fresh weight)
Kukatau	NI	89 6
	I	89 9
Significance level (F-test)		0 6185
Iole	NI	88 9
	I	85 0
Significance level (F-test)		0 0014
Waipio	NI	81 4
	I	90 1
Significance level (F-test)		0 0001

^aNI = Non-irrigated, I = Irrigated

4 2 Oxalate composition of amaranth tissue

4 2 1 Forms and amount of oxalate in amaranth

Two dominant fractions were found in amaranth. These were the water soluble and water insoluble fractions. Free oxalic acid (alcohol soluble fraction) could not be detected by the methods used in this study.

In an attempt to identify the accompanying cation in each oxalate fraction correlations between the oxalate concentration of each fraction with its cations and with the cations in the plant tissue were determined (Table 4 6, Figures 4 2-4 5)

The high correlation between the concentrations of tissue K and K in the soluble oxalate fraction with the soluble oxalate, r=0 6392 and 0 6403, respectively (Table 4 6, Figure 4 2), suggested that K oxalate was the main form of soluble oxalate. However, the higher correlation between the concentration of K plus Mg in the soluble oxalate fraction with the soluble oxalate, r=0 8558, suggested that Mg oxalate was an important part of the soluble oxalate (Figure 4 3)

Considering the insoluble oxalate fraction, the high and significant correlation between the concentrations of tissue Ca and Ca in the insoluble oxalate fraction with that of the insoluble oxalate (Table 4 6 Figure 4 4) shows that Ca oxalate was the main form of this fraction. However, the improved correlation with Ca plus Mg was substituted for Ca (Figure 4 5) suggested that Mg oxalate was also an important constituent of the insoluble oxalate fraction. In addition a portion of the Mg oxalate was probably extracted by hot water

Table 4 6

Correlation between soluble and insoluble fractions of oxalate with mineral composition of plant tissue and of fractionation extracts of amaranth

			Correlation co-	
	composit and fract		Soluble fraction of oxalate	Insoluble fraction of oxalate
Tissue K	<u> </u>		0 6392 (0 0001)	-0 2146 (0 1346)
C	Ça .		-0 3869 (0 0055)	0 6043 (0 0001)
" M	lg		-0 0436 (0 7639)	-0 1528 (0 2896)
" N	la		-0 0090 (0 9505)	-0 2031 (0 1571)
Soluble	fraction	K	0 6403 (0 0001)	-0 3465 (0 0128)
tt	1	Ca	-0 1689 (0 2360)	-0 1112 (0 4371)
	11	Mg	0 4087 (0 0029)	-0 3549 (0 0126)
ı	11	Na	0 0287 (0 8413)	-0 2538 (0 0723)
н	1	K + Mg	0 8558 (0 0001)	-0 5493 (0 0001)
Insolubl	e fractio	on K	-0 3240 (0 0204)	0 6980 (0 0001)
и	11	Ca	-0 3417 (0 0141)	0 6541 (0 0001)
it.	11	Мд	-0 0930 (0 5161)	0 5227 (0 0001)
11		Na	-0 1702 (0 2325)	0 3849 (0 0053)
	11	Ca + Mg	-0 3349 (0 0163)	0 7410 (0 0001)

^{*}Number of observations = 54

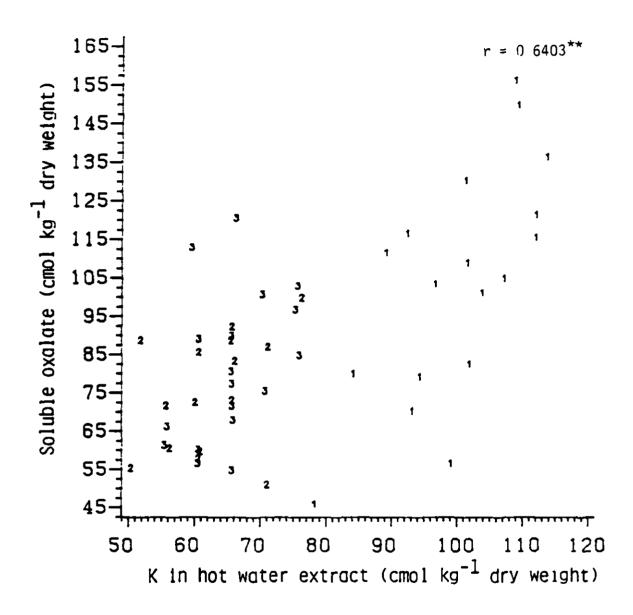


Figure 4 2 Relationship between soluble oxalate and K concentration in the extract of soluble oxalate fraction (hot water extract) of amaranth tissue (1=Kukaiau, 2=Iole, 3=Waipio)

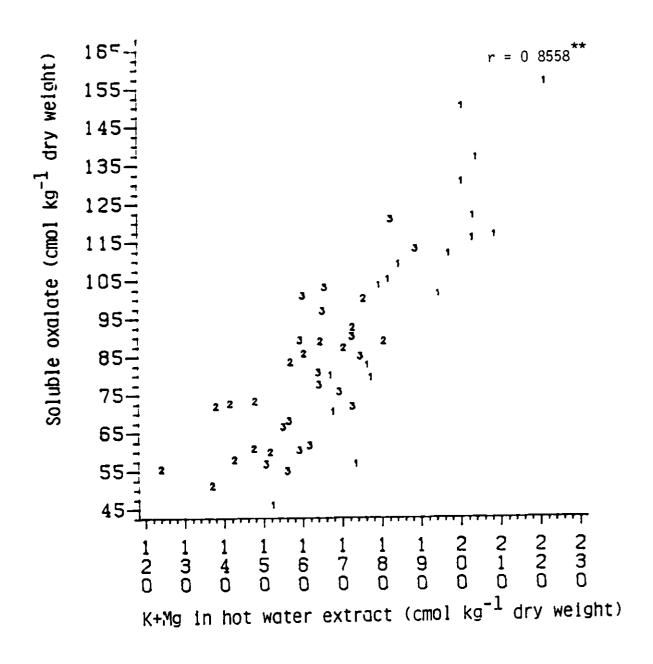


Figure 4 3 Relationship between soluble oxalate and the sum of potassium and magnesium concentration in the extract of soluble oxalate fraction (hot water extract) of amaranth tissue (1=Kukaiau, 2=Iole, 3=Waipio)

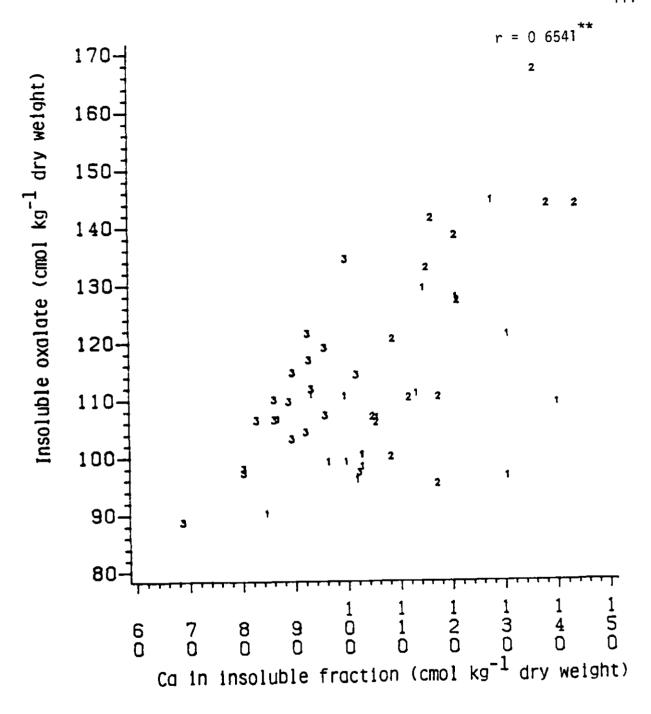


Figure 4 4 Relationship between insoluble oxalate and Ca concentration in the extract of insoluble oxalate fraction of amaranth tissue (1=Kukaiau, 2=Iole, 3=Waipio)

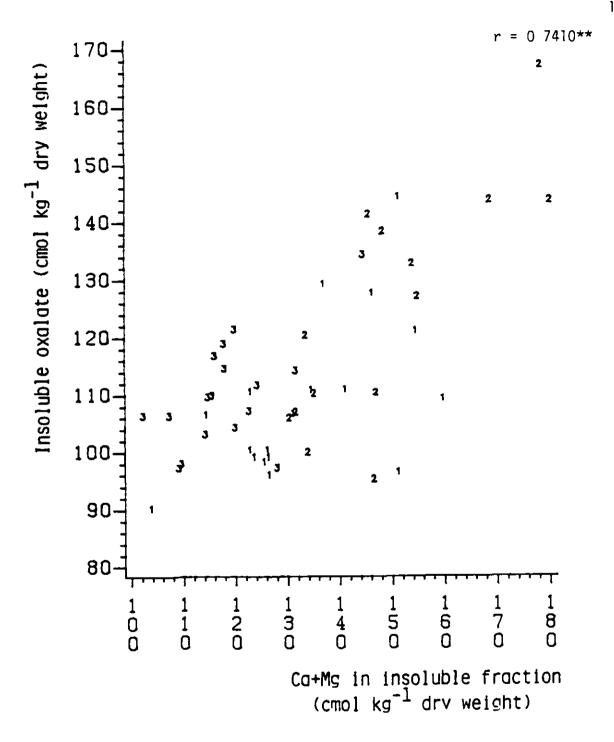


Figure 4.5 Relationship between insoluble oxalate and the sum of Ca and Mg concentration in the extract of insoluble oxalate fraction of amaranth tissue (1=Kukaiau, 2=Iole, 3=Waipio)

The analysis of cations, including Ca, Mg, K and Na, in the soluble and insoluble fractions showed that the dominant cations in the soluble and insoluble fractions were respectively K and Ca (Table 4.7). There was relatively little K in the insoluble fraction extract and little Ca in the soluble fraction extract (Table 4.7). In Table 4.7, the sum of the cations must be greater than the oxalate concentration because other organic and inorganic anions were present in the extract

The concentration of soluble oxalate in plants from all three sites were nearly the same as that of K. It is likely that the remainder of soluble oxalate was in the forms of Mg oxalate and Na oxalate. The small amount of Ca found in this fraction was probably associated with water soluble salts, such as $CaCl_2$ (Table 4.7a)

Considering the insoluble fraction (Table 4.7b), the high Ca concentration which almost matched the concentration of insoluble oxalate was strong evidence that Ca oxalate was the dominant form in the insoluble fraction. The remaining insoluble oxalate should be in the form of Mg oxalate. The correlation between Mg in the insoluble fraction and insoluble oxalate was significant (Table 4.6).

Total oxalate in amaranth grown in Kukaiau was more than 9%

It is interesting to note that higher concentrations of insoluble
oxalate were found in amaranth grown in all sites (Table 4-8) Plants
from Iole had 27% more insoluble oxalate than soluble oxalate, while
Waipio plants had 14% and Kukaiau plants had 2° more

Two values of total oxalate were obtained in this study. One was measured directly and the other was computed by adding the two

Table 4 7

Mineral concentrations in the (a) soluble, and (b) insoluble fractions of oxalate in amaranth from all plots in three sites

Site	(a) Minerals and oxalate concentration (cmol kg ⁻¹											dry weight)	
	Oxal at		Ca	Mg	K		N		SC		Total cat	l Ion -	
Kukanau	104 0	1	0 9	88 6	100	0	4	 5	194	1	90	1	
Iole	75 2	(8 0	91 6	62	5	3	7	158	6	83	4	
Waipio	81 6		1 0	99 9	65	6	9 !	9	176	4	94	8	

(b)

Site	Minera	ls and	oxalate	concent	ration	(cmol kg	dry weight)
	0xalat	e Ca	Mg	K	Na	SC*	Total cation - total oxalate
Kukanau	108 7	108 5	24 7	2 3	3 1 4	136 9	28 2
Iole	128 2	120 4	30 5	3 4	1 4	155 7	27 5
Waipio	108 8	89 8	27 3	1 4	1 3	119 8	11 0

^{*} Sum of cation concentration

Table 4 8

Relative concentrations of different fractions of oxalate produced in amaranth grown at three sites

Site		% dry v	veight		9		
	Total	Soluble+ insoluble	Soluble	Insoluble	Soluble	Insoluble	
Kukarau	9 2	4 7	9 6	4 9	49	51	
Iole	8 4	9 2	3 4	5 8	37	63	
Waipio	7 5	8 6	3 7	4 9	43	57	

oxalate fractions Since the two values were significantly different (Table 4 9), the sum of the two fractions was used to obtain percent of any fraction relative to total oxalate

Table 4 9

Comparison of the two means of total oxalate of amaranth obtained by two different methods

Mean of soluble+insoluble oxalate			
7			

Probability > |t| = 0 0035

4 2 2 Oxalate and calcium concentrations in amaranth tissue

Figure 4 6 shows that the concentrations of insoluble oxalate and tissue Ca were very close. This indicates that most Ca in amaranth tissue was bound in the form of Ca oxalate and was not available as a nutrient

4 2 3 Comparison of chemical composition in plants grown at different sites

Concentrations and productions of total soluble, insoluble and the combination of the soluble and insoluble oxalate were significantly different among the sites (Tables 4 10 and 4 11). Plants from Kukaiau had more total and soluble oxalate than those from Iole and Waipio. On the other hand, plants in Iole had higher insoluble oxalate concentration than Kukaiau and Waipio plants (Table 4 10). Iole plants produced significantly higher quantities of all forms of oxalate than Kukaiau or Waipio plants (Table 4 11).

Table 4 10

Oxalate concentrations in amaranth grown at three sites

Site			-	Oxalat	e c	oncer	itrations	(cmol	l kg ⁻¹ dry w	eight)	
	Total*			Soluble*		Insoluble*			Soluble + insoluble*		
Kukalau	204	3	Α	104	0	Α	108 7	В	212 7	Α	
Iole	186	9	В	75	2	В	128 2	Α	203 4	Α	
Waipio	166	3	С	81	6	В	108 8	В	190 4	В	

^{*} Means in the same column followed by the same letter are not significantly different at 0 05 probability level (Waller-Duncan's multiple range test)

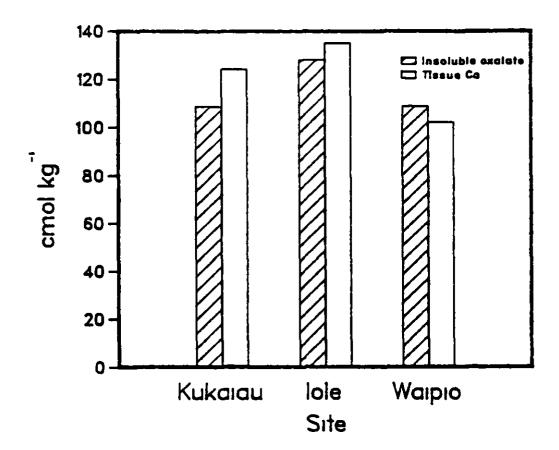


Figure 4 6 Comparative concentration of insoluble oxalate and tissue Ca in amaranth growth at three sites

Table 4 11
Oxalate production in amaranth grown at three different sites

Site	Oxalate production (g/10 plants)												
	Tota	il*	So	luble*	I	nso	luble*			le + luble*			
Kukarau	4 6	В	2	D B	2	7	В	4	7	В			
Iole	98	Α	3	7 A	7	0	Α	10	8	Α			
Waipio	2 3	В	1	1 В	1	5	В	2	6	В			

^{*}Means in the same column followed by the same letter are not significantly different at 0.05 probability level (Waller-Duncan's multiple range test)

Ionic and total N concentrations in plant tissue are thought to be factors affecting the oxalate concentrations in plants and Iole plants had higher tissue ionic concentrations and uptake values than Waipio plants (Tables 4 12 and 4 13) They also had higher total N concentration and uptake than Waipio plants (Tables 4 18 and 4 19) Schmidt et al (1971) reported that Amaranth species which were grown on soils with high soil carbon, nitrogen available phosphorus and potassium contents produced higher total oxalate relative to those grown on less fertile soils Although the Kukaiau and Iole soils were less fertile than the Waipio soil with respect to base status they had higher nitrogen contents than the Waipio soil Consequently plants from Kukaiau and Iole had higher concentrations and uptake of various ions than those from Waipio More importantly, Kukaiau and Iole plants had higher cation excess than Waipio plants (Table 4 12) According

Table 4 12

Ionic concentrations and cation excess contents of amaranth grown at three sites

Site	Ca	Mg	K 	Na	SC** cmol kg ⁻¹	P dry we	S eight	NO ₃	c1 	SA***	C-A
Kuka1au	124 4 B* (2 49)		* 147 6 A* (5 77)							49 9	326 3 A*
Iole		•	117 9 B (4 61)							43 0	333 3 A
Waipio	102 2 C (2 04)		108 2 C (4 23)							29 6	301 7 B

^{*} Means in the same column followed by the same letter are not significantly different at 0 05 level of probability (Waller-Duncan's multiple range test)

^{**} Sum of cation concentration

^{***} Sum of anion concentration

Table 4 13

Ionic uptake by amaranth grown at three sites

Site	Ca	Mg	K	Na		S	_{NO} 3	C1
				-g/10 plan	ts			
Kukatau	1 4 B*	0 6 B*	2 9 B*	0 06 B*	0 17 B*	0 23 B*	0 95 A*	0 14 B*
Iole	3 4 A	1 5 A	5 0 A	0 10 A	0 27 A	0 53 A	1 44 A	0 27 A
Waipio	0 6 B	0 4 B	1 3 В	0 06 B	0 09 B	0 14 B	0 18 B	0 08 B

^{*} Means in the same column followed by the same letter are not significantly different, at 0 05 level of probability (Waller-Duncan's multiple range test)

to the cation-amon balance concept, organic acids including oxalate are synthesized to balance excess cations

The significantly higher soluble oxalate concentration in Kukaiau plants (Table 4 10) was probably due to the higher equivalent concentration of tissue K in these plants compared with Iole and Waipio plants (Table 4 12) In the previous section (4 2 1) it was concluded that soluble oxalate was primarily K oxalate

Higher concentration and production of insoluble oxalate in plants grown in Iole relative to Kukaiau and Waipio plants (Tables 4 10 4 11) may be attributed to the higher Ca and Mg concentrations of Iole (Table 4 12)

Soluble oxalate concentrations of lole and Waipio plants were significantly different when only irrigated plants were considered (Table 4 14). This showed that when the variance due to irrigation effect was removed, the significant site effect emerged. The concentration of the sum of soluble and insoluble oxalate of the plants from Kukaiau and Iole were significantly different when only non-irrigated plants were considered as shown in Table 4 15

The plants from Waipio, irrigated plots also had higher tissue

K, Mg and Na but lower Ca concentration than those from Iole (Table

4 22) This was in accordance with the higher soluble oxalate in Waipio
irrigated plants than Iole plants

It appears from these findings that the amount and distributions of the various oxalate fractions were determined to a certain extent by the type and amount of cations in amaranth tissue. In plants from Iole, Ca was the dominant cation (Table 4-12) and insoluble oxalate

Table 4 14

Soluble oxalate concentration of amaranth grown in irrigated experiments of Iole and Waipio

Site	Soluble oxalate (cmol kg ^{-l} dry weight)
Iole	68 5
Waipio	85 0
Significance level (LSD)	0 0460

Table 4 15

Concentration of the sum of soluble and insoluble oxalate of amaranth grown in non-irrigated experiments of Kukaiau and Iole

Site	Soluble+insoluble oxalate (cmol kg ^{-l} dry weight)
Kukanau	212 2
Iole	191 0
Significance level (LSD)	0 0090

was the principal form of oxalate (Table 4 10) On the other hand,

K was the dominant cation in Kukaiau plants (Table 4 12), but there

was no significant difference between the concentrations of soluble

and insoluble oxalate (Table 4 10) The Waipio plants had approximately

equal concentrations of K and Ca (Table 4 12), but insoluble oxalate

was the dominant form of oxalate (Table 4 10) This last result together

with that from Kukaiau seemed to suggest that amaranth tended to

synthesize Ca oxalate in preference to K oxalate

Factors which affect ionic uptake by plants influence the kind and amount of oxalate produced by plants (Tables 4 10, 4 12). The plants grown in the high base, low N, Tropeptic Eutrustox did not have as high concentrations of bases in their tissue as those grown in the low base, high N Hydric Dystrandepts. This indicates that without adequate N, plants cannot make use of available resources, such as bases or phosphorus

Tonic concentrations and uptake of plants grown at different sites

The Hydric Dystrandept in Iole appeared to be richer in Ca Mg and K than the soil at Kukaiau (Table 3 17). This contributed to the higher concentrations of Ca and Mg in plants from Iole than in those from Kukaiau (Table 4 12). However, the significantly higher K concentration in the Kukaiau plants than in the Iole plants, in spite of the higher K concentration in the Iole soil (Table 3 17) may be due to dilution from higher biomass production in Iole.

It is likely that the significantly higher Na concentration in Waipio plants relative to Kukaiau and Iole plants was due to a high

Na concentration in the irrigation water This was also true for Cl concentration (Table 4 12)

With the exception of Na, the higher cation and anion uptake by Kukaiau and Iole plants relative to Waipio plants (Table 4 13) was likely due to the higher biomass and plant demand of Kukaiau and Iole plants than Waipio plants. Earlier studies have shown that ion uptake is closely related to plant growth and to the supply of sugar and metabolites translocated from the shoot to the root (Pitman, 1972, White 1973)

Relationship between oxalate concentrations and cation excess content

Total oxalate from all sites exhibited significant correlation with cation excess (Table 4 16, Figure 4 7). However, when the relationship was analyzed locationwise, only Waipio plants exhibited high and significant correlation between total oxalate and (C-A) (Table 4 16, Figure 4 8). The same situation was applicable to soluble+insoluble oxalate (Table 4 16, Figures 4 9 4 10). Significant correlations although not considered high, were also found between soluble oxalate and (C-A) in Waipio, and insoluble oxalate and (C-A) in Kukaiau (Table 4 16).

The results in Table 4 17 suggest that about 60% of the cation excess in amaranth grown at the three sites was balanced by oxalate

Total N and nitrate-N concentrations and uptake and crude protein contents

Total N concentration and uptake were significantly higher in Kukaiau and Iole plants than those from Waipio (Tables 4 18 and 4 19)

Table 4 16

Correlation between concentrations of different oxalate forms and cation excess

Oxalate	Correlation coefficient (Probability > r)								
	Combined ^a sites	Kukan au ^b	Iole ^b	Walpio ^b					
Total	0 3916	-0 3441	0 3158	0 6156					
	(0 0045)	(0 1620)	(0 2515)	(0 0065)					
Soluble	-0 0108	-0 3697	0 0940	0 4815					
	(0 9405)	(0 1311)	(0 7494)	(0 0431)					
Insoluble	0 4793	0 5229	0 3619	0 3905					
	(0 0004)	(0 0206)	(0 2036)	(0 1091)					
Soluble +	0 3442	-0 1751	0 4146	0 5790					
insoluble	(0 0144)	(0 4871)	(0 1405)	(0 0118)					

aNumber of observations = 54

^bNumber of observations = 18

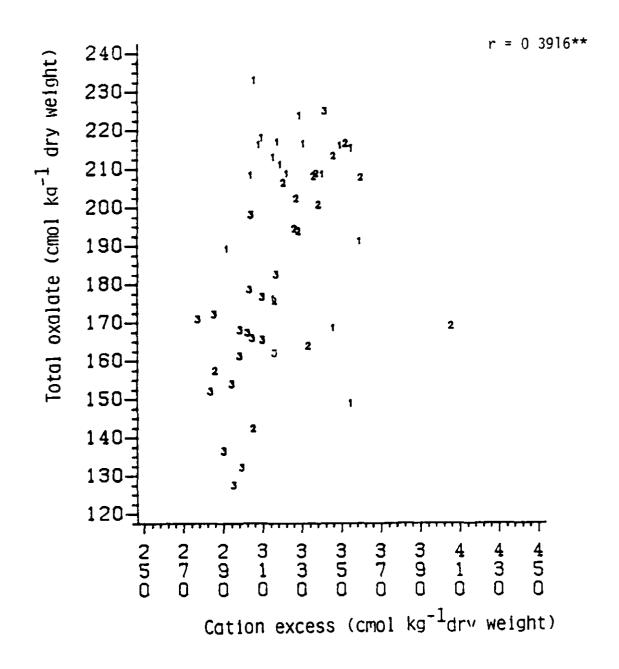


Figure 4.7 Relationship between total oxalate and cation excess in amaranth from all plots of the experiments at three sites (I-Kukaiau, 2=Iole, 3=Waipio)

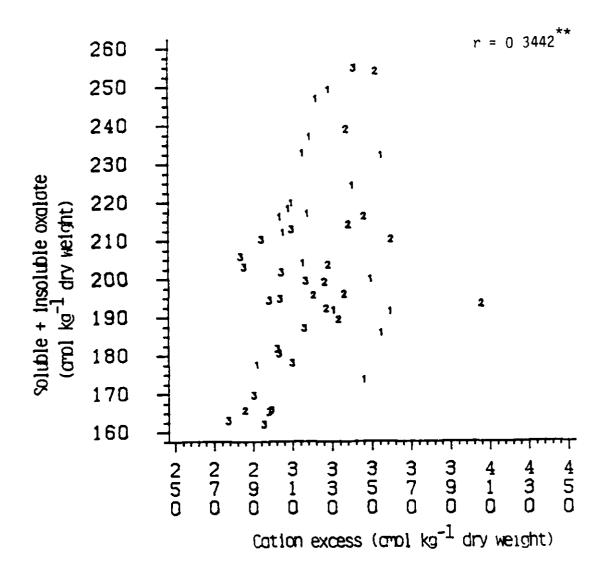


Figure 4.8 Relationship between the sum of soluble and insoluble oxalate and cation excess in amaranth from all plots of the experiments at three sites (1=Kukaiau, 2=Iole, 3=Waipio)

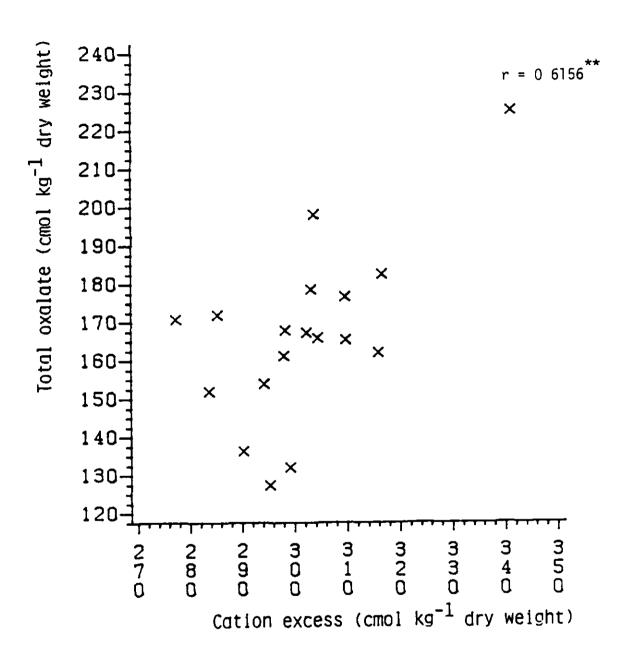


Figure 4.9 Relationship between total oxalate and cation excess in amaranth from Waipio site

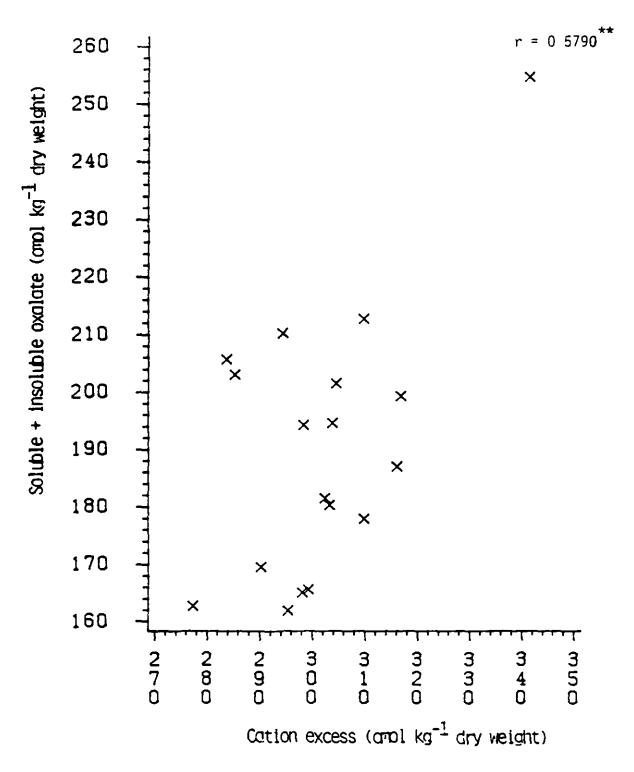


Figure 4 10 Relationship between the sum of soluble and insoluble oxalate and cation excess in amaranth from Waipio site

Proportion of total oxalate and the sum of soluble and insoluble oxalate relative to cation excess (C-A)

Site (C-A) (cmol kg ⁻¹)		Total oxal	ate_	Soluble+insoluble oxalate			
	Conc (cmol kg ⁻¹)	% (C-A)	Conc (cmol kg ⁻¹	% (C-A)			
Kukatau	326 3	204 3	62 6	212 7	65 2		
Iole	333 3	186 9	56 1	203 4	61 0		
Waipio	301 7	166 3	55 1	190 4	63 1		

Table 4 18

Total N, nitrate-N concentration and crude protein content of amaranth grown at three sites

Site	Total N*	Nitrate-N*	Crude protein*
Kuka 1 au	4 53 A	0 52 A	28 3 A
Iole	4 28 B	0 33 B	26 8 B
Waipio	3 28 C	0 13 C	20 5 C

^{*} Means in the same column followed by the same letter are not significantly different at 0 05 probability level (Waller-Duncan's multiple range test)

Table 4 19

Total N and nitrate-N uptake by amaranth grown at three sites

Site	Total	N*	Nit	rat	e-N*
		g/l	O plants ·		
Iole	4 77	Α	1	44	Α
Kukarau	2 24	В	0	95	Α
Waipio	0 99	В	0	18	В

^{*} Means in the same column followed by the same letter are not significantly different at 0.05 probability level (Waller-Duncan's multiple range test)

and this is related to soil nitrogen levels. When the tissue total N values were converted to crude protein (on a dry weight basis), they were comparable to the values of 27-33% reported by the National Research Council (1984) for four species of amaranth. However, the crude protein of Waipio plants was lower relfecting the low N content of the Tropeptic Eutrustox.

The higher total N uptake in Kukaiau and Iole plants (Table 4-19) reflected larger biomass production at Kukaiau and Iole. Even though the Waipio plants especially the irrigated ones, had the advantage of higher solar radiation and temperature relative to Kukaiau and Iole plants, low N prevented higher yields from being achieved in Waipio When soil N is deficient growth rate is low and plant nutrient demand is also low consequently the uptake of other nutrients becomes low

also, although they are available. White (1973) has pointed out that P uptake depends on the relative growth rate and on environmental factors, including the supply of other essential nutrients, such as nitrogen

The nitrate-N content of amaranth grown in Kukaiau and Iole (Table 4 18) were comparable to the content of nitrate-N in various varieties of Amaranthus gangeticus L (0 3-0 9% on a dry weight basis) reported by Der Marderosian et al (1981). However, the nitrate-N content in Waipio plants was unusually low only 0 13% (Table 4 18). This is additional evidence that the N content of the Waipio soil was too low to support the normal growth of plants. Nitrate accumulation is regarded as a natural and necessary process in plants and attempts to reduce nitrate accumulation can result in yield reduction (Lorenz, 1978).

Whether nitrate concentration found in amaranth in this study was potentially toxic depends on the amount of fresh vegetable consumed Given a moisture content of 90% and a nitrate-N content on a dry weight basis of 0 5% (e.g., the content in amaranth grown in Kukaiau, Table 4 18), the nitrate-N on a fresh weight basis would be 0 05% According to Gilbert et al (1946) 0 5 g is a toxic daily dose. Thus approximately 1000 g of fresh amaranth would need to be consumed each day to attain toxic levels of nitrate-N intake Der Marderosian et al (1981) did not think that more than 100 g fresh green vegetables would be consumed in a day. Their argument was probably based on an American The recommended quantity of vegetable diets for various groups diet of people in Africa is 150 g (Latham 1979) Therefore it appears that the nitrate-N content in amaranth will not be a health hazard unless an unlikely large quantity of this vegetable is consumed fresh

When green vegetables are cooked especially by boiling or blanching, a large part of nitrate is dissolved in cooking water Vegetables prepared this way will have less nitrate-N content, and less potential health hazard

4 2 4 Effect of irrigation on plant chemical composition

The uneven performance of amaranth did not permit the effect of irrigation to be interpreted clearly. As pointed out in Chapter III the irrigated plots at each experimental site were treated as if irrigation was the main plot of a split-plot design when in fact it was not. Thus, the irrigation effect should be interpreted with this in mind. In addition, the Kukaiau and Iole sites are naturally wet and, consequently, irrigation would not necessarily result in different plant performance. This is reflected in moisture contents of amaranth from Kukaiau and Iole as shown in Table 4.5

On the other hand, Waipio is a dry site and hence, the irrigation effect was anticipated to be greater. However, the effect of irrigation there was confounded by the fact that the non-irrigated and irrigated crops were harvested at different times which resulted in their being exposed to different weather. This was also true for the Iole, non-irrigated and irrigated plants where the irrigated crop was left in the field for a much longer period.

The significantly higher insoluble oxalate content in Tole irrigated amaranth relative to the non-irrigated treatment as shown in Table 4.20 went hand in hand with the higher Ca concentration in the amaranth from the irrigated plots (Table 4.22). This difference

Table 4 20

Effect of irrigation on oxalate concentration of amaranth grown at three sites

Oxalate	Irrigation	(сто	(cmol kg dry weight)			
	treatment	Kukalau	Iole	Waipio		
Total	non-irrigated	201 3	190 5	169 0		
	Irrigated	207 4	182 2	163 6		
Significance l	evel	0 5889	0 4755	0 4722		
(F-test)						
Soluble	Non-irrigated	99 0	78 4	78 2		
	Irrigated	108 9	70 4	85 0		
Significance le	eve1	0 4009	0 3383	0 5060		
(F-test)						
Insoluble	Non-irrigated	113 2	112 7	110 4		
	Irrigated	104 2	151 6	107 2		
Significance le	evel	0 0727	0 0235	0 5155		
(F-test)						
Soluble+Insoluble	Non-irrigated	212 2	191 0	188 6		
	Irrigated	213 1	221 9	192 2		
Significance lo	evel	0 9379	0 2476	0 7107		
(F-test)						

in insoluble oxalate content was most likely due to the difference in age and harvest time

The higher concentration of soluble oxalate in irrigated than the non-irrigated amaranth from Kukaiau (Table 4 20) appears to be associated with the higher K concentration in the irrigated plants (Table 4 22). On the other hand, soluble oxalate production and K uptake were lower in the irrigated Kukaiau plants than in the non-irrigated ones (Tables 4 21, 4 23). This suggests that the lower concentration of soluble oxalate in the non-irrigated amaranth relative to the irrigated ones was primarily due to dilution from high dry matter yield (Table 4 2).

Significant differences in Ca and P contents of plants from the irrigated and non-irrigated plots were observed (Table 4 22). These differences may be the consequence of factors other than the irrigation treatment. The significantly higher Ca concentration in irrigated Iole plants, and non-irrigated Waipio plants than their counterparts may be simply due to lower Ca in the counterpart soil (Table 3 17). The same argument can be applied to the higher P content in irrigated Waipio plants than the non-irrigated ones (Table 4 22).

Significantly higher Na and C1 concentrations in Waipio irrigated plants relative to the non-irrigated ones was probably due to the high Na and C1 in the irrigation water

Irrigation had significant effects on total N and nitrate-N concentrations only in Iole plants (Table 4 24). There are reasons to believe that this difference is not due to irrigation. It should be recalled that the irrigated plants in Iole emerged unevenly and at a

Table 4 21

Effects of irrigation on the production of oxalates in amaranth grown at three sites

Oxalate	Irrigation		g/10 plants					
	treatment	Kuk	Kukatau		Iole		Waipio	
Total	Non-irrigated	5	7	7	6 ^a	2	4	
	Irrigated	3	5	12	6	2	2 ^a	
Significance	e level	0	1402	0	3417	0	7688	
(F-tes	t)							
Soluble	Non-irrigated	2	5	3	$o^{\mathbf{b}}$	1	09	
	Irrigated	1	6	4	9	1	13 ⁵	
Significance level		0	1373	0	5850	0	8228	
(F-tes	t)							
Insoluble	Non-irrigated	3	6	4	6 ^C	1	54	
	Irrigated	1	8	10	7	1	48 ^C	
Significance	e level	0	0536	0	3996	0	8401	
(F-tes	t)							
Soluble +	Non-irrigated	6	0	7	6 ^d	2	63	
ınsoluble	Irrigated	3	4	15	5	2	61 ^d	
Significant	level	0	0764	0	4506	0	9652	
(F-test	t)							

a The two means are significantly different at 0 0436 probability level (LSD test)

Table 4 22

Effect of irrigation on ionic concentration of amaranth grown at three sites

Site	Irrı- gatıon*	cmol kg ^{-l} dry weight (% dry weight)							
		Ca	Mg	K	Na	Р	S	NO ₃	Cl
Kukarau	NI	125 9 (2 59)	101 3 (1 22)	144 5 (5 64)	5 0 (0 11)	3 1 (0 30)	8 7 (0 42)	7 6 (0 47)	7 2 (0 26)
	I	122 8 (2 45)	97 4 (1 17)	150 7 (5 89)	5 1 (0 12)	3 7 (0 36)	7 9 (0 38)	6 4 (0 40)	6 9 (0 24)
Probability > F		0 7961	0 5778	0 0017	0 7846	0 3786	0 2334	0 2088	0 4570
Iole	NI	126 5 (2 53)	119 4 (1 43)	126 1 (4 93)	5 2 (0 12)	2 7 (0 26)	9 9 (0 48)	6 9 (0 43)	6 8 (0 24)
	I	146 2 (2 92)	117 1 (1 41)	107 3 (4 19)	4 5 (0 10)	2 6 (0 25)	10 3 (0 50)	3 2 (0 20)	7 0 (0 25)
Probability > F		0 0445	0 7897	0 0222	0 0295	0 0454	0 4009	0 0222	0 5493
Waipio	N1	109 6 (2 19)	100 7 (1 21)	106 6 (4 16)	4 7 (0 11)	1 9 (0 18)	9 0 (0 43)	2 2 (0 14)	7 0 (0 25)
	I	94 8 (1 90)	124 1 (1 49)	109 9 (4 29)	12 4 (0 29)	4 5 (0 44)	9 7 (0 47)	2 2 (0 14)	8 3 (0 29)
Probability > F		0 0055	0 0176	0 4955	0 0001	0 0001	0 0184	0 9702	0 0033

^{*} NI = Non-irrigated I = Irrigated

Table 4 23

Effect of irrigation on ion uptake by amaranth grown at three sites

Site	Irrı- gatıon*								
	gueron	Ca	Mg	К	Na	Р	S	NO ₃	C1
Kukatau	NI	1 76	0 78	3 62	0 08	0 21	0 30	1 26	0 18
	I	1 02	0 42	2 18	0 04	0 14	0 15	0 65	0 10
Probability > F		0 1244	0 0523	0 1218	0 0885	0 1578	0 0611	0 1138	0 0917
Iole	NI	2 33	1 25	4 44	0 11	0 26	0 44	1 51	0 23
	I	4 86	1 87	5 70	0 13	0 28	0 64	1 36	0 33
Probability > F		0 2888	0 5218	0 6999	0 7507	0 9774	0 5609	0 7761	0 5596
Waipio	NI	0 68	0 38	1 28	0 03	0 06	0 13	0 19	0 08
·	I	0 58	0 43	1 30	80 0	0 12	0 14	0 18	0 09
Probability > F		0 4404	0 4848	0 9677	0 0261	0 0444	0 8752	0 9738	0 6504

^{*} NI - Non-irrigated I = Irrigated

Table 4 24

Effect of irrigation on total N and nitrate-N concentration in amaranth grown at three sites

Irrigation	Kukanau		Iole	Waipio
	Total N	NO3-N	Total N NO ₃ -N -(% dry weight)	Total N NO ₃ -N
Non-irrigated	4 52	0 47	4 68 ^a 0 43 ^b	3 15 0 14
Irrigated	4 55	0 40	3 77 0 20	3 41 ^a 0 13 ^b
Probability > F	0 8453	0 208	3 0 0167 0 022	2 0 3363 0 9702

a, b The two means superscripted by the same small letter are significantly different at 0 0001 probability level (LSD test)

much slower rate. This fact coupled with the lower N contents in the irrigated amaranth relative to their non-irrigated counterpart. in spite of the low soil N in the non-irrigated plots (Table 3-19), seems to indicate that other factors contributed to the poor growth and low utilization of nutrients.

4 2 5 Effects of N and P fertilizers on plant chemical compositions

Only P fertilizer had significant effects on oxalate content in amaranth grown in each site (Table 4.26). The significantly lower total oxalate concentration in P-treated plants in Kukaiau and Waipio and the apparent decrease in Iole plants are in accordance with previous findings by Peck et al. (1980) with table beets. The cation-anion balance concept was inadequate to explain this effect (Table 4.33) since

Table 4 25

Effect of irrigation on total N and nitrate-N uptake by amaranth grown at three sites

Irrigation	Kukalau		I	ole	Wa	1 p 10
	Total N	NO ₃ -N	Total N g/ 10 p	3	Total N	NO3-N
Non-irrigated	2 83	1 26	4 13 ^a	1 51 ^b	1 00	0 19
Irrigated	1 65	0 65	1 83	0 51	1 01 ^a	0 18 ^b
Probability > [F]	0 1025	0 1138	0 6235	0 7761	0 8576	0 973

a, b The two means superscripted by the same small letter are significantly different at 0 01 probability level (LSD test)

phosphorus application did not lower cation excess significantly relative to the non-P fertilizer treatments Peck et al (1980) found that P fertilizer lowered cation excess This study showed that in any particular site the sum of tissue cations was not affected by P fertilizer (Table 4 29) Plant K concentration, however, was significantly lowered by P fertilization relative to non-P treated plants in Kukaiau and Waipio On the other hand, Ca concentration was significantly higher in the P-treated Kukaiau plants and apparently increased in P-treated Iole plants Mg also decreased, but not significantly in the phosphorus plots in all sites Therefore P fertilizer in this study did not alter cation concentration in plants but did affect K and Ca and perhaps Mg differently

Table 4 26

Effects of fertilizers on various forms of oxalate in amaranth grown at three sites

Oxalate form	Fertilizer				late co ol kg ⁻	_				
		Kuka	n a	u*	Iole	<u>*</u>		Wall	010	*
Total	Basal	214	6	Α	190	7	Α	173	3	А
oxalate	Basal+N	213	5	Α	195	3	Α	179	6	Α
	Basa1+P	184	9	В	176	6	Α	145	9	В
Soluble	Basal	133	1	Α	86	0	Α	80	6	AB
oxalate	Basa1+N	105	5	В	73	8	AB	96	9	Α
	Basa1+P	73	3	С	65	6	В	67	5	В
Insoluble	Basa1	99	8	В	125	1	Α	103	5	Α
oxalate	Basa1+N	104	6	В	127	5	Α	115	5	Α
	Basa1+P	121	7	Α	132	1	Α	107	4	A
Soluble +	Basa1	232	9	Α	211	ı	Α	184	0	В
ınsoluble	Basal+N	210	1	В	201	3	Α	212	4	Α
oxalate	Basal+P	195	1	В	197	7	Α	174	9	В

^{*} Means of the same oxalate form and the same location followed by similar letter are not significantly different at 0 05 probability level (Waller-Duncan's multiple range test)

A significant decrease in soluble oxalate was observed in plants wherever phosphorus was applied (Table 4 26). This decrease was likely due to the effects of P fertilizer on decreasing K and probably Mg concentrations in the plants (Table 4 29). Significant increases in insoluble oxalate concentration in P-treated Kukaiau plants may be due to the additional Ca supplied to the plants by triple superphosphate. The increase in the insoluble oxalate in Iole was not significant, however this is probably related to the fact that the soil there was well supplied with Ca.

The insoluble oxalate content of amaranth in Waipio was not affected significantly by phosphorus fertilization (Table 4 26). The rate of phosphorus application was much less in Waipio (145 kg P ha⁻¹) than in either Kukaiau or Iole (225 kg P ha⁻¹)

Nitrogen fertilizer did not significantly affect various forms of oxalates in Kukaiau and Iole, but did increase concentrations of various forms of oxalate in Waipio (Table 4.26). Previous work showed that addition of N (particularly in the form of nitrate) tended to increase organic acid in plants (Kirkby and Knight, 1977). Ben-Zioni et al. (1970) observed the reduction of nitrate in leaves of corn tobacco and barley, resulted in build-up of cations in the tissue which in turn was neutralized by malic acid. They observed a stoichiometric relation between the amount of nitrate reduced and malate accumulated

Correlation of N and P fertilizers with oxalates shows that P has negative relationships with total soluble and insoluble oxalates in non-irrigated plants and with soluble oxalate in irrigated plants. N fertilizer exhibited a significant positive relationship with total oxalate in irrigated plants (Table 4 27)

Table 4 27

Correlation between N and P fertilizers with oxalates in irrigated and non-irrigated plots of amaranth

Irrigation	Fertilizer	С	a		
-		Total	Soluble	Insoluble	Soluble + insoluble
Non-	N	ns ^b	ns	ns	ns
ırrıgated	Р	-0 3809 (0 0500)	-0 5385 (0 0038)	0 6174 (0 0006)	ns
Irrigated	N	0 4363 (0 0292)	ns	ns	ns
	Р	ns	-0 4377 (0 0324)	ns	ns

^aNumber of observations = 27

Although irrigation did not have significant effects on oxalates in amaranth grown in the three experimental sites, the effects of irrigation X fertilizer interaction on oxalate, N and ionic concentrations and uptake were significant in Waipio plants (Appendix A). Figure 4 ll illustrates the significant irrigation X fertilizer effect on total oxalate content. Oxalate concentration was significantly higher in plants that received both nitrogen and irrigation. N-fertilized irrigated plants from Waipio also had higher N uptake than those in non-irrigated plots (Figure 4-12). The higher N uptake was probably a key factor leading to higher oxalate content in N-treated irrigated plants.

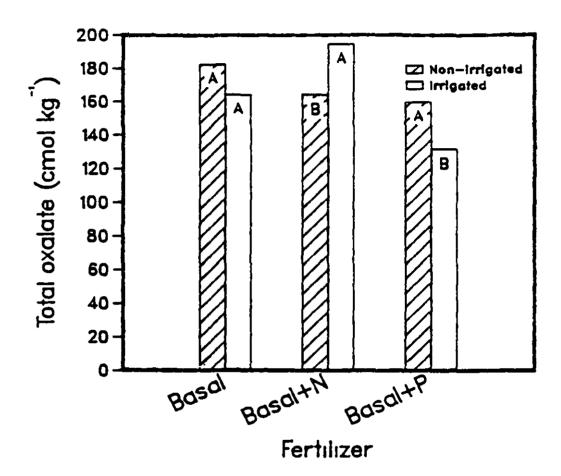
bns = Non-significant

Table 4 28

Effects of fertilizers on the production of various forms of oxalate in amaranth grown at different sites

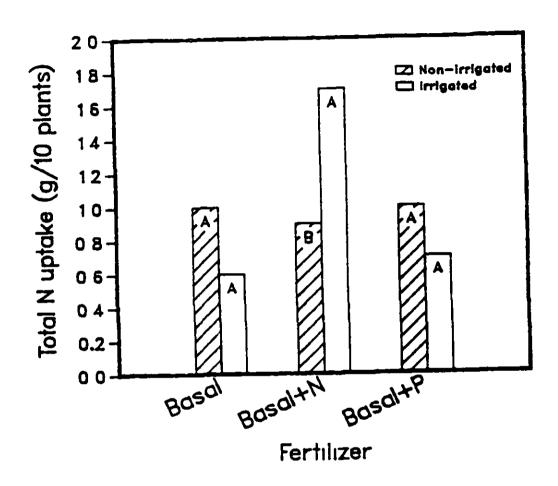
Oxalate	Fertilizer		0x	alate	on (g	(g/ 10 plants)					
		Kı	ıka	au*		Iol	e*	Waipio*			
Total	Basal+P	7	7	Α	8	8	Α	1	9	В	
oxalate	Basal+N	3	2	В	8	9	Α	2	9	Α	
	8asa1	2	7	В	11	9	Α	2	0	AB	
Soluble	Basal+P	2	9	Α	3	6	Α	0	9	В	
oxalate	Basal+N	1	5	Α	3	0	Α	1	6	Α	
	Basal	1	6	Α	4	6	A	0	9	В	
Insoluble	Basal+P	5	2	Α	7	2	Α	ı	4	Α	
oxalate	Basal+N	1	6	В	6	2	Α	1	9	Α	
	Basal	1	3	В	7	8	Α	1	2	Α	
Soluble +	Basal+P	8	1	Α	10	7	Α	2	3	В	
ınsoluble	Basal+N	3	1	В	9	2	Α	3	4	Α	
oxalate	Basal	2	9	В	12	4	Α	2	1	В	

^{*} Means of the same oxalate form and the same location followed by the same letter are not significantly different at 0 05 probability level (Waller-Duncan's multiple range test)



Means of the same fertilizer treatment with the same letter are not significantly different at 0 05 probability level (Waller-Duncan's multiple range test)

Figure 4 ll Effect of irrigation x fertilizer interaction on total oxalate concentration of amaranth grown at Waipio site



Means of the same fertilizer treatment with the same letter are not significantly different at 0 05 probability level (Waller-Duncan's multiple range test)

Figure 4 12 Effect of irrigation x fertilizer interaction on total N uptake of amaranth grown at Waipio site

Effects of N and P fertilizers on ionic concentration and uptake of amaranth

The effects of N and P fertilizers on cation concentrations in plants grown in different locations were not consistent (Table 4-29). The high Ca content in triple superphosphate fertilizer probably contributed to the higher plant Ca concentrations at both Kukaiau and Iole sites. Previous work (Peck et al. 1980) showed that P fertilization decreased cation concentrations in plants. The apparent reduction in Mg and K of P-treated Kukaiau plants and Mg in P-treated Iole plants was observed. It appeared that K concentrations in Iole plants were not affected by different fertilizer treatments. On the other hand K was the dominant cation in plants grown at the Kukaiau site. It is possible that the lower Ca and Mg concentrations in the soil at Kukaiau may have permitted K to be more readily absorbed.

The increase in K concentration in N-fertilized plants at Waipio was the only sign which indicated the effect of N fertilizer on cation concentration in plants. Plants at Kukaiau and Iole sites did not respond to N treatment suggesting that the soils already provided adequate N

The consistently higher cation uptake in P-treated plants of both Kukaiau and Iole sites suggests that the plant response to P created higher demand for these nutrients (Table 4 31)

N fertilization apparently led to higher cation uptake than other fertilizer treatments in Waipio plants (Table 4 31). This was because N created higher growth demand as indicated by higher dry matter production in N-treated Waipio plants (Table 4 2). Many studies show that

Table 4 29

Effects of N and P fertilizer applications on cation concentrations in amaranth grown at three sites

Site	Fertilizer	Ca*		Me	9*		1	(*		ı	۱a	*	\$0	SC**		
						—с	mol I	kg [°]	-1 c	iry	W	e1gh	t	_		
Kukan au	Basal	115 3	В	99	3	Α	154	6	A	5	2	A	374	3	A	
	Basal+N	115 4	В	104	4	Α	146	2	AB	4	7	Α	370	0	Α	
	Basal+P	142 4	Α	94	3	A	142	0	В	5	2	A	383	9	A	
Iole	Basal	131 9	Α	120	3	Α	119	9	Α	5	1	Α	377	2	A	
	Basal+N	131 6	Α	122	0	Α	115	7	Α	4	4	В	373	7	Α	
	Basal+P	140 8	Α	113	9	Α	118	0	A	5	2	Α	377	7	A	
Waipio	Basal	100 4	Α	116	8	Α	105	0	В	8	7	A	330	8	Α	
	Basal+N	103 4	Α	109	8	Α	117	7	Α	8	6	Α	339	5	A	
	Basal+P	102 8	Α	110	7	Α	102	0	В	8	4	Α	323	8	A	

^{*} Means in the same column of each location followed by the same letter are not significantly different at 0 05 probability level (Waller-Duncan's multiple range test)

^{**} Sum of cations concentration

N affects both absorption and translocation of ions (Barta, 1977)

Pretreatment of N deficient roots with nitrate-N resulted in stimulation of strontium and cesium uptake and increased transport of strontium to the shoots (Jackson and Williams, 1968) Anderson and Jackson (1972) reported that nitrate-N pretreatment greatly increased Ca uptake and translocation to shoots Ammonium and urea pretreatment also increased translocation of Ca

The increased P, S and C1 concentrations and uptake (Table 4 30 4 32) in P-treated plants from Kukaiau and Iole was likely due to the effects of P fertilizer on increasing plant growth demand. White (1973) found that the demand for P was associated with the rate of plant growth or the level of matabolic activity within the tissues, which appeared to have a marked influence on the rate of P uptake.

Effects of N and P fertilizers on cation excess (C-A)

In general cation excess was not affected by N and P fertilizers. The exception occurred in the N-treated plots in Kukaiau (Table 4 33). According to earlier workers (e.g., Joy, 1964, Kirkby, 1968. Kirkby and Knight, 1977, Kirkby and Mengel, 1967, Peck et al., 1980). nitrogen fertilizer can increase cation excess above that of the controls while P treatment can decrease cation excess. These trends can be seen in Waipio plants (Table 4 33). although the effects were not significant. The irrigation X fertilizer interaction effect on cation excess of plants was also not significant at any site. although there was a trend of N fertilizer to increase and P fertilizer to decrease oxalate in irrigated plants at Waipio.

Table 4 30

Effects of fertilizers on anion concentrations in amaranth grown at three sites

Site	Fertilizer	١	P*			S *		N	NO ₃ *			C1 ⁻	*	SA*		
		_				_	_	-cmol	kς	_] -1	dry '	we	ıght-			
Kukanau	Basal	3	4	AB	7	7	В	24	8	 В В	6	8	В	42	6	В
	Basal+N	2	9	В	7	3	8	47	8	3 A	6	0	C	64	0	Α
	Basal+P	4	0	Α	10	0	Α	20	5	5 B	8	4	Α	42	9	В
Iole	Basal	2	2	В	10	3	Α	20	7	7 8	6	9	В	40	2	В
	Basal+N	2	0	В	9	5	Α	33	6	5 A	6	3	С	51	4	Α
	Basal+P	3	5	Α	10	4	Α	17	3	3 B	7	3	A	38	5	В
Waipio	Basal	2	9	В	9	6	Α	8	3	Α	7	7	Α	28	4	A
	Basal+N	2	7	В	9	2	Α	12	7	Α	7	3	В	31	9	Α
	Basal+P	4	0	Α	9	2	Α	7	8	Α	7	9	Α	28	9	Α

^{*} Means in the same column of each location followed by the same letter are not significantly different at 0 05 probability level (Waller-Duncan's multiple range test)

^{**} Sum of anions concentration

Table 4 31

Effects of fertilizers on cation uptake by amaranth grown at three sites

Site	Fertilizer	(Ca*		ľ	Mg*			K*			\a*	
													
Kukanau	Basal	0	67	В	0	33	В	1	70	В	0	03	В
	Basal+N	0	81	В	0	41	В	1	97	8	0	04	В
	Basal+P	2	69	Α	1	05	Α	5	02	Α	0	11	A
Iole	Basal	3	42	Α	1	73	Α	5	64	Α	0	13	Α
	Basal+N	2	86	Α	1	41	Α	4	26	Α	0	10	Α
	Basal+P	3	92	A	1	44	Α	5	05	Α	0	13	A
Walpio	Basal	0	54	Α	0	35	Α	1	05	8	0	04	В
	Basal+N	0	73	Α	0	48	Α	1	66	Α	0	80	Α
	Basal+P	0	62	Α	0	38	Α	1	17	AB	0	05	AB

^{*} Means in the same column of the same location followed by the same letter are not significantly different at 0 05 probability level (Waller-Duncan's multiple range test)

Table 4 32

Effects of fertilizers on anion uptake by amaranth grown at three sites

Site	Fertilizer	F	>*		:	5*		NO	3*		(:1 *	
		_	·			g/1	0 p1	lants	5	<u> </u>			
Kukanau	Basal	0	08	В	0	10	В	0	51	A	0	07	В
	Basa1+N	0	10	В	0	12	В	1	06	Α	0	07	В
	Basal+P	0	33	Α	0	45	Α	1	29	Α	0	28	A
Iole	Basal	0	25	Α	0	58	Α	1	44	Α	0	31	Α
	Basal+N	0	17	Α	0	47	Α	1	66	Α	0	23	Α
	Basal+P	0	37	A	0	53	Α	1	27	Α	0	28	A
Waipio	Basal	0	07	Α	0	12	Α	0	13	В	0	07	Α
	Basal+N	0	10	Α	0	16	Α	0	28	Α	0	09	Α
	Basal+P	0	10	Α	0	13	Α	0	14	В	0	80	A

^{*} Means in the same column of the same location followed by the same letter are not significantly different at 0 05 probability level (Waller-Duncan's multiple range test)

Table 4 33

Effects of N and P fertilizers on cation excess in amaranth grown at three sites

Fertilizer	Cation	excess (cmol	kg ⁻¹ dry weight
	Kuka1au*	Iole*	Waipio*
Basal	331 6 A	337 O A	302 5 A
Basal+N	306 1 B	322 4 A	306 7 A
Basal+P	341 1 A	339 2 A	294 9 A

^{*} Means in the same column followed by the same letter are not significantly different at 0 05 probability level (Waller-Duncan's multiple range test)

There are many factors which might have prevented the expected response of cation excess to N and P fertilizers in Kukaiau and Iole plants to be detected but permit it to be expressed in the Waipio plants The first factors involve N metabolism N fertilizer applied in urea form was likely to be transformed to nitrate before being absorbed by The accumulation of nitrate was significantly higher in the plants N-treated plants at Kukaiau and Iole than those treated with the other fertilizers, but such accumulation was not significantly different among different fertilizer treatments in Waipio plants (Table 4 35) shows that in Kukaiau and Iole, high unassimilated nitrate content led to low cation excess content especially in N-treated Kukaiau and Iole On the other hand Waipio plants had lower nitrate accumulation plants (Table 4 35) suggesting that most of the nitrate was assimilated into protein resulting in increased cation excess

Amaranth grown at the Waipio site may have had higher nitrate reductase activity than those in Kukaiau and Iole. Nitrate reductase activity has been found to be affected by environmental factors, such as light intensity, temperature and soil moisture. Cantliffe (1972b) found that high light intensity promoted the high activity of nitrate reductase. Waipio plants received higher solar radiation than Kukaiau and Iole plants (Table 3 20)

The second factor for the weak causal relationship between cation excess and N and P fertilizer application may involve the use of triple superphosphate as the source of P P fertilizer was expected to reduce cation excess because it often reduces cation concentration example, Peck et al (1980) found that increasing rates of P fertilizer reduced cation concentration in table beets Triple superphosphate, however, contains high amounts of Ca resulting in high tissue Ca concentrations in P-treated Kukaiau and Iole plots. These high Ca concentrations are to have prevented P from reducing cation excess in Kukaiau and Iole On the other hand Waipio plants received a lower rate of P Moreover, the lower Ca concentration in these plants (Table 4 29) shows that the cation excess of these P-treated plants are not influenced by Ca to the same extent as P-treated Kukaiau and Iole plants Apparent response of cation excess to P fertilizer was shown in the Waipio plants (Table 4 33)

Effects of N and P fertilizers on total N and nitrate-N concentrations and uptake

Total N concentration was higher in N-fertilized plants in all sites, while P fertilizer reduced total N concentration (Table 4 34)

Total N uptake was significantly higher in P-fertilized plants in Kukaiau and Iole than in plants treated with basal fertilizer. On the other hand, in Waipio total N uptake was significantly higher in the N-treated plants (Table 4 34)

Table 4 34

Effects of fertilizers N and P on total N concentrations and uptake in amaranth grown at three sites

Fertilizer		Concentrat (% dry we	_		Uptake (g/ 10 plants)						
	Kuka1au*	Iole*	Walplo*	Kuka1au*	Iole*	Waipio*					
Basal+N	5 06 A	4 68 A	3 53 A	1 74 B	2 81 A	1 27 A					
Basal	4 47 B	4 15 A	3 26 AB	1 26 B	2 17 A	0 82 B					
Basal+P	4 06 C	4 06 A	3 06 B	3 71 A	4 18 A	0 88 B					

^{*} Means in the same column followed by the same letter are not significantly different at 0.05 probability level (Waller-Duncan's multiple range test)

Nitrate-N concentration varied with fertilizer treatments in the same way as total N concentration (Table 4 35) Nitrate-N uptake by Kukaiau and Iole plants was highest in P-treated plants and in N-treated plants in the Waipio plants (Table 4 35)

4 2 6 Relationships between oxalates, nitrate and agroenvironmental factors

All forms of oxalates with the exception of insoluble oxalate were significantly negatively correlated with subsoil temperature

Table 4 35

Effects of N and P fertilizers on nitrate-N concentration and uptake by amaranth grown at the three sites

Fertilizer		Concentration (% dry weight)										Uptake (g/ 10 plants)							
	Kı	ıka	ı au*	,	lol	e*	Wa	31 p	10*	Κι	ıka.	ı au*		Iol	e*	Wa	ııp.	10*	
Basal+N	0	67	Α	0	47	Α	0	10	Α	1	06	Α	1	27	Α	0	25	Α	
Basal	0	35	В	0	29	В	0	12	Α	0	51	Α	0	73	Α	0	13	В	
Basal+P	0	29	В	0	24	В	0	11	Α	1	29	Α	1	19	A	0	14	В	

^{*} Means from the same column followed by the same letter are not significantly different at 0 05 probability level (Waller-Duncan simultiple range test)

(Table 4 36) Significant correlations were found more often with soil temperature than with air temperature. All correlations were negative except for that between air temperature and soluble oxalate. Other studies on the relationship between temperature and plant oxalates although scarce, also indicate the negative relationship between the two variables. Kitchen et al. (1964b) postulated that at high temperatures more oxalic acid was used as a respiratory substrate.

Nitrate-N was also negatively correlated with air and soil temperature (Table 4 36). Temperature can increase nitrate content due to diminished nitrate reductase activities at high temperature (Cantliffe, 1972a). In this study other factors appeared to override the temperature effect. The most significant factor was soil

Table 4 36

Correlation between amaranth oxalates or nitrate content with air or soil temperature at three experimental sites

Correlation coefficient (Probability > |r|)

		Air tempe	rature			Topsoil t	emperature	1		Subsoil t	emperature	:
Plant Chemical	Max	Min	Av	Dif*	Нах	Min	Av	Dif	Max	Hin	Av	Dif
Total oxalate	ns	ns	ns	0 5267 (0 0001)	ns (0 0442)	0 2803 (0 0001)	ns (0 0001)	0 5181 (0 0001)	0 5646 (0 0001)	0 5636 (0 0001)	0 5653 (0 0001)	0 4332 (0 0001)
Soluble oxalate	ns	0 3449 (0 0132)	ns	ns	ns	ns	ns	0 3976 (0 0039)	0 3775 (0 0063)	0 3562 (0 0103)	0 3717 (0 0072)	0 4532 (0 0008)
insoluble oxalate	0 4256 (0 0019)	0 3375 (0 0154)	0 4240 (0 0091)	ns	0 3593 {0 0096}	0 3048 (0 0296)	0 3377	ns	NS	ns	ns	0 2707 (0 0547)
Soluble + Insoluble oxalate	-	ns	ns	0 3573 (0 0101)	ns	ns	ns	0 3190 (0 0225)	0 3620 (0 0091)	0 3619 (0 0091)	0 3620 (0 0090)	ns
Nitrate N	0 3046 (0 0281)	ns	ns	0 5439 (0 0001)	ns	0 4172 (0 0021)	0 3470 (0 0117)	0 6208 (0 0001)	0 6206 (0 0001)	0 6241 (0 0001)	0 6225 (0 0001)	0 4134 (0 0023)

Dif Difference between maximum and minimum values

ns non significant

N content $\$ For example, Waipio the site with the highest temperature had relatively lower soil N

Plant oxalate and nitrate were positively correlated with rainfall (Table 4 37) Adequate water supply provided by rains may facilitate ion uptake by roots which enhances oxalate synthesis and nitrate accumulation. Radiation on the other hand, exhibited negative correlation with both oxalates and nitrate (Table 4 37). Long exposure to high light intensity was found to decrease nitrate content due to an increase in nitrate reductase activity (Cantliffe, 1972a and b). The high plant nitrate levels in Iole and Kukaiau relative to those in Waipio can be attributed to the lower radiation and higher soil nitrogen in Iole and Kukaiau than in Waipio.

Nitrate content was also negatively correlated with wind speed

(Table 4 37) It has been postulated that factors that cause higher transpiration rates can lead to more rapid nitrate translocation to the site of reduction therefore, resulting in lower nitrate accumulation (Maynard et al. 1976)

Oxalates and nitrate were positively correlated with soil total N, but they showed a negative relationship with the other soil variables (Table 4 38, Figures 4 13-4 17) Earlier work has shown that N assimilation leads to synthesis of organic acid (e.g. Ikeda and Yamada 1981) In addition, high soil N leads to high N absorption and increased organic acid synthesis. The negative correlation of plant oxalates and nitrate with soil bases was most likely due to the inability of the plants to utilize the soil bases because of low soil N. For example, the Tropeptic Eutrustox in Waipio had a high base

Table 4 37

Correlation between oxalates intrate and some climatic factors, including rainfall, radiation wind speed and relative humidity in amaranth

Chemical composition	Correlation coefficient*** (Probability > r									
	Rainfall	Radiation	Wind speed	Relative humidity						
				Maximum	Minimum	Average	Dıf*			
Total oxalate	0 3740 (0 0063)	-0 4881 (0 0002)	-0 4824 (0 0003)	ns**	ns	ns	-0 4116 (0 0024)			
Soluble oxalate	ns	ns	ns	-0 2923 (0 0374)	ns	ns	-0 3062 (0 0289)			
Insoluble oxalate	0 4527 (0 0009)	ns	ns	0 4881 (0 0003)	0 5411 (0 0001)	0 5317 (0 0001)	ns			
Soluble + insoluble oxalate	0 3265 (0 0194)	-0 3321 (0 0173)	ns	ns	ns	ns	-0 3993 (0 0037)			
Nitrate N	0 3816 (0 0053)	-0 5727 (0 0001)	-0 6507 (0 0001)	ns	0 2720 (0 0511)	ns	-0 3064 (0 0272)			

^{*} Dif = Difference between maximum and minimum relative humidity

1

^{**} ns - non-significant

^{***} Number of observations = 54

Table 4 38 Correlation between amaranth oxalates, nitrate and some soil variables at three experimental sites

	<pre>Correlation coefficient** (Probability > r)</pre>								
Chemical composition	Soll N	Soil Ca	Soil Mg	Soll K	Soil Na	Soll P			
Total oxalate	0 5421 (0 0001)	-0 5094 (0 0001)	-0 5571 (0 0001)	-0 3320 (0 0162)	-0 3734 (0 0064)	-0 2477 (0 0766)			
Soluble oxalate	ns*	-0 3551 (0 0106)	ns	ns	ns	ns			
Insoluble oxalate	ns	ns	ns	ns	ns	ns			
Soluble + insoluble oxalate	0 3812 (0 0058)	-0 5075 (0 0001)	-0 4239 (0 0019)	-0 2706 (0 0548)	ns	-0 3042 (0 0300)			
Nitrate-N	0 5983 (0 0001)	-0 5495 (0 0001)	-0 6154 (0 0001)	-0 4742 (0 0004)	-0 3832 (0 0050)	ns			

^{*} ns = non-significant ** Number of observations = 54

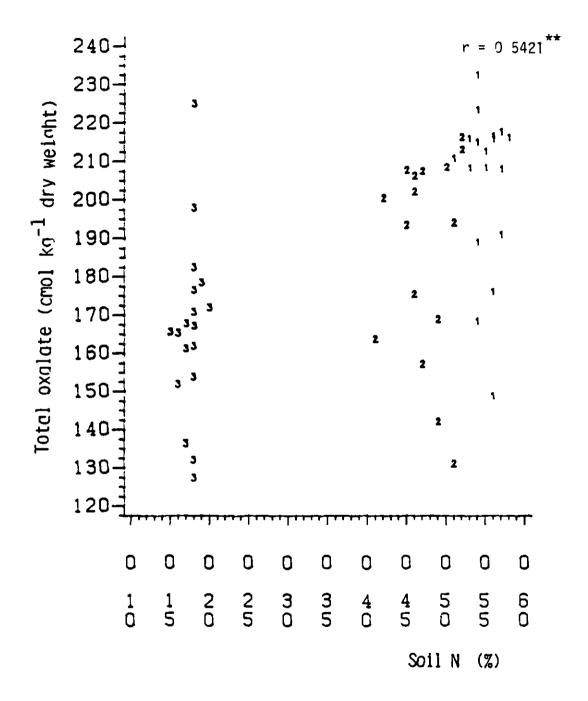


Figure 4 13 Relationship between tissue total oxalate of amaranth and soil nitrogen from experiments at three sites (1=Kukaiau, 2=Iole, 3=Waipio)

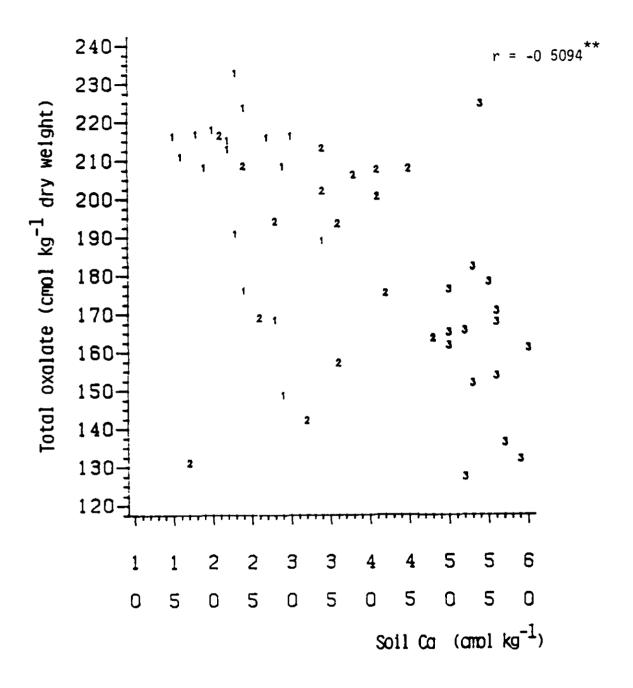


Figure 4 14 Relationship between tissue total oxalate of amaranth and soil Ca from experiments at three sites (1=Kukaiau, 2=Iole, 3=Waipio)

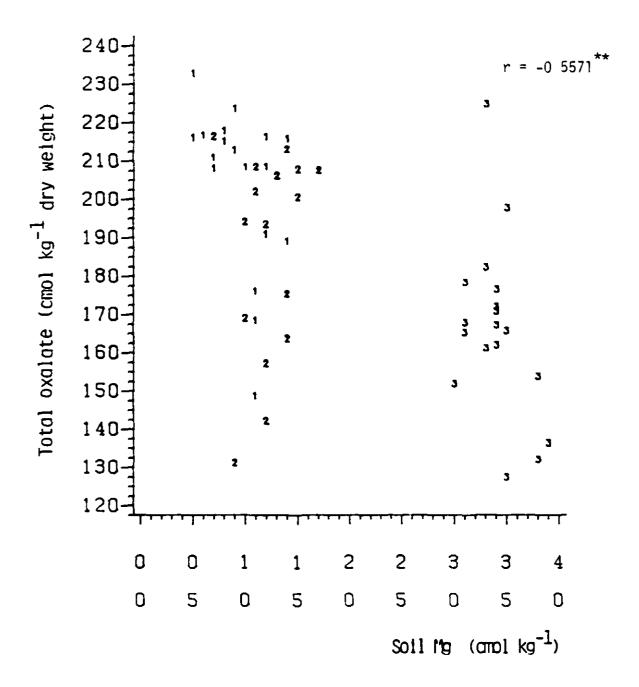


Figure 4 15 Relationship between tissue total oxalate of amaranth and soil Mg from experiments at three sites (1=Kukaiau, 2=Iole, 3=Waipio)

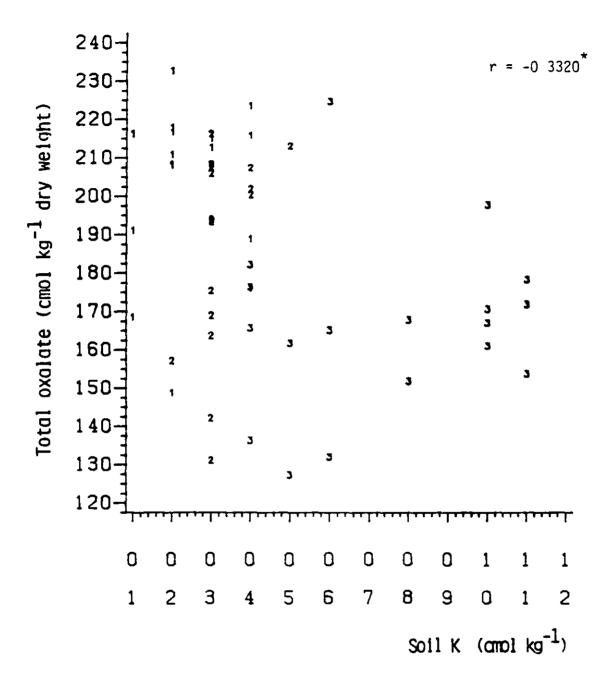


Figure 4 16 Relationship between tissue total oxalate of amaranth and soil K from experiments at three sites (1=Kukaiau, 2=Iole, 3=Waipio)

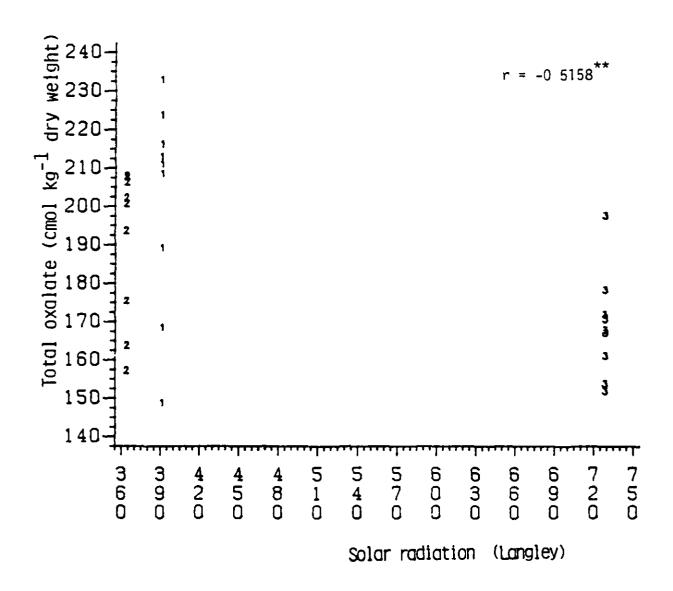


Figure 4 17 Relationship between tissue total oxalate of amaranth and solar radiation from non-irrigated plots of experiments at three sites (1=Kukaiau, 2=Iole 3=Naipio)

status but low N As a consequence, the plants at this site suffered mild N stress and did not absorb other nutrients efficiently. The low level of N and low cation uptake led to low contents of oxalate and nitrate despite the high base status of the soil

Soil P was negatively correlated with oxalates (Table 4 38) and this is consistent with the tendency of P to reduce the cation excess in plants

Multiple regression analysis of the relationships between oxalate, nitrate and agroclimatic factors

Multiple regression analysis of data from irrigated and nonirrigated plots were conducted separately to minimize the variance
associated with irrigation treatments described earlier in section 4 2 4
Irrigation treatments by themselves did not result in significant
effects on oxalate and nitrate concentrations in amaranth with the
exception of the insoluble oxalate and nitrate in Iole plants (Tables
4 20, 4 24) Significant interaction effects however, were found
For example, the effects of site x irrigation and site x irrigation
x fertilizer on nitrate-N content were significant (Appendix A)

The regression equation for total oxalate concentration in nonirrigated amaranth is presented in Table 4.39. The negative relationships of oxalate concentration with mean solar radiation and P
fertilizer rate were as shown in Figures 4.17. 4.18. P fertilizer has
decreased oxalate in table beets (Peck et al. 1980) apparently because
P fertilizer decreased uptake and concentration of cations by plants
and therefore decreased cation excess. Soil Mg and K contributed
positively to oxalate production since their uptake increase cation.

Table 4 39

Multiple regression equation for total oxalate concentration in amaranth from non-irrigated plots of three experimental sites

Variable*	Regression coefficient	Standard error	t	Probability > t
Intercept	225 261	7 4549	30 217	0 0001
SRSQ	-0 000025	0 000061	-0 400	0 6934
PLEVEL	-0 0894	0 0421	-2 121	0 0454
SOLMG	36 3598	13 5733	-2 679	0 0137
SOLK	84 7203	42 4200	1 997	0 0583

Model $r^2 = 0$ 6123 Model Adj $r^2 = 0$ 5419

*SRSQ = Square of solar radiation

PLEVEL = Fertilizer P rate SOLMG = Soil Mg content SOLK = Soil K content

excess The negative correlations of soil Mg and K with oxalate (Figures 4 15 and 4 16) were probably due to their interaction with soil N. Although soil Mg and K were high at the Waipio site the plants did not absorb a large amount of Mg and K due to low N supply

In the irrigated treatments, solar radiation gave rise to a positive parameter estimate (Table 4 40) in spite of its negative correlation with total oxalate (Figure 4 19). The difference between maximum and minimum air temperature, however, gave rise to negative regression coefficient. This is consistent with the negative

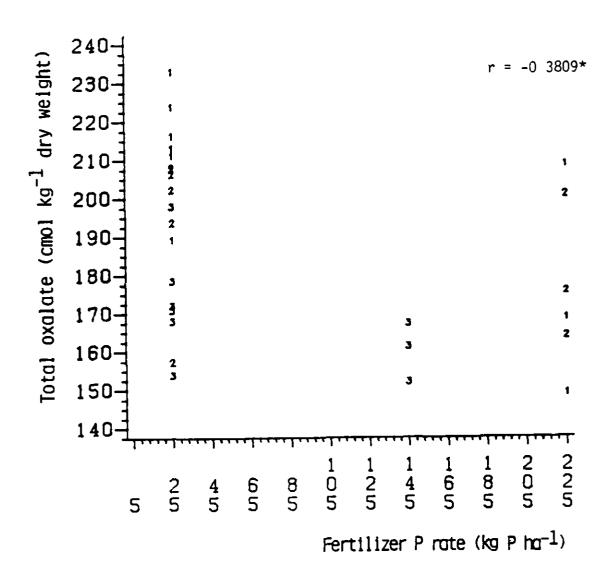


Figure 4 18 Relationship between tissue total oxalate of amaranth and fertilizer P rate from non-irrigated plots of experiments at three sites (l=Kukaiau, 2=Iole 3=Waipio)

relationship between this variable and total oxalate (Appendix D) The difference between maximum and minimum air temperature exhibited curvilinear relationship with total oxalate. To take into account the non-linear relationship in the regression studies the difference between maximum and minimum air temperature was squared. Solar radiation and the square of the difference between maximum and minimum temperature were highly correlated (r = 0.9996, Appendix D). This may be due to clear skies giving rise to high radiation and warm temperature during the day and low temperature at night. The fact that the two variables gave rise to opposite signs of the regression coefficient (Table 4.40) indicated that they contributed to oxalate production in different ways

Fertilizer N rate also gave rise to a positive regression coefficient which was consistent with the positive correlation between this variable and total oxalate (Figure 4 20)

The regression model for soluble oxalate in non-irrigated amaranth with the related statistics is shown in Table 4 41 unexpectedly soil K explained a significant amount of variance in soluble oxalate as soluble oxalate occurred primarily as potassium oxalate P fertilizer rate and soil Ca were negatively correlated with soluble oxalate and the relationship was in accordance with their negative relationships with soluble oxalate (Figures 4 21 4 22 Appendix C) P fertilizer is also a source of Ca for the plants The regression coefficient of soil P was positive (Table 4 41) however it was negatively related to soluble oxalate as shown in Figure 4 14 P usually brings about a reduction in cation excess which in turn, results in a decrease in oxalate concentration It is likely

Table 4 40

Multiple regression equation for total oxalate concentration in amaranth from irrigated plots at three experimental sites

Varıable*	Regression coefficient	Standard error	t	Probability > t
Intercept	1991 572	659 811	3 018	0 0068
SRSQ	0 0059	0 0022	2 688	0 0141
NLEVEL	0 1791	0 0503	3 557	0 0020
SOLK	105 048	54 8168	1 916	0 0697
AIR4SQ	-30 9963	11 2800	-2 748	0 0124

Model $r^2 = 0.6076$ Model Adj $r^2 = 0.5291$

* SRSQ = Square of solar radiation

NLEVEL = Fertilizer N rate

SOLK = Soil K concentration

AIR4SQ = Square of the difference between maximum and minimum temperature

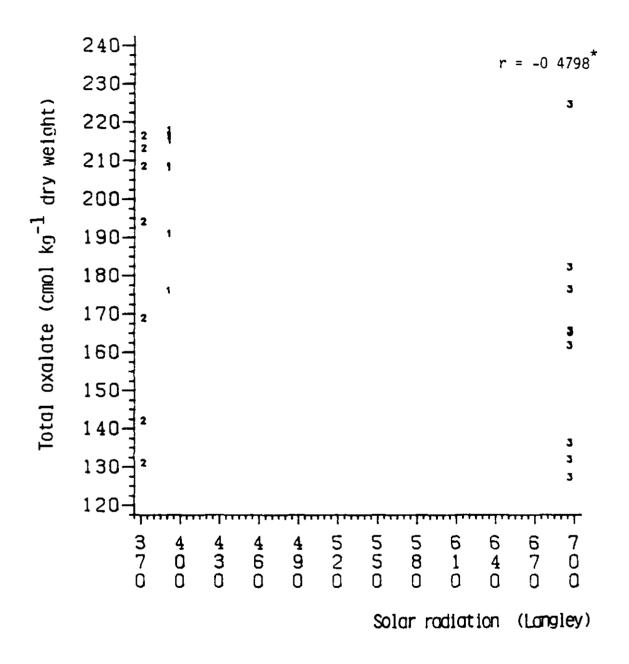


Figure 4 19 Relationship between tissue total oxalate of amaranth and solar radiation from irrigated plots of experiments at three sites (1=Kukaiau, 2=Iole 3=Waipio)

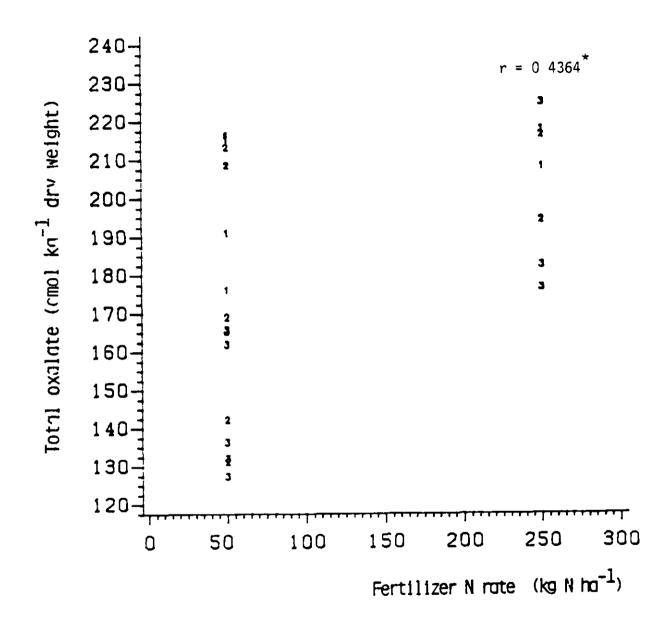


Figure 4 20 Relationship between tissue total oxalate of amaranth and fertilizer N rate from irrigated plot of experiments at three sites (1=Kukaiau, 2=Iole, 3=Waipio)

Table 4 41

Multiple regression equation for soluble oxalate concentration in amaranth from non-irrigated plots of three experimental sites

Variable*	Regression coefficient	Standard error	t	Probability > t
Intercept	113 253	20 3643	5 561	0 0001
SOLK	56 5055	21 8189	2 590	0 0167
PLEVEL	-0 1698	0 0603	-2 818	0 0100
SOLCA	-18 7353	4 8909	-3 831	0 0009
SOLP	0 4673	0 2815	1 660	0 1110

Model $r^2 = 0.6359$ Model Adj $r^2 = 0.5698$

* SOLK = Soil K concentration

PLEVEL = Fertilizer P rate

SOLCA = Soil Ca concentration
SOLP = Soil P concentration

that fertilizer P affected plant growth more than initial soil P and this is supported by the fact that soil P did little to explain the variance in soluble oxalate (Table 4 41). Because soil P was measured at harvest it included both the P applied as fertilizer and initial soil P. In the multiple regression analysis, both variables were retained but only P level was significant. This is due to the fact that P level and soil P were highly correlated.

The regression analysis of soluble oxalate in irrigated plants is shown in Table 4.42. In accordance with the positive relationship

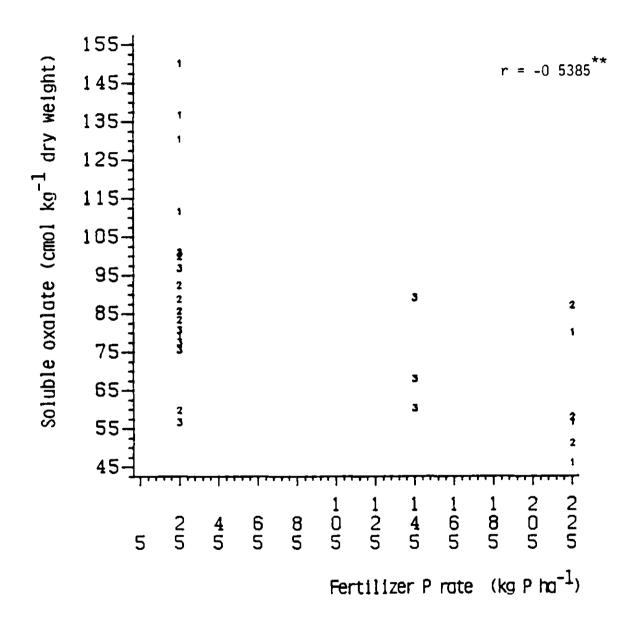


Figure 4 21 Relationship between tissue soluble oxalate of amaranth and fertilizer P rate from non-irrigated plots of experiments at three sites (l=Kukaiau 2=Iole 3=Waipio)

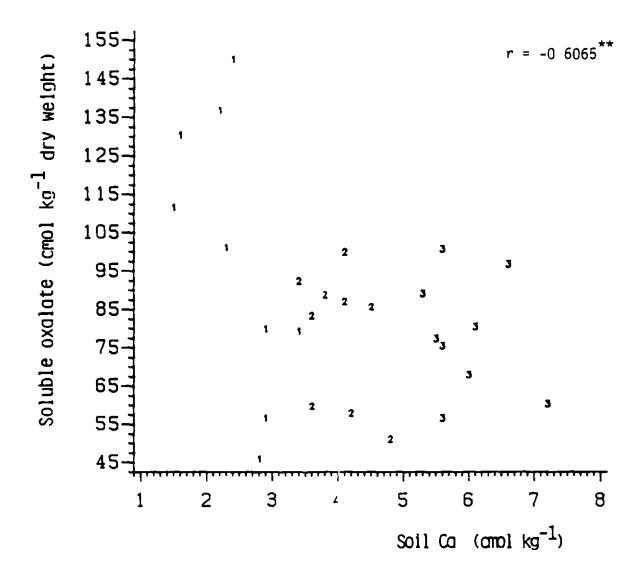


Figure 4 22 Relationship between tissue soluble oxalate of amaranth and soil Ca from non-irrigated plots of experiments at three sites (1=Kukaiau 2=Iole 3=Waipio)

Table 4 42

Multiple regression equation for soluble oxalate concentration in amaranth from irrigated plots of three experimental sites

Variable*	Regression coefficient	Standard error	t	Probability > t
Intercept	-109 043	47 0516	-2 318	0 0306
AIR2	13 9927	3 1128	4 495	0 0002
PLEVEL	-0 1439	0 0430	-3 350	0 0031

Model
$$r^2 = 0.5880$$

Model Adj $r^2 = 0.5488$

* AIR2 = Minimum air temperature PLEVEL = Fertilizer P rate

between soluble oxalate and minimum air temperature (Figure 4 23)
this variable also gave rise to a positive parameter estimate. The
effect of air temperature on soluble oxalate concentration was positive
while the effect on total and insoluble oxalate was negative
Fertilizer P rate also produced negative regression coefficient in
accordance with its negative relationship with soluble oxalate
(Figure 4 24)

The regression model for insoluble oxalate concentration in nonirrigated plants is shown in Table 4.43. Only variables related to

P supply significantly influenced insoluble oxalate. Although soil

P was not significantly related with insoluble oxalate (Appendix C)

the regression coefficient was significantly negative in this equation.

The reason put forward earlier that P being an anion tends to decrease

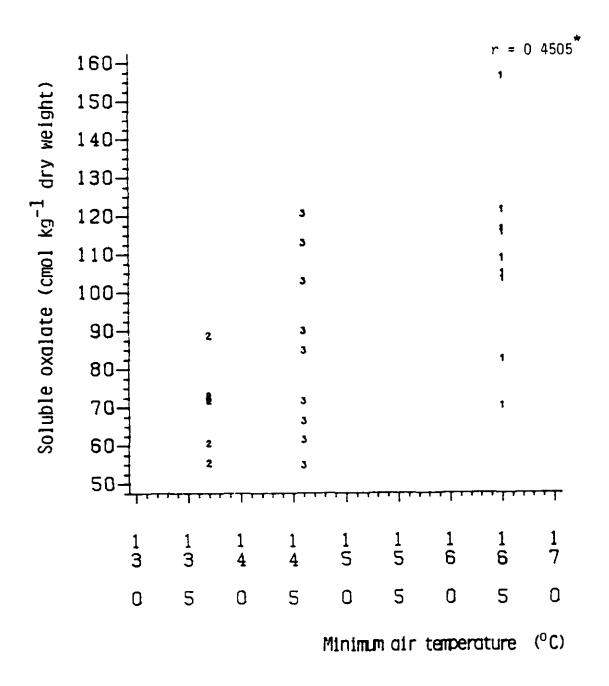


Figure 4 23 Relationship between tissue soluble oxalate of amaranth and minimum air temperature from irrigated plots of experiments at three sites (l=Kukaiau, 2=Iole, 3=Waipio)

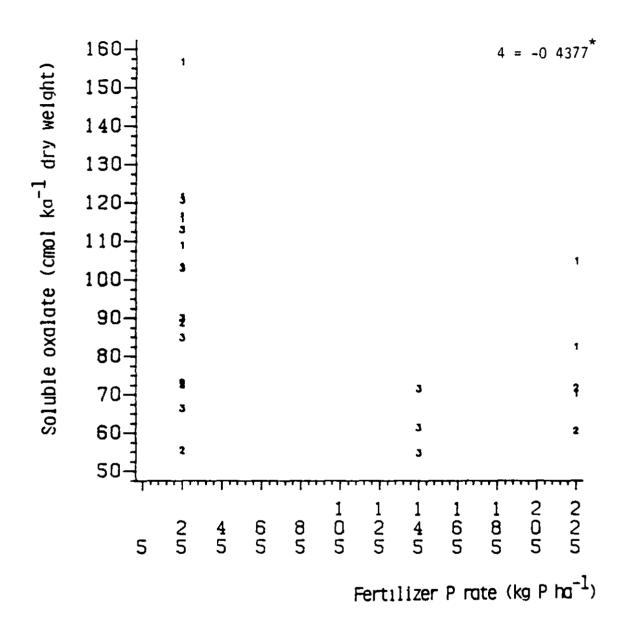


Figure 4 24 Relationship between tissue soluble oxalate of amaranth and fertilizer P rate from irrigated plots of experiments at three sites (1=Kukaiau, 2=Iole, 3=Waipio)

Table 4 43

Multiple regression equation for insoluble oxalate concentration in amaranth from non-irrigated plots of three experimental sites

Variable*	Regression coefficient	Standard error	t 	Probability > t
Intercept	135 991	8 8214	15 516	0 0001
SOLP	-0 5121	0 1386	-3 696	0 0011
PLEVEL	0 1659	0 0277	5 997	0 0001

Model $r^2 = 0.6057$ Model Adj $r^2 = 0.5728$

the cation excess and therefore, oxalate production applies here as well. The regression coefficient of fertilizer P rate was significantly positive which is consistent with the significant positive relationship between insoluble oxalate and fertilizer P rate (Figure 4 25). The additional Ca supplied to plants by P fertilizer probably led to high Ca oxalate content in plants receiving this treatment.

The regression model for insoluble oxalate in irrigated plants is shown in Table 4.44. Seventy-five percent of the variance in insoluble oxalate was explained by this model in contrast to 57% for soluble oxalate in non-irrigated plants. 55% for soluble oxalate in irrigated plants and 57% for insoluble oxalate in non-irrigated plants. P-related variables were significantly related as were fertilizer N rate and average air temperature. Fertilizer N rate was not

^{*} SOLP = Soil P concentration PLEVEL = Fertilizer P rate

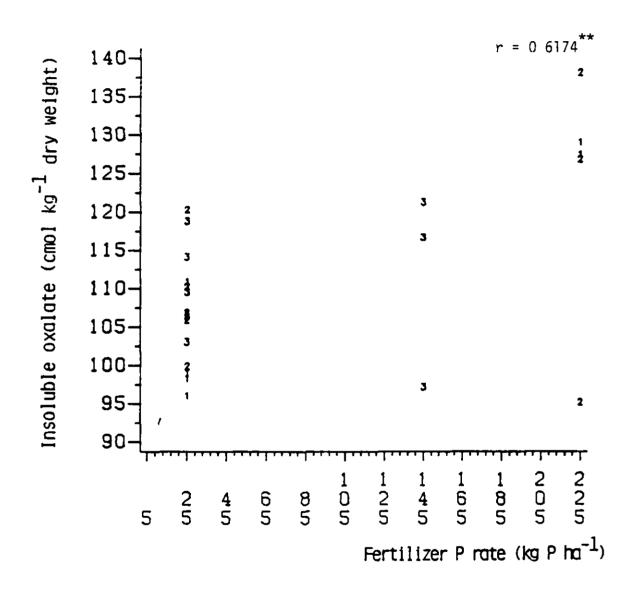


Figure 4 25 Relationship between tissue insoluble oxalate of amaranth and fertilizer P rate from non-irrigated plots of experiments at three sites (1=Kukaiau, 2=Iole 3=Waipio)

Table 4 44

Multiple regression equation for insoluble oxalate concentration in amaranth from irrigated plots of three experimental sites

Variable*	Regression coefficient	Standard error	t	Probability > t
Intercept	441 555	42 6866	10 346	0 0001
AIR3	-16 5982	2 0681	-8 026	0 0001
NLEVEL	0 0754	0 0295	2 552	0 0190
PLEVEL	0 0512	0 0335	1 528	0 1422

Model $r^2 = 0.7840$ Model Adj $r^2 = 0.7516$

NLEVEL = Fertilizer N rate PLEVEL = Fertilizer P rate

regression coefficient was significantly positive. The role of N in organic acid synthesis in plants is well known. The process of N assimilation leads to the synthesis of organic acid as shown for example by the study by Ikeda and Yamada (1981). For this regression average air temperature was negative as was the case for simple correlation (Figure 4-26).

The regression model for nitrate-N concentration in non-irrigated plants is shown in Table 4-45. Fertilizer N contributed most to explaining the variance in nitrate-N concentration. The regression

^{*} AIR3 = Average air temperature

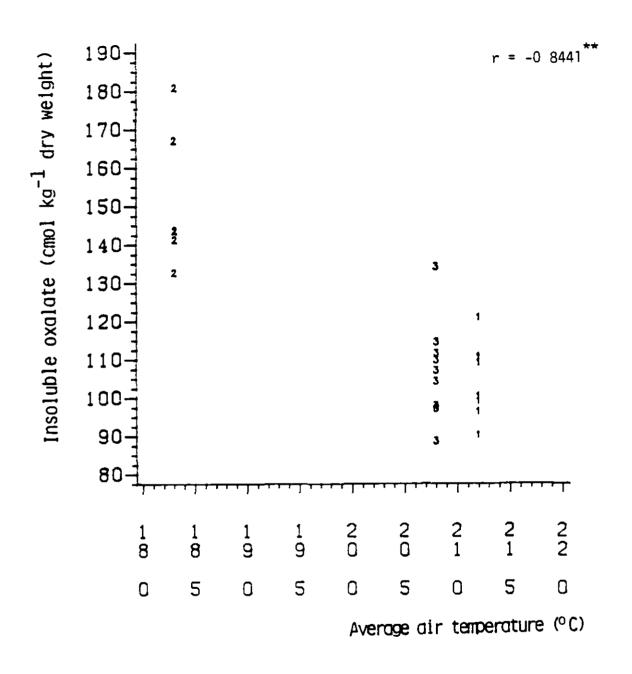


Figure 4 26 Relationship between tissue insoluble oxalate of amaranth and average air temperature from irrigated plots of experiments at three sites (1=Kukaiau, 2=Iole, 3=Waipio)

Table 4 45

Multiple regression equation for nitrate-N concentration in amaranth from non-irrigated plots of three experimental sites

Variable*		gression efficient	-	tandard rror		t	Proba	ability > t
Intercept	39	8414	4	6198	8	624	0	0001
WSPSQ	-0	3053	0	2506	-1	218	0	2360
NLEVEL	0	0764	0	0164	4	657	0	0001
SOLMG	-18	5415	5	6715	-3	269	0	0035
SOLK	39	0297	18	5464	2	104	0	0470

Model $r^2 = 0.7861$ Model Adj $r^2 = 0.7472$

* WSPSQ = Square of wind speed
NLEVEL = Fertilizer N rate
SOLMG = Soil Mg concentration
SOLK = Soil K concentration

coefficient was positive, and consistent with the relationship between the two variables (Figure 4 27). Initial soil N did not significantly influence plant nitrate concentration probably due to its low level compared with applied fertilizer N. The regression coefficient of the square of wind speed was negative in accordance with its negative relationship with nitrate-N (Figure 4 28). High wind speed usually increases transpiration rate. According to Maynard et al. (1976) transpiration also accelerates the translocation of nitrate to the site of reduction. The regression coefficient of soil Mg was negative in

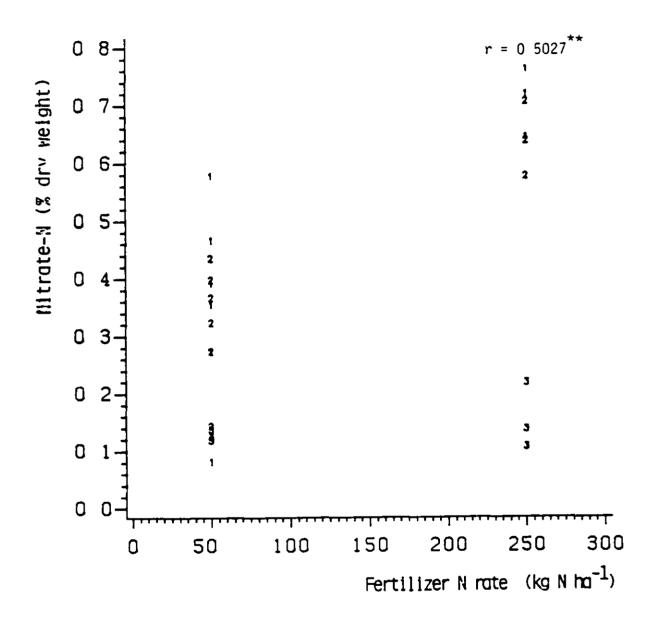


Figure 4 27 Relationship between tissue nitrate-N concentration of amaranth and fertilizer N rate from non-irrigated plots of experiments at three sites (l=Kukaiau, 2=Iole, 3=Waipio)

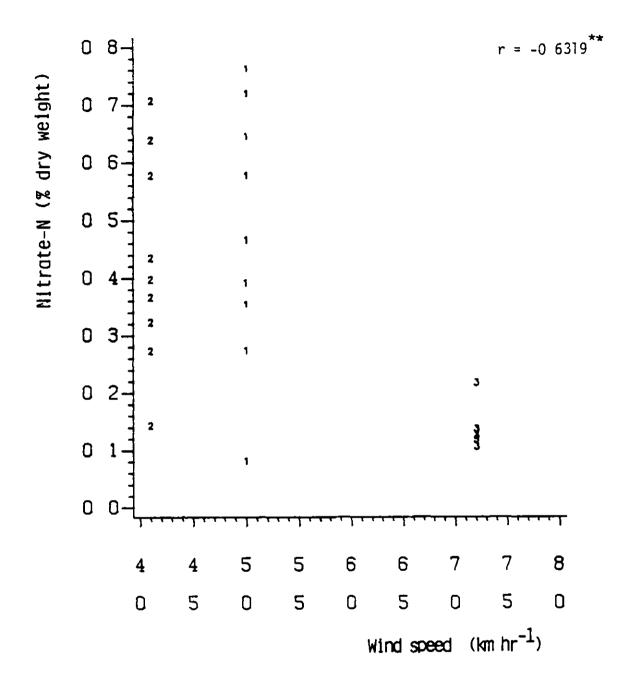


Figure 4 28 Relationship between tissue nitrate-N concentration of amaranth and wind speed from non-irrigated plots of experiments at three sites (1=Kukaiau, 2=Iole, 3=Waipio)

accordance with its negative relationship with nitrate-N (Figure 4 29)

On the other hand, the regression coefficient of soil K was positive despite its negative relationship with nitrate-N (Figure 4 30). The change in sign is probably due to the highly correlated nature of soil Mg and soil K (Appendix C). Moreover, K is often the cation accompanying nitrate movement from the root to the shoot (Minotti, 1968). K fertilizer also increased nitrate content in some vegetables (Cantliffe, 1973).

The results of the multiple regression analysis of nitrate-N concentration in irrigated amaranth are shown in Table 4 46. In addition to N fertilizer rate (Figure 4 31) and soil N (Figure 4 32), minimum air temperature also helped to explain the variance in the nitrate-N concentration. Minimum air temperature was squared because it showed a curvilinear relationship with nitrate-N (Figure 4 33). The positive relationship between minimum air temperature and nitrate-N concentration shown in Figure 4 33 was apparent in the multiple regression analysis. This is consistent with the observation that high temperatures increased plant nitrate content. This effect was attributed to a combination of reduced nitrate reductase activity and higher availability of soil nitrate due to increased microbial activity.

4 3 Response of cassava to environmental factors

In this section emphasis is placed on the results concerning the effects of environmental factors on nutrient and antinutrient contents of cassava leaves

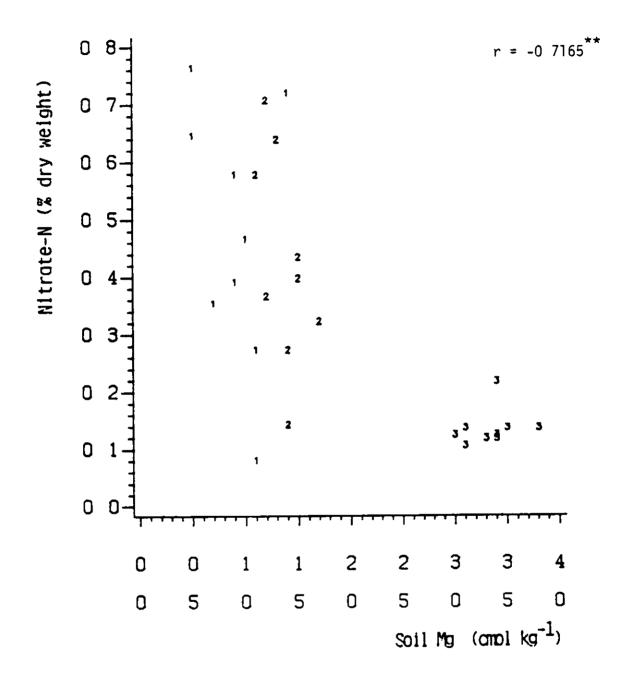


Figure 4 29 Relationship between tissue nitrate-N concentration of amaranth and soil Mg from non-irrigated plots of experiments at three sites (1=Kukaiau, 2=Iole, 3=Waipio)

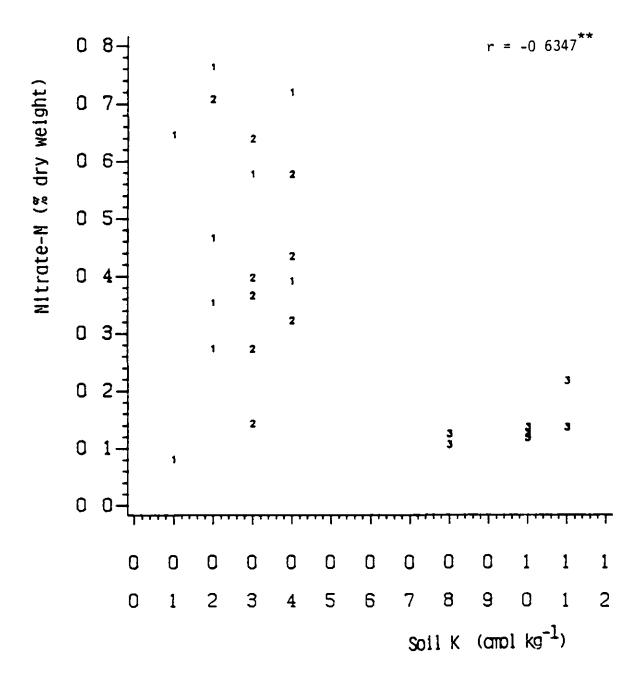


Figure 4 30 Relationship between tissue nitrate-N concentration of amaranth and soil K from non-irrigated plots of experiments at three sites (1=Kukaiau, 2=Iole, 3=Waipio)

Table 4 46

Multiple regression equation for nitrate-N concentration in amaranth from irrigated plots of three experimental sites

Varaible*		ression fficient		andard ror		t	Proba	ability > t
Intercept	-31	6711	10	4777	-3	023	0	0065
AIR2SQ	0	1377	0	0498	2	763	0	0116
NLEVEL	0	0603	0	0169	3	559	0	0019
SOLTN	27	4725	9	9755	2	754	0	0119

Model $r^2 = 0.6595$

Model Adj $r^2 = 0.6108$

* AIR2SQ = Square of minimum temperature

NLEVEL = Fertilizer N rate

SOLTN = Soil N concentration

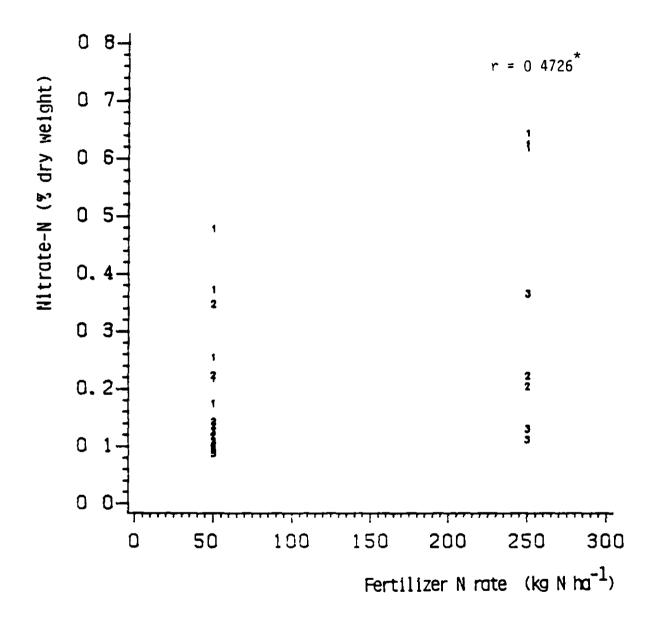


Figure 4 31 Relationship between tissue nitrate-N concentration of amaranth and fertilizer N rate from irrigated plots of experiments at three sites (l=Kukaiau, 2=Iole, 3=Waipio)

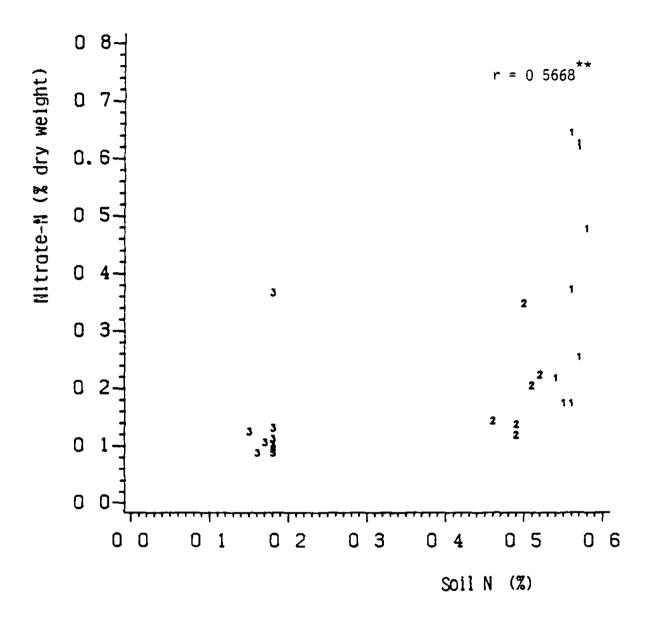


Figure 4 32 Relationship between tissue nitrate-N concentration of amaranth and soil N from irrigated plots of experiments at three sites (l=Kukaiau, 2=Iole, 3=Waipio)

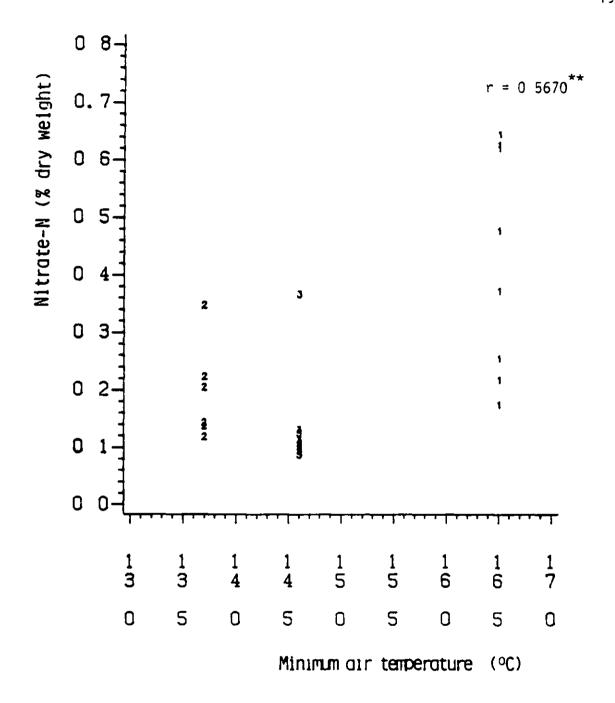


Figure 4 33 Relationship between tissue nitrate-N concentration of amaranth and minimum air temperature from irrigated plots of experiments at three sites (l=Kukaiau, 2=Iole 3=Waipio)

4 3 1 Forms and content of oxalate in cassava leaves

The significant positive correlations between concentrations of total and insoluble oxalate and tissue Ca and Mg suggest that oxalate in cassava leaves was in the form of Ca oxalate or Mg oxalate or both However, the fact that total and insoluble oxalate had significant positive correlations only with Ca in the insoluble fraction (Table 4 47 Figures 4 34 4 35) and not with Mg (Table 4 47) suggests that oxalate in cassava was mostly in the form of Ca oxalate. Another suggestion of the dominance of Ca oxalate over Mg oxalate is that the sum of Ca and Mg was not as well correlated with oxalates as Ca alone (Table 4 47)

Soluble oxalate was detected in only one sample from the Molokai non-irrigated plot which had a soluble oxalate content of 6.5 cmol kg-1 The insoluble oxalate concentration, from Molokai and Iole plants was consistently lower than total oxalate concentration (Appendix E) and consequently the difference between the two was taken as the soluble oxalate concentration (Table 4 48, Appendix E) Molokai plants had a relatively higher content of soluble oxalate than the plants from the other sites while Waipio plants had virtually no soluble oxalate (Table 4 48, Appendix E) It cannot be stated for certain from this study what form of soluble oxalate occurred in cassava leaves It probably was Mg or K oxalate or both Nevertheless, the fact that Waipio plants had significantly higher tissue Ca concentration and lower tissue K concentration than the plants from the other three sites (Table 4 49) probably accounts for the absence of soluble oxalate

Table 4 47

Correlation between oxalates and various cations in cassava leaves

		Correlation coefficients* (Probability > r)						
Element	Total	oxalate	Insoluble oxalate					
Tissue Ca		5883 0064)	0 6444 (0 0022)					
Mg		4222 0637)	0 5898 (0 0062)					
K		1998 3984)	-0 4482 (0 0475)					
Na		3700 1083)	-0 2642 (0 2604)					
Insoluble fraction C		6239 0033)	0 7751 (0 0001)					
M	J	0141 9529)	0 1935 (0 4138)					
к		3167 1737)	-0 3785 (0 0998)					
N.	-	7414 0002)	0 7415 (0 0002)					
Ca+M	3	5835 0069)	0 7472 (0 0001)					

^{*} Number of observations = 20

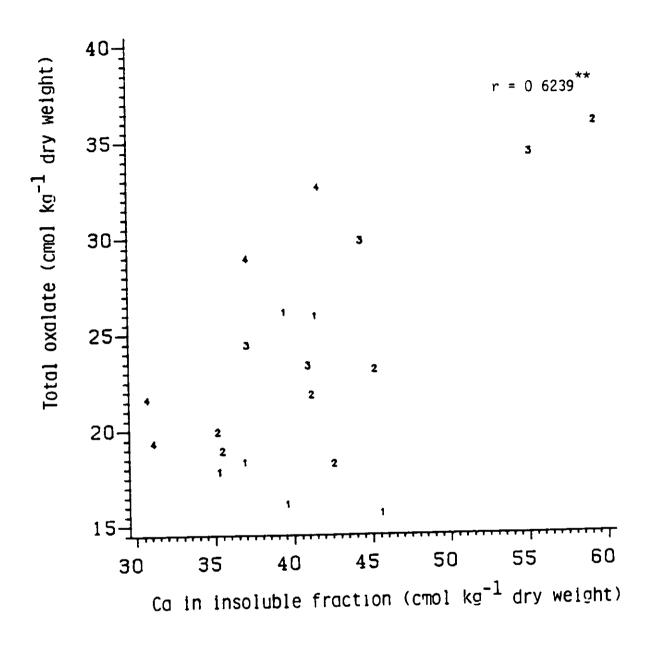


Figure 4 34 Relationship between total oxalate and Ca in the extract of insoluble oxalate fraction of leaf tissue of cassava grown at four sites (l=Kukaiau, 2=Iole 3=Waipio)

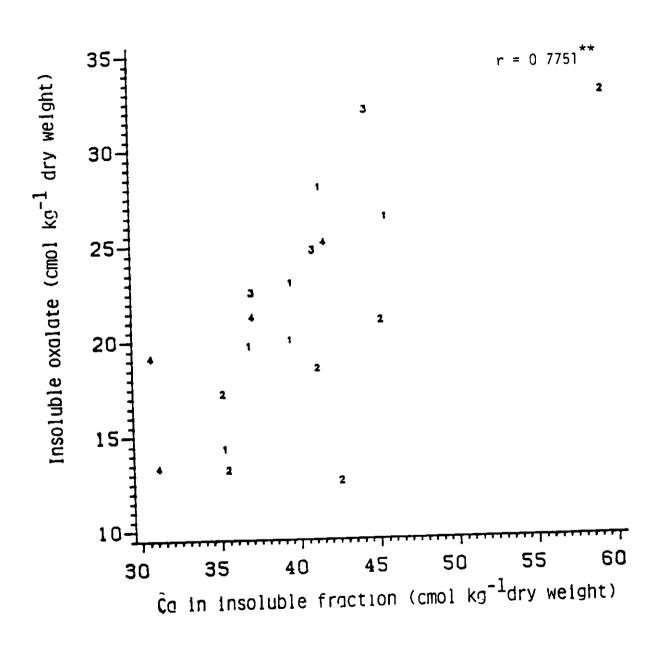


Figure 4 35 Relationship between insoluble oxalate and Ca in the extract of insoluble oxalate fraction of leaf tissue of cassava grown at four sites (1=Kukaiau, 2=Iole, 3=Waipio)

Table 4 48

Oxalate concentration in cassava leaf tissue of plant grown at four different sites

Site	Total oxalate	Insoluble oxalate -1	Soluble oxalate ^d
		——(cmol kg ^{-l} dry	weight
Molokaı	25 6 A ^b	19 7 B ^b	5 9
	(1 2) ^C		
Waipio	28 0 ^a A	29 4 A	-
	(1 3)		
Iole	23 O A	19 2 B	3 8
	(1 0)		
Kukatau	20 0 ^a A	21 9 AB	-
	(0 9)		

^aPair comparison using LSD show that the two means are significantly different (Probability > |t| = 0 0464)

bMeans in the same column followed by the same capital letter are not significantly different at 0 05 probability level (Waller-Duncan's multiple range test)

 $^{^{\}text{C}}$ Oxalate concentration in the unit of $^{\text{7}}$ on a dry weight basis

d Soluble oxalate was obtained by subtracting insoluble oxalate from total oxalate

in Waipio plants and that if soluble oxalate did occur it would do so in the form of K oxalate

The content of total oxalate in cassava leaves ranges from 0 9-1 3% on a dry weight basis (Table 4 48). This range was considerably less than that in amaranth

4 3 2 Chemical compositions of plants grown at different sites

Waipio plants had higher concentrations of total and insoluble oxalates than the plants from the other sites (Table 4 48). For Waipio plants, the results showed that all oxalate was in the forms of insoluble oxalate while some soluble oxalate occurred in plants from Molokai and Iole (Table 4 48). The higher Ca concentration in Waipio plants (Table 4 49) probably led to the higher Ca oxalate concentration in these plants. The relatively higher K concentrations in plants from the Molokai and Iole sites (Table 4 49) probably led to the occurrence of small quantities of soluble oxalate.

It appears that the oxalate form was determined by the dominant cations in the plants. Ca was the dominant cation in plants from Waipio (Table 4-49), which probably resulted in Ca oxalate being the primary form of oxalate. Molokai and Tole plants had about equal concentrations of Ca and K which may have promoted the formation of K oxalate although Ca oxalate was still dominant.

Relationship between oxalate concentrations and cation excess

Oxalate constituted only 18 6-25 9% of the cation excess in cassava leaves (Table 4 51) It is probable that oxalate was not the dominant organic acid in cassava. This is different from amaranth in which more than 60% of cation excess was accounted for by oxalate

Table 4 49

lonic concentrations and cation excess contents in cassava grown at four experimental sites

Site	Ca	 Mg	K	Na	sc ^a	P	S	мо3	C1	SA^{b}	C-A
				(cr	nol kg ^{-l} d	ry weigh	t)				
Molokai			49 7 A ^C (1 8)		123 6 A ^C	3 6 B ^C (0 3)	5 2 B ^C (0 2)	7 7 A ^C	5 8 A ^C (0 20)	22 2 A ^C	101 4 A ^C
Waipio	63 9 A (1 3)	22 7 A (0 3)	42 7 B (1 7)	1 2 C (0 03)	130 2 A	3 9 B (0 4)	4 8 C (0 2)	7 8 A	5 8 A (0 19)	22 2 A	108 1 A
Iole	54 4 AB	18 9 B (0 2)	50 2 A (2 0)	1 3 BC (0 03)	124 9 A	5 6 A (0 5)	5 5 A (0 3)	2 9 C	4 7 B (0 17)	18 6 B	106 3 A
Kukalau	55 8 AB	18 6 B (0 2)	50 5 A (2 0)	1 6 A (0 04)	126 5 A	4 2 B (0 4)	5 2 B (0 2)	4 9 B	4 9 AB (0 17)	19 2 B	107 4 A

^aSum of cations

bSum of anions

^CMeans of each ion with the same capital letter are not significantly different at 0 05 probability level (Waller-Duncan's multiple range test)

 $^{^{\}rm d}$ Ionic concentrations in the unit of % on a dry weight basis

Table 4 50

Insoluble oxalate and calcium concentrations in cassava grown at four sites

	Insoluble o	Tissue Ca		Ca in the form	of	
Site	(cmol	kg ^{-l} dry	weigh	t)	(% tissue Ca)	}
Molokai	19 7	<u></u>	53	6	37	
Walpio	29 4		63	6	46	
Iole	19 2		54	4	35	
Kukanau	21 9		55	8	39	

Total oxalate was positively correlated with cation excess (Figure 4 36) Oxalate exhibited a higher positive correlation with Ca than with cation excess, therefore, it can be said that oxalate was more closely related with Ca concentration than with cation excess. Since oxalate was probably not the primary organic acid in cassava, the lack of a significant response to cation excess was not surprising. It might very well be that in cassava total organic acid responded to cation excess, whereas, oxalate responded to Ca. The literature is not consistent with regard to the relationship between oxalate synthesis and Ca absorption. Olsen (1939) and Rasmussen and Smith (1961) reported a positive relationship between Ca and oxalic acid in some plants. On the other hand. Osmond (1967) found no relationship between oxalate synthesis and Ca absorption in Atriplex. He also found that oxalate content correlated with cation content rather than Ca alone.

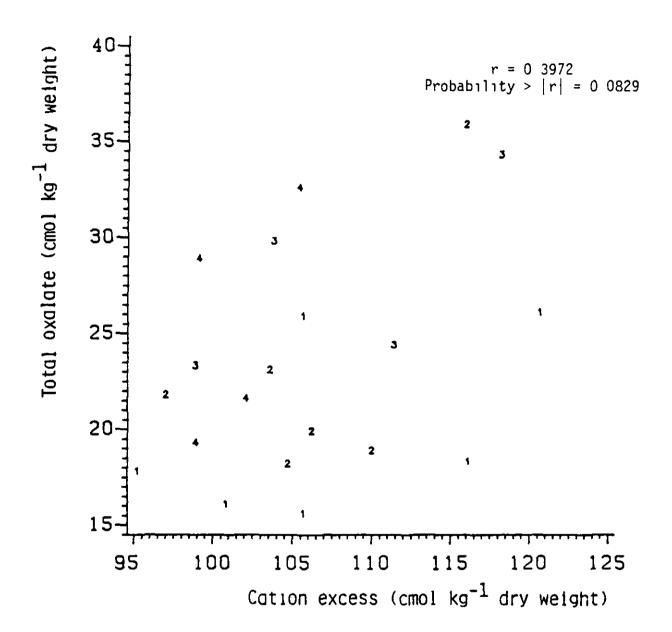


Figure 4 36 Relationship between total oxalate concentration and cation excess in leaf tissue of cassava grown at four sites
(l=Kukaiau, 2=Iole 3=Waipio)

Table 4 51

Oxalate content as percentage of cation excess (C-A)
in cassava leaves

Site	Total oxalate ————(cmol kg	Cation excess -1 dry weight)————	Total oxalate (% C-A)
Molokai	25 6	101 4	25 2
Waipio	28 0	108 1	25 9
Iole	23 0	106 3	21 6
Kukarau	20 0	107 4	18 6

Total N and nitrate-N concentrations and crude protein content

Higher total N and crude protein contents occurred in plants grown at the Hydric Dystrandept sites than at the Tropeptic Eutrustox sites (Table 4 52) This also occurred in amaranth plants and can be attributed to the higher soil N content in Hydric Dystrandept soils On the other hand, nitrate-N was significantly higher in plants grown in the Tropeptic Eutrustox than in plants from the Hydric Dystrandept The reason behind the higher nitrate-N in Tropeptic Eutrustox plants despite the lower soil N may be due to the climatic effects on nitrate assimilation Nitrate concentration was positively correlated with air and soil temperatures (Table 4 57) High temperature can decrease the nitrate reductase activity which results in the accumulation of nitrate (Cantliffe 1972a) The different results for nitrate-N between amaranth and cassava may also be caused by differences in rooting depth and length of growing period. Cassava roots permeate

Table 4 52

Total N, nitrate-N and crude protein contents in the leaves of cassava grown at four sites

Site	Total N*	Nitrate-N* (% dry weight)	Crude protein*
Molokai	4 60 B	0 11 A	28 8 B
Waipio	4 29 C	0 11 A	26 8 C
Iole	5 18 A	0 04 C	32 4 A
Kukatau	4 72 8	0 07 B	29 5 B

^{*} Means in the same column followed by the same letter are not significantly different at 0 05 probability level (Waller-Duncan's multiple range test)

a larger volume of soil for a longer period of time than do amaranth roots. Nitrate-N found in cassava leaves in this study is not considered high and did not present a health hazard.

Crude protein content of cassava leaves was 26 8-32 4% on a dry weight basis. This is in the high range of protein content reported in the literature (Rogers and Milner 1963)

4 3 3 Effects of irrigation on chemical composition of plants grown at Molokai and Waipio sites

Significantly higher total and insoluble oxalates were in nonirrigated plants at Molokai but not at Waipio (Table 4 53). Both
Molokai and Waipio are considered dry sites, but Molokai was even drier
than Waipio as shown by the nearly two-fold less precipitation received
at Molokai (Table 3 21)

Total N concentration was significantly lower in non-irrigated plants at Molokai (Table 4 54) Nitrate-N concentrations were not affected by irrigation

Table 4 53

Effect of irrigation on oxalate concentration of taro grown at Molokai and Waipio

	Site				
	Mole	okaı	Wan	p10	
	Total oxalate	Insoluble oxalate	Total oxalate	Insoluble oxalate	
Irrigation*		Cmo	ol kg ^{-l} dry	we1ght	
NI	45 9	39 5	31 8	25 6	
I	25 6	19 7	28 0	29 4	
Probability > F	0 0426	0 0030	0 3792	0 4516	

^{*} NI = Non-irrigated I = Irrigated

4 3 4 Influence of plant soil and climatic factors on oxalate and nitrate concentrations

Insoluble oxalate concentrations were negatively correlated with total N but positively correlated with nitrate-N (Table 4 55). The negative relationship between oxalates and total N was rather surprising because nitrogen metabolism usually promotes the synthesis of oxalate. Total N was negatively correlated with tissue Ca (Figure 4 37). This

Table 4 54

Effect of irrigation on total N and nitrate-N concentrations in taro grown at Molokai and Waipio

	Site						
		Molokai	Warpio				
	Total N	Nitrate-N	Total N	Nitrate-N			
Irrigation*			% dry weight				
NI	4 08	0 11	4 30	0 10			
I	4 60	0 11	4 29	0 11			
Probability > F	0 0045	0 7718	0 9213	0 0887			

^{*} NI = Non-irrigated, I = Irrigated

Table 4 55

Correlation between oxalates and total N and nitrate-N in cassava leaves grown at four sites

	Correlation coefficient* (Probability > r)					
Oxal ate	Total N	Nitrate-N				
Total	-0 4129 (0 0704)	0 4317 (0 0574)				
Insoluble	-0 6393 (0 0024)	0 4082 (0 0740)				

^{*}Number of observations = 20

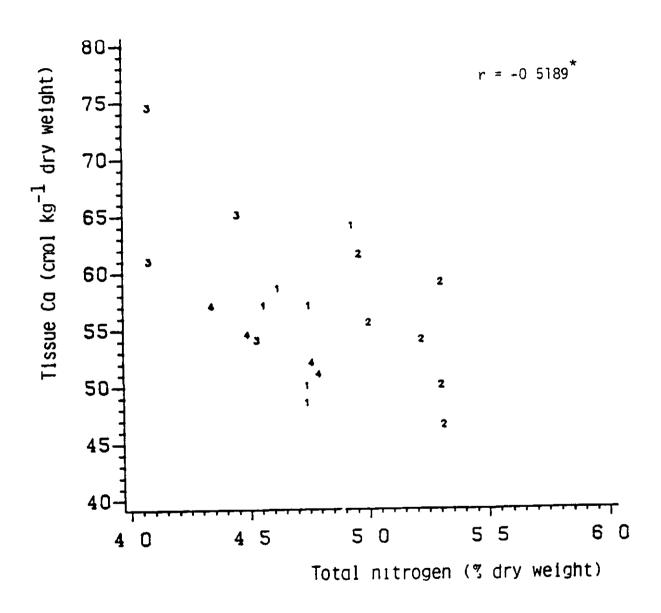


Figure 4 37 Relationship between Ca and total N concentration in leaf tissue of cassava grown at four experimental sites (1=Kukaiau, 2=Iole, 3=Waipio)

suggests that total N and Ca are also correlated and therefore the relationship between total N and oxalates may not be a causal one

Oxalate and nitrate were positively correlated with soil cations while nitrate was negatively correlated with soil P (Table 4 56). The uptake of cations can increase the cation excess in plants which, in turn, leads to the synthesis of organic acid

Table 4 56

Correlation between oxalate in cassava and soil variables from four sites

Soil	<pre>Correlation coefficients* (Probability > r)</pre>					
variables	Total oxalate	Insoluble oxalate	Nitrate			
Soil Ca	ns**	ns	ns			
Mg	0 4727 (0 0353)	0 4400 (0 0522)	0 6243 (0 0033)			
К	0 4743 (0 0346)	ns	0 7148 (0 0004)			
Na	0 4528 (0 0450)	ns	0 8190 (0 0001)			
p	ns	ns	-0 4745 (0 0345)			

^{*} Number of observations = 20

The positive correlations between temperature and oxalates

(Table 4 57) in cassava were different from the relationship found
in amaranth. Kitchen et al. (1964b) postulated that in spinach, oxalate

^{**} ns = non-significant

Table 4 57 Correlation between oxalate and nitrate in cassava with some climatic variables from four sites

Climatic variable	Corre (Pr		
	Total Insoluble oxalate		Nitrate-N
Average air temperature	ns**	ns	0 8510 (0 0001)
Average relative humidity	ns	-0 4581 (0 0422)	ns
Average topsoil temperature	ns	0 4701 (0 0365)	0 7362 (0 0002)
Average subsoil temperature	0 4626 (0 0400)	0 4481 (0 0475)	0 7198 (0 0003)
Rainfall	-0 4583 (0 0421)	ns	-0 6953 (0 0007)
Radiation	ns	ns	0 8485 (0 0001)
Wind speed	ns	ns	ns

^{*} Number of observations = 20 ** ns = non-significant

was used as a substrate for respiration at high temperature. The positive correlations of nitrate with temperature and radiation were also found in amaranth. Cantliffe (1972a) and Maynard et al. (1976) found similar relations in other plants.

4 4 Response of taro to environmental factors

As in the case with the section on cassava, this section emphasizes the results concerning the effects of environmental factors on nutrient and antinutrient contents of taro leaves

4 4 1 Forms of oxalate in taro

Taro contained both soluble and insoluble oxalates in contrast to cassava where most oxalate was in the insoluble form. The positive correlation between insoluble oxalate and Ca (Table 4-58, Figure 4-38) suggests that insoluble oxalate was primarily Ca oxalate. Moreover, the higher correlation of Ca plus Mg concentrations with insoluble oxalate (Table 4-58, Figure 4-39) suggests that a part of the insoluble oxalate was in the form of Mg oxalate.

There was a positive correlation between both tissue K and K in the soluble oxalate fraction with soluble oxalate (Table 4.58. Figure 4.40). This suggests that the soluble oxalate was primarily K oxalate Mg oxalate probabily constituted a significant part of soluble oxalate as suggested by high correlation where Mg was also considered r=0.7898 (Table 4.58). The correlation between K+Mg in the soluble fraction and soluble oxalate was highly significant (Figure 4.41) suggesting that K and Mg oxalate were important constituents in taro

Table 4 58 Correlation between oxalates and mineral concentration ın taro leaves

Element		Correlation coefficient* (Probability > r)					
		Soluble oxalate	Insoluble oxalate	Soluble+Insoluble oxalate			
Tissue Ca		-0 7881 (0 0001)	0 3282 (0 0053)	ns			
Mg		ns**	ns	0 3435 (0 0323)			
К		0 6931 (0 0001)	ns	0 3412 (0 0335)			
Na		-0 5675 (0 0002)	0 3526 (0 0277)	ns			
Soluble fraction	Ca	-0 6012 (0 0001)	ns	ns			
	Mg	0 4531 (0 0038)	ns	0 4240 (0 0071)			
	K	0 6712 (0 0001)	ns	ns			
	Na	ns	ns	ns			
	K+Mg	0 7898 (0 0001)	ns	-			
Insoluble fraction	Ca	-0 7637 (0 0001)	0 4755 (0 0022)	ns			
	Mg	ns	0 3741 (0 0190)	0 4652 (0 0028)			
	K	0 3415 (0 0334)	-0 3201 (0 0470)	ns			
	Na	-0 6858 (0 0001)	0 3360 (0 0365)	ns			
	Ca+Mg	-0 7270 (0 0001)	0 5036 (0 0001)	ns			

^{*} Number of observations = 42 ** ns = Non-significant

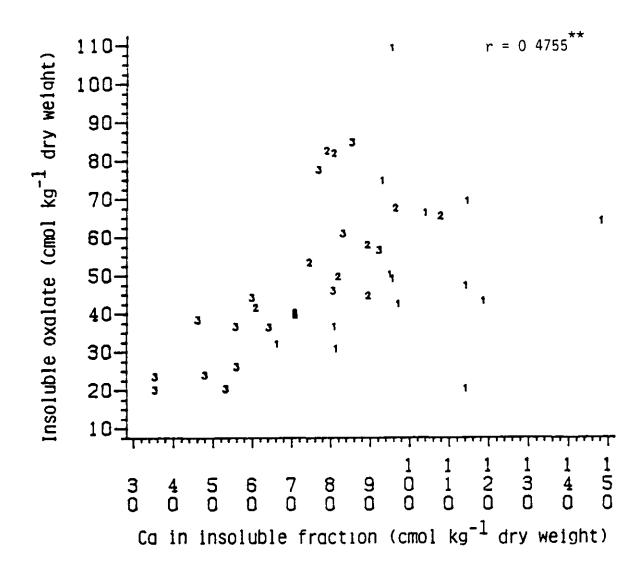


Figure 4 38 Relationship between insoluble oxalate and calcium in the extract of insoluble oxalate fraction of leaf tissue of taro grown at three sites (l=Kukaiau, 2=Iole, 3=Waipio)

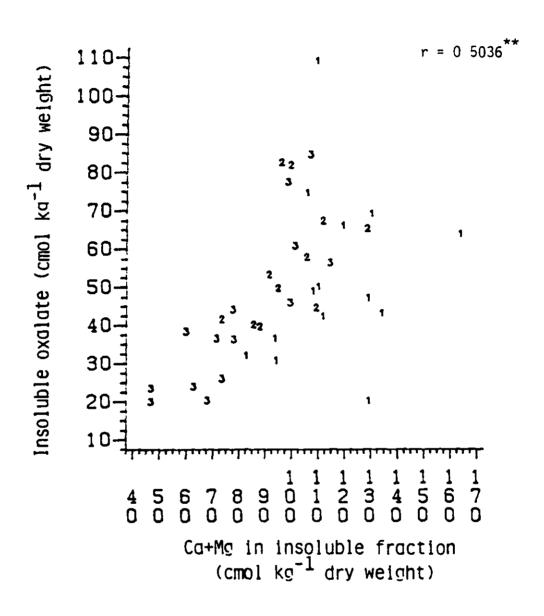


Figure 4 39 Relationship between insoluble oxalate and the sum of calcium and magnesium concentration in the extract of insoluble oxalate fraction of leaf tissue of taro grown at three sites (l=Kukaiau, 2=Iole, 3=Waipio)

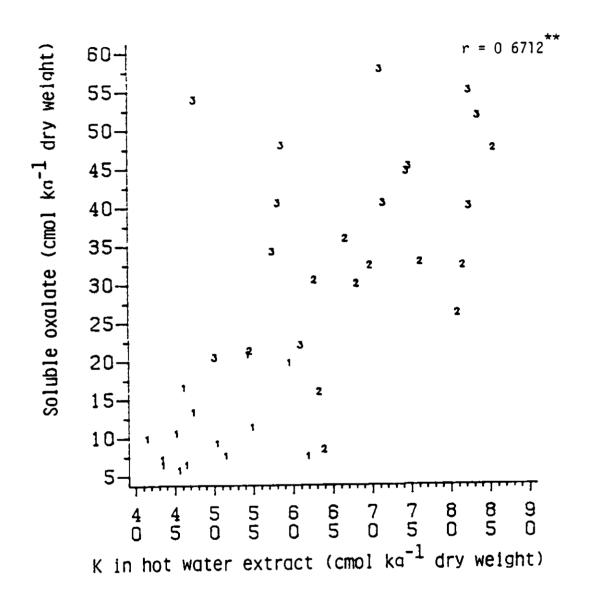


Figure 4 40 Relationship between soluble oxalate and potassium concentration in the extract of soluble oxalate fraction (hot water extract) of leaf tissue of taro grown at three sites

(l=Kukaiau, 2=Iole 3=Waipio)

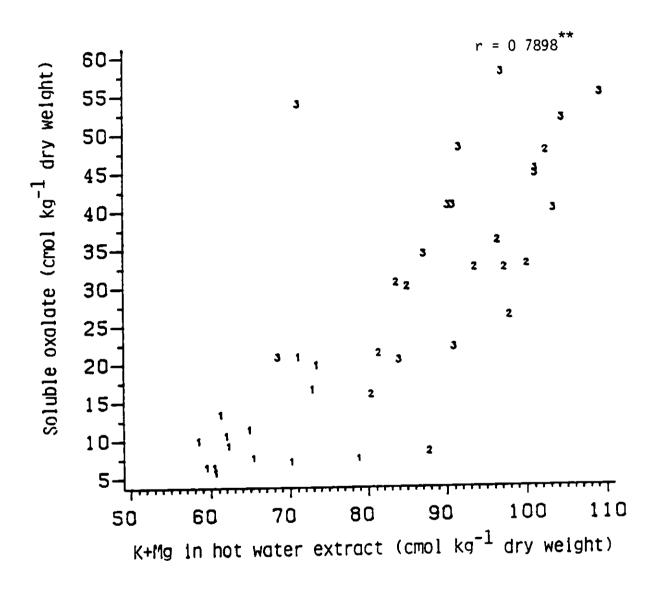


Figure 4 41 Relationship between soluble oxalate and the sum of potassium and magnesium concentration in the extract of soluble oxalate fraction (hot water extract) of leaf tissue of taro grown at three sites (1=Kukaiau, 2=Iole 3=Waipio)

The analysis of mineral concentrations, including Ca Mg, K and Na in the soluble and insoluble fraction also shows that the dominant cation in the soluble fraction was K while Ca appears to dominate in the insoluble fraction (Table 4 59) Little Ca was found in the soluble fraction and little K was found in the insoluble fraction

Ca oxalate crystals are known to be plentiful in taro leaves

(Sunell and Arditti, 1983) These crystals have been implicated in
the irritant quality of taro. Sunell and Arditti (1983) suggested that
Ca oxalate plays a role in Ca balance within the plant. Sunell and
Healey (1979) reported that Ca oxalate in taro was not metabolically
inactive because crystal numbers and density changed during plant
development. The finding of soluble oxalate in taro implies that Ca
from other foods can be bound and rendered unavailable.

4 4 2 Comparison of chemical composition of plants grown at different sites

Significantly higher soluble oxalate was found in plants from the Tropeptic Eutrustox soil at the Waipio site than in plants grown in the Hydric Dystrandept soils at Kukaiau and Iole (Table 4 60)

Furthermore for soils of the same family of Hydric Dystrandepts, plants from Iole had significantly higher soluble oxalate than plants from Kukaiau (Table 4 60) Plants grown at Waipio and Iole had significantly higher tissue K and Mg concentrations than did plants at Kukaiau (Table 4 61) Higher soluble oxalate was also found in Waipio and Iole plants Although Iole plants had almost equal concentrations of tissue K as the Waipio plants, they had significantly higher tissue Ca. This was

Table 4 59

Mineral concentrations in the soluble and insoluble fraction of extracts of oxalate in taro grown at three sites

Site		·	······································		cmol kg	-l dry weigh	nt			- -
	Soluble fraction				Insoluble fraction					
	Oxalate	Ca	Mg	K	Na	0xalate	Ca	Mg	K	Na
Kukatau	10 9	2 6	16 4	49 4	0 22	52 5	101 4	14 6	2 1	0 90
Iole	28 5	2 3	20 8	71 2	0 31	56 7	78 6	16 3	2 4	0 84
Waipio	41 1	1 5	27 3	65 0	0 25	42 4	62 3	16 7	2 3	0 62

Table 4 60

Oxalate concentrations of taro leaves grown at three different experimental sites

	cmol kg ⁻¹ dry weight								
Site	Soluble* oxalate	Insoluble* oxalate	Soluble + Insoluble* oxalate						
Kukalau	10 9 C	52 5 A ^a	63 4 B						
Iole	28 5 B	56 7 A ^b	85 2 A						
Waipio	41 1 A	42 4 A ^C	83 5 A						

^{*} Means in the same column followed by the same capital letter are not significantly different at 0 05 probability level (Waller-Duncan's multiple range test)

probably the reason for the lower soluble oxalate in plants grown at Iole than at Waipio

Plants from the Hydric Dystrandept soils contained higher insoluble oxalate than plants grown in the Tropeptic Eutrustox soil. This was associated with the significantly higher tissue Ca in plants from the Hydric Dystrandepts (Table 4-61)

Table 4 60 also shows that while the insoluble oxalate did not vary with site the soluble fraction did. Tissue Ca was higher in plants from Kukaiau than from Iole and Waipio while tissue K was higher in Iole and Waipio than in plants from Kukaiau (Table 4 61). This probably led to the higher proportion of insoluble oxalate and a lower proportion

^a12 7 mg/g FW

b₁₃ 7 mg/g FW

^C10 3 mg/g FW

Table 4 61

Ionic concentrations and cation excess in taro leaves grown in three experimental sites

					c	mol kg (% dry w	dry weight eight)				
Site	Ca	Mg	К	Na Na	SCª	P	S	NO ₃	CI	SAb	C A
Kukalau				1 1 A ^C (0 03)	256 8 A ^C	3 2 c ^c (0 31)	5 9 A ^C (0 3)	7 1 A ^C	36 6 A ^C (1 3)	52 7 A ^C	204 1 A ^C
lole	110 3 B (2 2)	43 7 B (0 5)	111 1 A (4 3)	1 1 A (0 03)	266 2 A	3 8 B (0 37)	5 6 A (O 3)	7 2 A	28 9 B (1 O)	45 5 B	220 7 A
Walpio	90 5 C (1 8)	54 2 A (0 7)	111 5 A (4 3)	0 9 B (0 02)	257 1 A	4 5 A (0 44)	5 6 A (O 3)	6 8 A	39 9 A (1 3)	51 8 A	205 3 A

^aSum of cations

b Sum of anions

C Means in the same column followed by same capital letter are not significantly different at 0 05 probability level (Waller Duncan's multiple range test)

d lonic concentrations in the unit of % on a dry weight basis

of soluble oxalate in plants grown at Kukaiau (Table 4 62) On the other hand Waipio plants had lower tissue Ca and higher tissue K than Kukaiau plants (Table 4 61) which probably led to a higher proportion of soluble oxalate and a lower proportion of Ca oxalate than at Kukaiau (Table 4 62) Plant Mg and soluble oxalate contents at Iole were lower than in plants from Waipio while Ca and insoluble oxalate contents were higher

Table 4 62

Comparative concentrations of different forms of oxalate as percentage of soluble + insoluble oxalate in taro leaves from three sites

Site	% of soluble + insoluble oxalate						
	Soluble* oxalate	Insoluble* oxalate					
Kuka1 au	17 2 C	82 8 A					
Iole	33 5 B	66 5 A					
Waipio	49 2 A	50 8 A					

^{*} Means in the same column followed by the same letter are not significantly different at 0 05 probability level (Waller-Duncan's multiple range test)

de la Pena and Plucknett (1969) found that addition of K to upland and lowland taro reduced the concentration of Ca as well as Mg Simple correlation (Appendix F) of data from all sites shows that soil Ca was negatively correlated with tissue Ca while tissue Ca was, in turn negatively correlated with tissue K Tissue K and soil K however,

were positively correlated indicating that K may be the most important soil variable influencing taro soluble oxalate content in Table 4 67

The high concentration of Cl (1 0-1 3%) in taro leaves (Table 4 61) is of interest. It was more than 5 times higher than in leaves of amaranth and cassava (0 2-0 3% dry weight). According to Mengel and Kirkby (1978), Cl content of most plants generally range from 0 2-2 0% dry weight. For Cl sensitive crops, the reduction in yields was associated with tissue levels of 0 5-2 0% Cl. Spinach and beetroot are considered halophytes and contain Cl content ranging from 0 5-2 2° dry weight (Vityakon, 1979). These facts suggest that taro is of high Cl plant.

Oxalate and calcium concentration in taro leaves

Approximately 40 to 50 percent of Ca was in the forms of Ca oxalate (Table 4 63) Therefore, about half of the total tissue Ca in taro leaves should not be available to humans. The plant maintained a sizable soluble oxalate pool despite a large quantity of Ca unbound by oxalate. The mechanism by which taro maintains a high soluble oxalate pool in the presence of high plant Ca concentration cannot be explained at this time.

Relationship between oxalate and cation excess

Oxalate constituted from 30 to 40 percent of the cation excess in plants at the various sites (Table 4 64). Significant relationships between oxalates and cation excess were found in insoluble oxalate from combined data of three experimental sites and soluble and insoluble oxalate in Waipio site (Table 4 65). The sum of soluble and insoluble oxalate was not significantly correlated with cation excess. In

Table 4 63

Calcium in the form of calcium oxalate in taro leaves grown at three sites

Site	cmol		
	Insoluble oxalate	Tissue Ca	Ca oxalate (% tissue Ca)
Kukanau	52 5	131 7	39 9
Iole	56 7	110 3	51 4
Waipio	42 4	90 5	46 9

Table 4 64

Total oxalate as percentage of cation excess in taro leaves from three experimental sites

Site	% cation excess
Kukanau	31 1
Iole	38 6
Waipio	40 7

contrast to other plants, it appears that oxalates in taro were more closely related with specific cations (Table 4 58) than with cation excess

Total N nitrate-N and crude protein contents

Significantly higher total N was found in taro leaves from the Iole site than in leaves from Kukaiau and Waipio (Table 4 66). This is likely because Iole soil has higher N content. The levels of nitrate-N in the leaves were below the dangerous level in all cases.

At Iole site crude protein content of taro leaves was similar to that of amaranth and cassava while at Kukaiau the levels were lower (Tables 4 66, 4 52, and 4 18)

Table 4 65

Correlation between oxalates and cation excess in taro leaves

oxalate	<pre>correlation coefficient (Probability > r)</pre>						
	combined ^a 3 sites	Kuka 1 au ^b	Iole ^b	Waipio ^b			
Soluble	ns*	ns	ns	-0 7170 (0 0039)			
Insoluble	0 3854 (0 0154)	ns ns	ns ns	0 7747 (0 0011)			
Soluble+ Insoluble	ns	ns	ns	ns			

^{*} ns = non-significant

^aNumber of observations = 42

bNumber of observations = 14

Table 4 66

Total N, nitrate-N and crude protein concentrations of taro leaves grown at three sites

Site	 	% dry weig	ht
	Total N*	Nitrate-N*	Crude protein*
Kukarau	3 9 B	0 10 A	24 4 B
Iole	4 6 A	0 10 A	28 8 A
Waipio	3 8 B	0 09 A	23 8 B

^{*} Means in the same column followed by the same letter are not significantly different at 0 05 probability level (Waller-Duncan's multiple range test)

4 4 3 Relationships between oxalates in taro leaves and soil and climatic variables

Soluble oxalate in taro leaves was positively correlated with soil Ca, Mg, K, and Na and negatively correlated with soil P (Table 4 67). This is consistent with the cation-anion balance concept in which increasing cation content leads to an increase in cation excess and consequently to an increase in the synthesis of organic acid. On the other hand, increasing anion concentration leads to a decrease in cation excess and a consequent decrease in organic acid content.

Soluble oxalate was correlated with climatic variables while no correlation was observed for insoluble oxalate or the sum of soluble and insoluble oxalate (Table 4 68). Soluble oxalate in taro leaves was positively correlated with air and soil temperature as was the case with amaranth and cassava. Both rainfall and relative humidity

however, were negatively correlated with soluble oxalate. A correlation matrix (Appendix F) shows that radiation, air temperature soil temperature, relative humidity, rainfall, and wind speed were all highly correlated. Thus when radiation was high temperature was high, rainfall was more likely and the air was more turbulent. It is likely that one variable serves as a surrogate for all others. In any case, the climate was sufficiently different among sites to have caused differences in the soluble oxalate content.

Table 4 67

Correlation between taro oxalates and soil variables from three experimental sites

Element	<pre>Corrleation coefficient* (Probability > r)</pre>							
	Soluble oxalate	Insoluble oxalate	Soluble+insoluble oxalate					
Soil Ca	0 7020 (0 0001)	ns**	0 4756 (0 0022)					
Mg	0 7400 (0 0001)	ns	ns					
К	0 7161 (0 0001)	ns	ns					
Na	0 6747 (0 0001)	ns	ns					
Р	-0 7618 (0 0001)	ns	-0 4714 (0 0025)					

^{*} Number of observations = 42

^{**} ns = non-significant

Table 4 68 Correlation between taro oxalates and climatic variables from three experimental sites

Climatic	<pre>Correlation coefficient* (Probablity > r)</pre>								
variables	Soluble oxalate	Insoluble oxalate	Soluble+Insoluble oxalate						
Average air temperature	0 5168 (0 0008)	ns**	ns						
Average relative humidity	-0 5626 (0 0002)	ns	ns						
Average topsoil temperature	ns	ns	ns						
Average subsoil temperature	0 7113 (0 0001)	ns	ns						
Rainfall	-0 7219 (0 0001)	ns	ns						
Radiation	0 6227 (0 0001)	ns	ns						
Wind speed	-0 5412 (0 0004)	ns	ns						

^{*} Number of observations = 42 ** ns = non-significant

CHAPTER V

SUMMARY AND CONCLUSIONS

This study provides new evidence to support the hypothesis that plant composition is strongly influenced by the same environmental factors that regulate plant growth and development While it is well known that soil fertility affects the mineral nutrition of plants, the effect of soil fertility on the antinutrient content of plants has heretofore not been well documented This study also shows that the effect of soil fertility on plant composition in general, and antinutrient in particular, is strongly modified by atmospheric conditions The situation is further complicated by the fact that plant utilization of soil phosphorus and bases is controlled first by soil nitrogen and only secondarily by the amount of phosphorus and bases present in the soil Furthermore, plant composition is determined to a larger extent by plant genotype than by environmental factors

Plant oxalate and cation excess is significantly related although in some cases better relationships are found between oxalate and individual cations. The results of this study support earlier studies which show that oxalate is produced in response to cation excess. In addition, the forms of oxalate produced depend on the dominant cations in the plant tissue.

Tables 5 1 and 5 2 summarized the effects of soil and climatic factors and cation excess on the oxalate and nitrate contents of three crops. Soil and climate affect cation excess (C-A) which in turn affect oxalate content.

Table 5 l summarizes the important relationships that exist between soil-climate factors and oxalate and in trate contents. The results show that these relationships differ for different crops. Through multiple regression analysis (Table 5.2) the key factors influencing individual plant constituents are identified. Formation of different forms of oxalate in the same plant is influenced by different environmental factors. The fact that solar radiation air temperature and wind speed appear in the equations show that weather variables contribute measurably to plant composition.

These findings suggest that nutritional quality of crops can be controlled through crop selection and management of the environment. If as much attention is given to improving nutritional quality of food crops as is now given to increasing yields—the goal of meeting the nutritional needs of people will be more readily served.

Table 5 l

Soil climatic factors and cation excess correlated with the concentration of oxalates and nitrate N in amaranth cassaya and taro

Positive and negative signs are those of the correlation coefficients—Asterisks represent significance level where there is no asterisk—the correlation is not significant

Crop	Plant	Cation excess		Soil				1		Climate			
	composition	(C A)	N	Р	Ca	Hg	K	Av te	Subsoil	Rainfall	Radiation	Wind speed	Relative humidity
Ą	Soluble oxalate		+		*] •		•			
4	Insol oxalate	+**	+							+**			+*
A	Total oxalate		+ *		**	**	*	}		+**	**	**	+
R A	Sol + Insol oxalate	+ *	+*	*	**					+ *	##		+
N	NO3 N		+**		**	**	**	i *		+**	*	**	+
ī								1					
Н								1				_	
c	Insol oxalate	+		+		+*	+	1 +	+*		+	+	*
A	Total oxalate	+			+	+*	+*	1 +	+*	*	+		
S	NO3 N			*	+	+**	+**	+	+ *	-++	†**	+	
S								1					
A								1					
٧								1					
A								<u> </u>					
T	Soluble oxalate			**	+ *	+**	+**	+*	+**	**	+**	**	**
A	Insol oxalate	**		+				1		+		+	+
R O	Sol + insol oxalate	•			+	+		+	+		+		
-	NO3 N			+				1		+		+	+

Table 5 2

Variables identified through multiple regression analysis to contribute significantly to the oxalate and nitrate contents of amaranth

Plant composition (Y) ^a	X variables ^b	Contributions ^C		
Total oxalate (NI)	SRSQ PLEVEL SOLMG SOLK	- - + +	ns * **	
Total oxalate (I)	SRSQ NLEVEL SOLK AIR4SQ	+ + +	** ** NS **	
Soluble oxalate (NI)	SOLK PLEVEL SOLCA SOLP	+ - - +	** ** ** ns	
Soluble oxalate (I)	AIR2 PLEVEL	+	** **	
Insoluble oxalate (NI)	SOLP PLEVEL	- +	** **	
Insoluble oxalate (I)	AIR3 NLEVEL PLEVEL	- + +	** * ns	
Nitrate-N (NI)	WSPSQ NLEVEL SOLMG SOLK	- + - +	ns ** **	
Nitrate-N (I)	AIR2SQ NLEVEL SOLTN	+ + +	** ** **	

Table 5 2 (continued) Variables identified through multiple regression analysis to contribute significantly to the oxalate and nitrate contents of amaranth

Notes

aNI = non-irrigated plants
 I = irrigated plants

^bsrsq = Square of solar radiation PLEVEL = Fertilizer P rate SOLMG = Soil magnesium content = Soil potassium content SOLK NLEVEL = Fertilizer N rate = Square of the difference between maximum and AIR4SQ minimum temperature SOLCA = Soil Calcium content SOLP = Soil phosphorus content AIR2 = Minimum air temperture AIR3 = Average air temperature WSPSO = Square of wind speed AIR2SQ = Square of minimum air temperature SOLTN = Soil total nitrogen content

CPositive and negative signs are those of the parameter estimates or regression coefficients in the regression equations. Asterisks indicate the significance level (Probability > /t/)

Appendix A Analysis of variance of various dependent variables using combined data of amaranth experiments from three sites

DEPENCENT VARIABLE D	₩					
SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE C V
MODEL	29	161983 94320755	5585 65321405	1 76	0 0529	0 664963 90 6982
ERACH	23	72788 15000000	J164 70217391		ROUT MSE	DW MEAN
COINFLIED FOTAL	52	234772 09320755			56 25568570	61 88867925
SO HCF	D۴	TYPE 1 55	F VALUE PR > F	DF	TYPE 111 55	F VALUE PR > F
LOC REP(LOC) IRR LOCOIMM REPOIMM(LO) FER LOC FER IRR FER LOC IRR FER	6 1 2 6 4 2 4	53900 48863238 20205 65624183 4 90925030 7744 04052128 33153 24519841 6017 66287562 10367 14401307 11415 57969841 11535 01674603	8 52 0 0017 1 49 0 2272 0 00 0 9609 1 14 0 3359 1 75 0 1553 0 75 0 4011 0 83 0 5198 1 80 0 1872 0 91 0 4740	2 6 2 6 2 4 2 4	46752 66914530 28750 16394444 30 69160000 5937 61060000 31278 7372778 5605 98299145 10201 1919413 11715 56418803 11535 01674603	7 39 0 0033 1 51 0 2179 0 01 0 9224 0 94 0 4024 1 65 0 1765 0 89 0 4260 0 61 0 5341 1 65 0 1797 0 91 0 4740

SIRRICE DF 14PE 111 SS F VALUE PH > F

LLC 467 / 66914530 4 68 0 0552

TF 15 C H 01 C C 51No 1 L LV 111 MS FUR MEPOTRHICUC) AS AN ENRUR 1LIM

SO CE F 14PE 111 SS F VALUE PH > F

THR 1 30 69160000 0 0 01 0 9413

LIC 14 37 C1000000 0 7 0 937

TESTS (F F T) 1 SES USING THE TYPE IFI MS FOR REP(LOC) AS AN ERROR TERM

DEPENDENT VARIABLE	DX							
SOURC E	ρf	SUM OF SQUARES	MEAN SQUARE	WRE	F VALUE	•	R-SOUARE	> U
MODEL	29	30674 42102030	1057 73065567	5567	2 32	0 0 2 3 0	0 753286	11 5023
EAROR	22	10046 39507778	456 65432172	21.12		ROOT MSE		DX MEAN
CONRECTED TOTAL	15	40720 81609808				21 36947172	103	8 78480769
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JAR FEB	r 04	-			N			
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TESTS OF HYPOTHESES USING THE TYPE	USING THE TYPE	SM TIT	FOR REPILOC) AS AN ERHOR	TERM				
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רסכ	N	13063 06060556	X 01	0 0024				
TESTS OF HYPOTHESES USING THE TYPE III MS	US ING THE TYPE		FDA REPOIRALLOC) AS AN ERROR TERM	RROR TERM				
SOURCE	DF	1YPE \$11 \$5	F VALUE	£				
JRR LOC 1RA	→ №	74 55628929	0 28	0 6163				

DEPENDENT VARIABLES O	0 % 2							
SOURCE	, ,	SUM OF SOUARES	HEAN SOUARE		F VALUE	¥ ^ £	R-SQUARE	>
MODEL	5.0	29545 43161765	1018 8079868	Q.	9 9	1000 0	0 685238	15 4143
ERROR	21	3030 27563333	162 39406730	•		ROOT MSE		DAZ NEAN
CURRECTED TOTAL	96	33375 70745098				13 50633551		67 61568627
SOURCE	0.F	TYPE I SS	F VALUE PR	L ^ 4	ō	TYPE 111 S\$	F VALUE	PR > F
LOC REP1LOC	N 4 -	7750 28033987 3478 80744444	90 00	1220	N-0-	7673 92118056 3360 22598291 80 18038714	21 56	1977
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SOURCE	ò	TYPE 111 55	F VALUE PR	F V &				
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TESTS OF HYPOTHESES L	USING THE T	TYPE III MS FOR REPOIL	RRELLOC) AS AN ERROR	R TERM				
SOURCE	à	TTPE III SS	F VALUE PR	F 4				
IAR LOC•IAR	-N	50 16035714	99 98 98 98	5534				
DEPENDENT VARIABLES C	0 x 3							
SOURCE	0	SUM OF SQUARES	MEAN SOUARE	w	F VALUE	£	R-SOUAHE	> •
MODEL	5.8	15119 35227124	521 3569 7487		• 03	9000 0	0 647740	0 0340
EARUR	2.1	2715 53361111	129 31112434	•		ROOT MSE		CK 3 HEAN
CORRECTED TOTAL	99	17034 80580235				11 37150493	•	14 47056824
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ME PO I RRILOCI	10 0		2 ~ .		N 10 4	065 74046		
	1 4 N	1084 94628595 875 70054762	900	1172	4 4 4	01440004 ULO1	90 M	0 0 0
LOCO I RRO FER	•	Ľ	2		1∢	1994	•	
TESTS OF HYPOTHESES USING THE		TYPE III MS FOR REPIL	OC) AS AN ERROR TER	# E				
SOURCE	10	TYPE III SS	F VALUE PR	. α α				
301	N	3762 33027778	13 20 0	000				
TESTS UP HYPOTRESES USING THE TYPE	JS ING INE 1	III MS FOR REPO	RATIOC) AS AN ERER	RIERM				
SOURCE	90	INPE III SS	F VALUE PR	F 4 8				
198 LOC 188	-8	569 70321429 J534 00250000	3 25	0000				

SPURICE	DEPENDENT VARIABLE:	012 3							
MODEL 29 20		_	SUM OF SQUARES	MEAN S	Q LIARE	F VALUE	PR > F	R-SQUARE	c v
SOURCE OF TYPE I SS F VALUE PR > F OF TYPE II									
SOURCE OF 17PE 155 F VALUE PR > F DF 17PE 111 55 F DF 17PE 111 55 F VALUE PR > F DF 17PE 111 55	ERROR	21	5118 03888889	243 716	1 3757		ROOT MSE		DX2_3 MEAN
REPLICATION	CORRECTED TOTAL	50	31887 16039216				15 61141049	2	02 08627451
REPLICATION									
REPLICAL 6									
LOSS BREADOR 2 1282 05555100	REP(LOC)	6	7541 51672222	5 16	0 0021	6	5738 09350427	3 92	0 0007
REPHIENTLOCY 6 1143 30760193 0 70 0 5903 6 1140 07401700 0 70 0 5903 6 1140 07401700 0 70 0 5903 6 1140 07401700 0 70 0 00001 1 1 1 1 1 1 1 1 1 1 1	IRA LOCATER		905 13124699	3 71	0 0676	ļ	996 03571429		
The content	REPOIRRILOC)	6	1143 39780193	0 76	0 5935	6	1149 07401709	0 79	0 5908
The content	FER	Š	4452 20049020		0 0014	\$		6 32	0 0071
TESTS OF HYPOTHESES USING THE TYPE 111 MS FOR REPILOC) AS AN ENROR TERM SOURCE	IRAOFER		1212 04104762			2			0 1430
SOURCE OF TYPE 111 SS F VALUE PR > F LOC	LOCO I RHO FER			0 90	0 4020	•	876 72550794	0 90	0 4826
The color C	TESTS OF HYPOTHESES	USING THE TY	PE 111 MS FOR REPILO	C) AS AN ERHO	R TERM				
TESTS (F Y)DTILSES USING THE TYPE III MS FOR REP RR(LUC) AS AN ERROR TERM SULUCE OF TYPE III 53 P VALUE PR > F IAR	SOURCE	DF	TWE 111 55	F VALUE	PR > F				
SULUCE OF 17PE 111 55 P VALUE PR > F IAR	FOC	Ł	4466 84090278	2 34	0 1776				
IRR 1 996 03571429 5 20 0 0628	TESTS OF YEOTHESES	USING THE TYP	PE III MS FOR AFP IA	RELUCT AS AN	ERROR TERM				
DEPENDENT VARIABLE IN BOLRCE OF SUM OF SOURCES MEAN SOURCE F VALUE PR > F R-SQUARE C V MODEL 29 26 49339419 0 91356532 10 14 0 0001 0 930392 7 4624 REROR 22 1 90212889 0 0 00009677 ROOT MSE IN MEAN CORRECTED FOIAL 31 20 47552300 0 30016124 4 02230769 SOURCE DF TYPE I 55 F VALUE PR > F DF TYPE I 11 55 F VALUE PR > F LDC LDC REFILOC) 2 15 60727933 67 06 0 0001 2 15 22261719 84 48 0 0001 REFILOC) 2 15 60727933 67 06 0 0001 2 15 22261719 84 48 0 0001 REFILOC) 0 1 147340708 2 23 0 0440 4 1 24043475 2 31 0 0699 REFILOC) 0 0 0 147340708 2 23 0 0440 6 1 1 24043475 2 31 0 0699 REFILOC) 0 0 0 00270812 1 65 0 0001 2 2 15 22261719 84 48 0 0001 REFILOC) 0 0 00270812 1 62 0 1418 6 0 0 0947717 1 106 0 0701 REFIREDCO 2 3 77162004 20 93 0 0001 LOCUPER 2 3 77162004 20 93 0 0001 LOCUPER 4 0 0760050 1 60 0 20577 LOCUPER 5 0 0 0004 TESTS OF HYPOTHESES USING DIE TYPE III MS FOR REPOIRRILDC) AS AN ERROR TERM SOURCE DF TYPE III SS F VALUE PR > F LOC 2 15 22261719 36 55 0 0004	SULMCE	OF	146E 111 22	P VALUE	PR > F				
## SOURCE OF SUM OF SQUARES MEAN SQUARE F VALUE PR > F R-SQUARE C V MODEL 29 26 49339419 0 91356332 10 14 0 0001 0 930392 7 4624 ### REAR 22 1 96212889 0 09009677 ROOT MEE TYPE 111 SS F VALUE PR > F	IAR Loc iar								
MODEL 29 26 49339419 0 91356532 10 14 0 0001 0 930392 7 4624 ERROR 22 1 98212889 0 0 9909677 RODY MSE TH MEAN CORRECTED FOLAL 51 28 47552300 0 30016124 4 02230769 SOURCE OF TYPE I 55 F VALUE PR > F DF TYPE II1 55 F VALUE PR > F LDC 2 15 68727933 87 06 0 0001 2 15 22261719 80 48 0 0001 REPLOC) 0 1 42340708 2 63 0 0445 6 1 24943475 2 31 0 0699 IRA 1 0 37758519 4 10 0 0526 1 0 34522230 6 05 0 0222 LOC-IRR 2 1 0 2 90283335 16 55 0 0001 2 2 90079235 16.54 0 0001 REPOIRRILLOC) 0 0 0 092706112 1 82 0 1448 6 0 09547717 1 66 0 1791 FEA FER 2 2 3 77162004 20 93 0 0001 2 2 90079235 16.54 0 0001 RESIST OF HYPDIHESES USING DR. TYPE III MS FOR REPOIRRILLOC) AS AN ERROR TERM SOUNCE DF TYPE III SS F VALUE PR > F LOC 2 15 22261719 36 55 0 0004 TESTS OF HYPDIHESES USING DR. TYPE III MS FOR REPOIRRILLOC) AS AN ERROR TERM SOUNCE DF TYPE III SS F VALUE PR > F LOC 2 15 22261719 36 55 0 0004	DEPENDENT VARIABLE	TH							
ERROR 22 1 98212889 0 99099677 ROOT MSE TH MEAN CORRECTED FOIAL 51 28 47552308 0 30016124 4 02230769 SOURCE DF TYPE ESS F VALUE PR > F DF TYPE 111 SS F VALUE PR > F DF TYPE 111 SS F VALUE PR > F DF TYPE 111 SS F VALUE PR > F DF TYPE 111 SS F VALUE PR > F DF TYPE 111 SS F VALUE PR > F DF TYPE 111 SS F VALUE PR > F DF TYPE 111 SS F VALUE PR > F DF TYPE 111 SS F VALUE PR > F DF TYPE 111 SS F VALUE PR > F DF TYPE 111 SS F VALUE PR > F DF TYPE 111 SS F VALUE PR > F DF TYPE 111 SS F VALUE PR > F DF TYPE 111 MS FOR REPOIRRILACY AS AN ERROR TERM SOUNCE DF TYPE 111 MS FOR REPOIRRILACY AS AN	SOURCE	OF	SUM OF SQUARES	MEAN S	GUARE	F VALUE	PR > F	R-SQUARE	c v
CORRECTED FDIAL 31 20 47352308 0 30016124 4 02230769 SOURCE DF TYPE I SS P VALUE PR > F DF TYPE II1 SS P VALUE PR > F LDC 2 15 60727933 87 00 0 0001 2 15 22201719 84 48 0 0001 REP(LOC) 0 1 42340708 2 63 0 0448 6 1 124934775 2 31 0 0699 IRR 1 0 37758519 4 10 0 0528 1 0 3492220 6 05 0 0221 LOC-IRR 2 1 0 37758519 4 10 0 0528 1 0 3492220 6 05 0 0221 REPPIRALLOC) 6 0 09276012 1 82 0 1418 6 0 09547717 1 56 0 0701 REPPIRALLOC) 6 0 09276012 1 82 0 1418 6 0 09547717 1 56 0 1701 FER REPUBLICOCH 0 0 05276012 1 82 0 1418 6 0 09547717 1 56 0 1701 FER REPUBLICOCH 0 0 05276012 1 82 0 1418 6 0 09547717 1 56 0 1701 LOC-PER 4 0 0 57809050 1 86 0 2007 4 0 04015731 1 69 0 1375 LOC-IRROFER 3 2 0 19644356 1 09 0 3555 2 0 20135164 1 12 0 3475 LOC-IRROFER 3 2 0 19644356 1 09 0 3555 2 0 20135164 1 12 0 3475 LOC-IRROFER 4 0 49336495 1 37 0 2700 4 0 49336495 1 37 0 2769 TESTS OF HYPDIHESES USING PIE TYPE III MS FOR REPO[RAILDC) AS AN ERROR TERM SOUNCE DF TYPE III SS F VALUE PR > F LOC 2 15 22261719 36 55 0 0004	MODEL	29	26 49339419	0 913	56532	10 14	0 0001	0 930392	7 4624
SOURCE DF TYPE E 55 F VALUE PR > F DF TYPE E 11 S5 F VALUE PR	ERROR	22	1 98212889	0 090	09677		ROOT MSE		TN MEAN
LÜC 2 15 68727933 67 06 0 0001 2 15 22261719 64 40 0 0001 REPILOC) 6 1 42340708 2 63 0 0445 6 1 24943475 2 31 0 0699 1	CORRECTED FOTAL	51	20 47552300				0 30016124		4 02230769
REPILOC) 1 42340708 2 3 0 0448 1 0 37758519 4 19 0 0528 1 0 34522255 2 31 0 0629 1 0 04076255 1 0 04076255 1 0 04076255 1 0 04076255 1 0 04076255 1 0 04076255 1 0 04076255 1 0 04076255 1 0 04076255 1 0 04076255 1 0 04076255 1 0 04076255 1 0 0 0629 1 0 0 04076255 1 0 0 0629 1 0 0 04076255 1 0 0 0629 1 0 0 04076255 1 0 0 0629 1 0 0 04076255 1 0 0 0629 1 0 0 04076255 1 0 0 0629 1 0 0 04076255 1 0 0 0629 1 0 0 04077655 1 0 0 0629 1 0 0 04076255 1 0 0 0629 1 0 0 0607 1 0 0 0 0607 1 0 0 0 0607 1 0 0 0 0607 1 0 0 0 0607	SOURCE	DF	TYPE t 55	F VALUE	PR > F	DF	14PE 111 55	/ VALUE	PR > F
IRA 1			15 60727933		0 0001				
COC-1RR 2 2 9883335 16.55 0.0001 2 2 98079235 16.54 0.0001 2 2 98079235 16.54 0.0001 2 2 98079235 16.54 0.0001 2 2 98079235 16.54 0.0001 2 2 98079235 16.54 0.0001 2 2 98079235 16.54 0.0001 2 2 98079235 16.54 0.0001 2 2 98079235 16.54 0.0001 2 2 98079235 16.54 0.0001 2 2 98079235 16.54 0.0001 2 2 98079235 16.54 0.0001 2 2 98079235 16.54 0.0001 2 2 98079235 16.54 0.0001 2 2 98079235 16.54 0.0001 2 2 98079235 16.54 0.0001 2 2 98079235 16.54 0.0001 2 2 98079235 16.54 0.0001 2 2 98079235 16.54 0.0001 2 2 98079235 16.54 0.0001 2 98079235 16.55 0.0001 2 98079235 16.55 0.0001 2 98079235 16.55 0.0001 2 98079235 16.55 0.0001 2 98079235 16.55 0.0001 2 98079235 16.55 0.0001 2 98079235 16.56 0.0001 2 98079235 16.56 0.0001 2 98079235 16.56 0.0001 2 98079235 16.56 0.0001 2 98079235 16.56 0.0001 2 98079235 16.56 0.0001 2 98079235 16.56 0.0001 2 98079235 16.56 0.0001 2 98079235 16.56 0.0001 2 980	IRA					ì		2 JI 6 05	0 0099
FER 2 3 77162004 20 3 0 0001 2 3 36070001 18 70 0 0001 18 70 0 0001 18 70 0 0001 18 70 0 0001 18 70 0 0001 18 70 0 0001 18 70 0 0001 18 70 0 0001 18 70 0 0001 18 70 0 0001 18 70 0 0001 18 70 0 0001 18 70 0 0 0001 18 70 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0		2		16 55	0 0001	2	2 98079235	16.54	1000
LOCOFER) 62 20 93		9		1 66	
1989 FER 2 0 19644556 1 09 0 3536 2 0 20136184 1 12 0 3450 LOCOTRROFER A 0 49336495 1 37 0 2769 TESTS OF HYPOTHESES USING THE TYPE III MS FOR REPILOC) AS AN ERROR TERM SOUNCE DF TYPE III SS F VALUE PR > F LOC 2 15 22261719 36 55 0 0004 TESTS OF HYPOTHESES USING THE TYPE III MS FOR REPOTRAILDC) AS AN ERROR TERM SOURCE DF TYPE III SS F VALUE PR > F 188 1 0 5452250 3 65 0 1045	LOC+FER	Ā	0 57809056			•	0 61015731	1 69	0 1875
SOUNCE DF TYPE III SS F VALUE PR > F LOC 2 15 22261719 36 55 0 0004 TESTS OF HYPOTHESES USING THE TYPE III MS FOR REPOTRALLOC) AS AN ERROR TERM BOURCE DF TYPE III SS F VALUE PR > F IRR I 0 54522250 3 65 0 1045									
LOC 2 15 22261719 36 55 0 0004 TESTS OF HYPOTHESES USING THE TYPE 181 MS FOR REPOTRALLOC) AS AN ERROR TERM BOURCE OF TYPE 111 SS F VALUE PR > F IRR 1 0 54522250 3 65 0 1045	TESTS OF HYPOTHESES	USING THE TY	PE III MS FOR REPILO	C) AS AN ERRO	M TERM				
TESTS OF HYPUTHESES USING THE TYPE III MS FOR REPOTRATILOC) AS AN ERROR TERM SOURCE OF TYPE III SS F VALUE PR > F IRR I 0 54522250 3 65 0 1045	SOURCE	DF	TYPE III SS	F VALUE	PA > F				
SOURCE DF TYPE III 53 F VALUE PR > F 1RR I 0 54522250 3 65 0 1045	FOC	2	15 22261719	36 55	9 9994				
IRR I 0 54522250 3 65 0 1045									
	TESTS OF HYPOTHESES	USING THE TY	PE III MS FOR REPOLA	RRILDC) AS AN	ERROR TERM				

DEPENDENT VARIABLE (6. 20							
SOURCE	ģ	SUM OF SQUARES	MEAN SOUARE	LAE	P VALUE	¥ ^ ¥	A-SQUARE	>
MODEL	53	23627 73762294	821 646124	2493	2 69	9600 0	0 779954	0 4040
ERMOR	N 14	6722 62040574	305 573654	191		ROOT MSE		CC_A MEAN
CORRECTED TOTAL	5	30550 35802868				17 40006517	916	69121154
SOURCE	OF	IYPE 1 SS	F VALUE	7 .	ŏ	TYPE 111 55	F VALUE	P
LOC REP(LOC) 188	0.0=N	9576 85374351 838 15596393 1733 29643932 222 01662514	10000000000000000000000000000000000000	000000000000000000000000000000000000000	~~~	10074 49258838 174 86893659 1858 85738195 1858 85738195	6 4 5 4 5 4 6 6 6 6 4 6 6 6 6	20000
REPORTION FER LOC FER BRROFER LOC OF BROFER	0N#N#	3642 07152111 1409 22027253 3527 39921805 994 60591746 1662 03381889	3.233 -aa	2000 2000 2000 2000 2000	P# # 10 C	2000000		M & -M
TESTS OF HYPOTHESES USING	#	TYPE III MS FOR REPILC	DC) AS AN ERROR	TERM				
SOUNCE	90	TYPE 111 SS	F VALUE	£				
70 1	N	10073 49256838	39 00	0 000				
1651S (P H DIMESES	DIMESES USING 14	TYPE ILL MS FOR REPORT	RR(LDC) AS AN ER	MOR TERM				
SOURCE	UF	TYPE 111 55	F VALUE	r. ?				
18H 18H	-	1858 55735195 748 4540P 141	2 93 0 0	11 1 0				
DEPENDENT VARIABLE	5							
SOURCE	6	SUM OF SOUARES	HEAN SOUARE	ARE	FVALUE	£	R-SQUARE	> U
MODEL	5.0	19933 0006661	673 55402299	299	011	6000	0 655661	10 1970
ERROR	22	3294 43333333	149 74696970	970		ROOT MSE		CA NEAN
CORRECTED TOTAL	ā	22827 50000000				12 23711444	120	000000000
SOURCE	ă	TYPE 1 88	F VALUE	£	ò	TYPE III SS	P VALUE	* *
LOC REP(LOC)	N -9 -	9109 21777778 1416 43055556			N-G-	1707 1777778	3	0 - 6
LOC+1RA REP-1RR(LUC)	- O- O				- er e	2586 30849673	-	
LOCOTER LOCOTER LOCOTER LOCOTRA	W # N #	15561 65659507 1556 69604326 1562 59456657 1036 27500000	7.00 7.00 7.00 7.00 7.00 7.00 7.00 7.00	0000 0000 0000 0000 0000	1 444	1624 48657967 8640 61059026 656 66905269 1056 2750000	77.87 	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
1HE 5ES	US ING THE	_	OC) AS AN ERROR	TERM				
SOURCE	ě	17PE 111 SS	F VALUE	8				
707	N	10222 69738562	17 96	0 0029				
TESTS OF HYPUT RESES USING THE TYPE	US ING THE	ALL MS FOR REPOR	RRILDCI AS AN ER	AROH TERM				
SOURCE	N G	TYPE 111 55	F VALUE	£				
IRR LOC•IRH	-~	8 63350694 2566 30849673	9 S	0 0604				

DEPENDENT VARIABLE MG									
SCANCE	90	SUM OF	SQUARES	MEAN SOL	SOUARE	F VALUE	F & E	R-SOUARE	>
MODEL	29	9405 24	******	324 31868774	1774	5 63	1000 0	0 666633	1961 9
ERROR	22	1202 51	7555556	54 66252	2525		ROOT MSE		HG NEAN
CORRECTED TOTAL	15	10607 61	1750000				1 39341093	0	9 72500000
SOURC E	90	1 A	PE I SS F	VALUE	ž	ă	TYPE 111 55	F VALUE	F .
1700	N 4	3279 6(0361111			æ 4	3249 06611111	86	
LAR) - -	915	27.0045	10) —		10	
LOC+IRR Repojaniloc)	N 40	2020	1104256			N O	200 000	0	
FER LOCOFER	N 4	4 C	3276190			e e	386 14247824	#) =	
IRROFER LUCOIRE FER	N 4	20 40	10309026	P 8	4664	N #	39 94057348	000	0 6661
TESTS OF HYPOTHESES USING THE TYPE	NG THE TYPE	111	FOR REP(LOC) A	ş					
SOUNCE	* 0	IYPE	111 SS F	. VALUE	PR V R9				
707	٧	3244 0	08611111	7 60	0 0209				
TESTS OF HEW THESES USING THE TWEE BELLING	ING THE TYPE		FOR MEP TRAILE	LOC) AS AN EI	RHOR YERM				
	3	IYPE	111 55 F	. VALUE	¥.				
188	٠.	90		70	0 0000				
DEPENDENT VARIABLE K	4								
BOARCE	ı.	SLOH OF 5	SQUARES	MEAN SOL	SOURRE	F VALUE	, æ	R-SUUARE	>
HODEL	20	19100 60	50673077	658 641611	1141	11 98	1000 0	0 940457	5 9394
FIRE	22	1209 32	32000000	24 9690909	1604		ROOT MSE		K MEAN
CORRECTED TOTAL	5	20309 93	92673077				7 41411430	124	62 00 46 15
Sounce	ğ	17	FPE # 85 F	VALUE	£	2	TYPE 111 55	P VALUE	P 4
10C 301	Nd		1007521			a <	0245424		0 1
) (90000) —(114 1076736) (U	-
REPORTED OF	4 0 1		0665612			NO	1919110		94
LOCOFER	u		**************************************	0 2 2		N - (1	1068 91277778 1068 91277778	7 4 9	
LOC+IRR+FEA	•		1100694			:•	0810069		7
TESTS OF HYPOTHESES USING THE TYPE	ME THE TYPE	======================================	FOR REPILOC) A	AS AN ERROR	TERM				
Sounce	•	1 YPE	111 SS F	. VALUE	£ .				
701	a	15249 03	2454248	102 94	0 0001				
TESTS OF MYLOTHESES US ING THE TYPE	NG THE TYPE	111 #5	FOR REPOTARILE	LLAC) AS AN EI	RRON TERM				
	96	TYPE	F 88 111	. VALUE	P .				
188 100 • 1 HR	-0	10 44 1	18767361	*# ***	0 2605				

DEPENDENT VANTABLE	1							
SOURCE	96	SUM OF SQUARES	MEAN SQUARE	8	# T	8		
HODEL	60	429 65070065	4 A224 V79A				3400c-	>
ERROR	6				<u>e</u>	1000	0 903627	9 1296
	;	777700	161125 0	20		ROOT MSE		NA MEAN
COMPET NEW TOTAL	Ť	436 91692308				0 56673796		6 20 7692 31
SOURCE	4	TYPE I SS	F VALUE	^ %	90	TYPE III SS	FVALUE	4
100 98 Pås OCS	N 1	149 74477030	7		~		ž	•
	-		7 0		•	~	3	5
LOCOLNA REPONDED OF S	~ -	91195510 601	0		→ ~		223	ò
201	۰ م	1 34206404	•		•	•		5 2
1000FFR	1 47 1		25		~ •		N	2
LOCOTAR FER	• •	0 35969689	200	0 6223	~	9163636	-0	0 5576
TESTS OF HYPOTHESES	HYPOTHESES USING THE TYPE	III MS FOR REBIL	FREEDR		•		•	8
SOURCE	ų.							
2	; '	L	L AALUE	P. C.				
<u> </u>	~	151 06677778	205 49	1000 0				
IESTS OF MYPOTHESES	A THE SUM SAME SAME OF A STATE OF	1	,					
		S TOR KEP	INRILOCI AS AN ERROR	DR TERM				
and the	D.	1 VPE 111 55	F VALUE	PR V F				
184	-~	71 69006250	467 52	10000				
DEPENDENT VANTABLE	۵							
\$DONEC E	DF	SUM OF SOUARES	MEAN SOLAHE	뿔	FVALUE	£ ^ £	R-SUUARE	> U
MDDEL	5.0	68 79967521	2 37240259	30	7 39	1000 0	0 906939	16 2959
EMROR	22	7 05955556	0 3208669	60		R001 MSE		P MEAN
COMPECTED TOTAL	4	75 65923077				0 5664 7055		3 09615365
SOURCE	å	TYPE 1 5S	F VALUE	PR V F	à	86 111 39YT	FVALUE	4
501	•	***************************************			•		•	
REPLICOL)	۰.	5 07315276	6.0		• •		~	
	- 0	14 74140410	19		4		9 6	
REPOSENCE OC.	1-04	5927776	2		1 6		•	
LOCOFER	•	101490000	25	0 000	≈	10 4005050	200	1000
TANGER TO STREET	~ <	2 87102273 7 0000000	24		N		•	9000
TESTS OF HYPOTHESES USING	USING THE TYPE	III MS FOR REPAR	N ERROR 1	, ü	•		,	
BOWRCE	50	TYPE 111 55	FVALUE	P				
רסע	~	5 62799346	9K 10	0 1048				
			:					
5	MYPOT ESES USING THE TYPE 331 M	201	v	-				
BOARCE	ă	ā	FVALUE	Œ				
168 Locotar	- ~	16 66067320	53 95 36 20	0000				

DEPENDENT VARIABLE	5						
SOURCE	D#	SUM OF SQUARES	MEAN SQUARE	F VALUE	An		
MODEL	29	83 19647863	2 8688 44 09	4 09	PR > F	R SQUARE	C A
EHROR	22	15 4204444	0 70092929		0 0006	0 643633	9 0926
COMMECTED TOTAL	51	98 61692308	0 10072729		ROOT MSE		S MEAN
		10 01072300			0 83741550		9 20769231
SOURCE	DF	TYPE E SS	F VALUE PR >	F OF	TYPE III SS	F VALUE	PR > F
LUC REP(LOC)	\$	27 65143697	19 72 0 00	01 2	28 44043791		
IRR LOC+IAR	Ī	6 01348611 0 10793798	2 10 0 09 0 15 0 69	51 6	9 13591919	20 29 2 17	0 0001 0 0852
REP IRR(LOC)	\$	6 27609620	4 46 0 02	šá ž	0 18000694 6 36279085	0 26 4 54	0 6174
FER	ž	3 13796581 11 49900433	0 75 0 61 8 20 0 00	6 6	2 67248485	0 64	0 0224 0 7007
LDC FER IRR#FER	•	14 97968254	5 20		10 57339060	7 54	0 0032
LOCOTRHOFER	2	5 38030619	5 90 0 00	2	15 19139583 6 20251510	5 42 5 85	0 0034
	-	2 35056250	0 84 0 51	56 4	2 35056250	0 84	0 0092 0 5156
	USING THE IN	PE III MS FOR REPELO	C) AS AN EHHOR TERM				
SOUNCE	D F	TYPE III SS	F VALUE PR >	•			
LOC	2	28 44043791	9 34 0 01	14			
TESTS OF HYPOT & ES	USING THE TH	PE 111 MS FOR REPOIR	ALLUCT AS AN ERHOR T	: RM			
SOURCE	DF	TYPE 111 55	F VALUE PR >				
IRR LOC IRR	<u>.</u>	0 38000694	0 40 0 54	54			
	2	6 36279085	7 14 0 02	59			
DEPENDENT VARIABLE	CHO3						
SOURC E	OF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C V
MODEL	29	10085 69494687	347 78258437	7 93	0 0001	0 912687	31 1067
ERRUA	22	964 85602796	43 85709218		ROOT MSE		CHO'S MEAN
CORRECTED TOTAL	51	11050 55097403			6 62246074		21 20955769
SOURCE	0F	TYPE I 65	F VALUE PR >	F DF	TYPE ILI SS	F VALUE	PR > P
LOC REPILOC)	2	4253 13023626	46 49 0 00		4195 01888053	47 03	0 0001
IRA	6	434 94311532 594 60845560	1 65 0 18 13 56 0 00	00 6 13 1	364 12768103 677 11915126	1 46	0 2377 0 0007
LOC+ I AR	ž	529 18158622	6 03 0 00		542 17062720	15 44 6 18	0 0007 0 0074
REP+IRR(LOC) FER	6 2	293 08194678	1 11 0 10		305 98509740	1 16	0 3011
LÖCOFER	į.	2367 03758561 871 9486362 9	27 21 0 00 4 97 0 00		1955 [1465509 923 23447702	22 29 5 26	0 0001
IRR+FER LOC+1RR+FER	2	185 71796149	2 12 0 14	i Ž	219 87918835	2 51	Ŭ 1045
_	•	536 04542132	3 06 0 03	32 4	536 04542132	3 06	0 0342
	·	PE III MS FOR REP(LO		_			
SOURCE	DF	TYPE [11 55	F VALUE PR >	•			
FOC	2	4195 01888053	32 76 0 00	06			
TESTS OF HYPOTHESES	USING THE IY	PE III MS FOR REPOIR	H(LDC) AS AN ERROR T	E RM			
SOURCE	OF	TYPE 111 SS	F VALUE PR >	•			
IRA							

DEPENDENT VARIABLE	CL						
SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	c v
MODEL	29	40 72136752	1 40418509	9 37	0 0001	0 925130	5 3013
ERROR	22	3 2955556	0 14979798		ROOT MSE		CL HEAN
CORMECTED TOTAL	51	44 01692308			0 38703744		7 19230769
SOURCE	DF	TYPE SS		> F 0F	TYPE III SS	F VALUE	PR > F
LOC Rep(LDL)	2 6	5 21692308 2 5870000		0001 2 0317 6	5 19588235 2 41797980	17 34 2 69	0 0001
IRA LOC+ I RR	1 2	2 16931006 5 81883522		0010 1 0001 2	1 95069444 5 84477124	13 02 19 51	0 0016
REPOIRR(LOC) FER	6	1 17318803 15 48659307	1 31 0.	2960 6 0001 2	0 78969697	0 88 49 49	0 5265 9 0001
LOCOFER IRROFER	4 2	5 70739683 1 17183475	9 53 0	0001 4	5 77674479 1 08763953	9 64	0 0001 0 0434
LOCOLAN FER	•	39028646		0352 2 0888 4	1 39028646	5 25 3 93	0 0888
TESTS OF HYPOTHESES	USING THE TY	PE III MS FOR REP(LO	C) AS AN ERROR TER	M			
SUURCE	DF	14PE []] SS	F VALUE PR	> F			
LOC	2	5 19588235	6 45 0	0320			
TESTS OF H FOTHESES	US ING 1 Y	FE 111 M5 FON REP 18	RELUCE AS AN ERROR	TERM			
SOURCE	DF	TYPE 111 \$5	F VALUE PR	> F			
IRR LUC IRH	1 2	1 95069444 5 84477124		00 #\$ 00 1 7			
DEPENDENT VARIABLE	OXPRO						
\$DURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR → F	R-SQUARE	C V
MODEL	29	1370 32547083	47 2526 0244	1 68	0 1060	0 689230	98 3141
EHROR	22	617 87267008	28 08512137		ROOT MSE		UXPRO MEAN
CORRECTED TOTAL	51	1986 19814091			5 24953973		5 39041644
SOURCE	OF	TYPE SS	F VALUE PR	> F DF	TYPE 111 55	F VALUE	PH > F
LOC REP(LOC)	2 6	495 04570986 192 24677916		0015 2 3722 6	463 44082323 136 04367554	8 25 0 81	0 0021 0 5752
IRR	1	2 69880554 90 25966452	ō 10	7595 i 2232 2	11 35267560 103 57776817	0 40 1 64	0 5315 0 1818
LOC+1RR REP+1RR(LUC)	ş 6	241 54006843	j 43 - Ö	2468 6	186 25425348	1 11	0 3407
FER LOC+FER	2	20 87636827 95 52468136	0 85 0	6938 2 5067 4	8 72135303 125 80635907	0 16 1 12	0 8571 0 3725
IRR FER Loctire fer	2	89 57848280 142 55491069		2255 2 3121 4	112 41489910 142 55491089	2 00 1 27	0 1590 0 3121
	USING THE TY	PE III MS FOR REP(LO	C) AS AN EHROR TER	н			
SOURCE	OF	14PE 1 55	F VALUE PR	> F			
LDC	2	463 44082323	10 22 0	0117			
TESIS OF 1 5 11 1E 5E5	USING THE IY	PE III MS FOR REPOIR	RELOC) AS AN ERROR	TERM			
SOURCE	DF	TYPE 111 SS	F VALUE PR	> F			
IRR LUÇ⊕IRH	1 2	11 35267560 103 57776817		5675 2654			

DEPENDENT VARIABLE	OXZPRU							
SOURCE	96	SUM OF SQUARES	MEAN SC	SQUARE	F VALUE	£ ^ £	M SOUARE	> 0
MODEL	5.8	160 92517959	6 238	3879930	•	0 0003	0 605060	52 6942
ERROR	2 3	28 21612910	1 3436	52520		ROOT MSE		DK2PRO MEAN
CORRECTED TOTAL	00	209 14130669				15014848		2 19984946
SDURCE	OF	TYPE I SS	F VALUE	9 0	9	TYPE 111 SS	F VALUE	7
100	N 4	57 35090060			Ne	60	0	00
200) —	•) 		N	0
LOCOLAR REPolar(LDC)	N 40				∾	70	4 (1	9 9 9
	~ ∢	7 44869945			~	69 6	•	900
INDOFEN LOCAL DOLLER	· N •		**************************************	100	• W •	0 04720909	100	200
TESTS OF HYPOTHESES USING	, ¥	S FOR REPIL	z	TER	,		,	,
SOUNCE	90	1YPE 111 55	F VALUE	PH > F				
100	~	47 29569053	9 6	7001 0				
TESTS (P. MYFOTHESES	HYF OTHESES USING THE T	TYPE III MS FOR REPOLA	RRILOC) AS AN E	E PATOR TERM				
	Þ	17PE 111 55	F VALUE	P8 V F				
1RA LOC+1RA	- N	0 33370103	3 %	0 8460				
DEPENDENT VAHLABLE	Онжхо							
SOURCE	9	SUM OF SQUARES	MEAN S	SOUARE	F VALUE	¥ ^ Æ	R-SOUARE	> J
MODEL	5.0	909 69247103	31 360	36870590	10 52	1000 0	0 935590	48 5498
ERROR	12	62 62660261	2 9622	21917		R001 MSE		DX3PRO MEAN
CORRECTED TOT L	90	972 31907364				1 72691030		3 55696701
SOURCE	DE	TYPE 1 SS	F VALUE	P & Aq	ŏ	TYPE III SS	F VALUE	F < Eq.
707	OI I	-			CH :	231 624 93311	Of P	94
JAR 1	o -		0 4		o -	11 654 79605	· ·	90
LOCO BRA	~ •				N •	211 79528326	7 -	00
	N	-	N 4		N	78 32234465	24	99
IAR FER	~ ~	3 52202707	900	0 5630	N	1 67691309	00	0 1517
TESTS OF HYPOTHESES USING	3	FOR REPIL	7	I				
SOURCE	Đ	TYPE 111 55	P VALUE	PR > m				
707	∾	231 82493311	3 07	0 1205				
	•			!				
	MINOR ESES USING INC. IVE.	III MO FUN NEPEL	< ; C :	_				
	Š		r value	ř				
IRR LDC+IRR	- 0.	11 65479605	25.	0 5864 0 3846				

DEPENDENT VARIABLE OX	2_3PR0						
SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	c v
MODEL	29	1871 19986692	64 52413334	0 16	0 0001	0 918476	48 8574
ERAGR	51	166 08891053	7 90899574		ROOT MSE	Ox	HASH OFRE
CORRECTED TOTAL	50	2037 28877745			2 81229368		5 75613059
SOURCE	DF	TYPE I SS	F VALUE PR > F	OF	TYPE 11 SS	F VALUE	PH > F
LOC REP(LUC) IRA LOC+IRA REP+IRK(LUC) FER LOC+FER IRR+FER LOC+IRA FER	2 6 1 2 6 2 4	574 72583796 317 52566634 17 84671518 222 13973772 456 71654666 123 79445234 146 90205599 4 82291971 26 72513500	36 33 0 0001 6 69 0 0005 2 26 0 1479 14 04 0 0001 9 62 0 0001 7 53 0 0029 4 01 0 0143 0 30 0 7404	1 2 6 2 4	485 73760941 457 79418759 15 93270179 141 85786538 405 10602651 140 16759132 131 78225917 2 33236113 26 72513500	30 71 9 65 2 01 8 97 9 86 4 17 0 15	0 0001 0 0001 0 1705 0 0015 0 0016 0 0122 0 6632
TESTS OF HYPOTHESES US	ING TIE T	TYPE III MS FOR REPILU	C) AS AN ERHOR TERM				
SOURCE	DF	TYPE 111 55	F VALUE PR > F				
LOC	2	465 73760991	3 16 0 1142				
TESTS OF YOUTHESES US	ING THE T	TYPE III MS FOR REPOLR	RELOCE AS AN ERROR TER	H			
SOUPCE	DF	TYPE 111 SS	F VALUE PR > F				
IHR LOC IHH	1 2	15 93270179 141 85786538	0 21 0 6662 0 92 0 4500				
DEFENDENT V AL ELE TA	iLP1						
SUURCE	DF	SUM OF SQUARES	MEAN SQLAPE	F VALUE	PR > F	F SCUAFE	C V
MUDEL	3.0	315 40643097	10 6760636	2 13	0 0356	0 737467	87 4313
ENROH	2	115 1106550	5 1035 010		FCCT MSE		THUPT MEAN
CURRECTED TOTAL	51	427 67749 23			2 2 503300		2 5637611
SOURCE	OF-	TYPE I SS	F VALUE PR > (F OF	TYPE LIL SS	F VALUE	PH > F
LLL REFILIC) IMI LGCOIMH REP IMMILU I FER LOC FEN IMMOFER ILC IN FE	2 1 2 0 4 2	124 03925765 35 02411 46 0 01779 25 11 87712 57 60 04253057 6 0 2666 0 19 834624 3 21 46275367 37 0 516 77	1 15 C CCC 1 14 0 70 0 00 0 9.3 1 16 0 G 1 96 0 11 C 9 0 61 0 97 0 442 2 10 0 146 1 e2 0 161	7 6 6 2 6 2 9 9	118 22979106 41 43848854 9 01349362 1 063 1585 55 6657769 26 60615528 25 26720711 37 04516577	11 6 1 0 0 00 1 18 1 95 0 5 1 0	0 0004 0 2764 0 559 0 32 4 0 1174 0 8 6 0 2995 0 1072 0 1616
IL IS OF THE LESES O	[66]	TYPE LLE M. FOR REPILE	C) A N LRHOR TERM				
S CHCE	O	LANE TIT 2	F V LIE PR >	F			
FOC	2	11 22979106	e 56 0 017	5			
TE IS O T &	161 1		GILLECT 5 AN ERHUF TE				
SULH 1	L	1 (111 72	+ VALUE FI >				
FOC 11	ı	U 01347 62 12 06351585	0 61 0 57				

DEPENDENT VARIABLE	CAUPT							
SOURCE	ò	SUM OF SQUARES	NCAN S	SQUARE	F VALUE	. ^ £	A-SOUARE	>
MUDEL	45	185 98212530	6 413	41317673	1 73	0 95	0 604834	100 7404
EHRUR	22	81 68204721	3 7126	02033		_		
CORRECTED TOTAL	15	267 66417251				9		•
SOURCE	O.	TYPE I SS	F VALUE	PR V	ņ	TYPE 111 SS	F VALUE	ع م
701	٠,	ă			Q	F 45 8500	•	
IRR	۰-	ĕ			i o	9296877	• •	2 %
LOCe 188	α,	ž			~ ~	0224080	•	7
FER	۰ ۸				•	1571754	• 4	2
LOCOFER	•	ř			V 4	1351588	٥.	~
LOCOTRR FER	N 4	1 49651270	- 0 - 0 - 0 - 0	0 3807	N 4	9 2230297 9 2230297 9 304364	- 75	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
TESTS OF HYPOTI ESES USING THE	USING THE TYPE	III MS FOR REPOR	OC) AS AN ERROR	TEX	•		•	ñ
SOURCE	96	y)	F VALUE	PR >				
701	N	69 90563436	00 9	0 0370				
TESTS OF MYPOTHESES	HYPOINESES USING THE TYPE 111 MS	FOR	EPOTRACLOC) AS AN	EAROR TERM				
SOURCE	P	TYPE 111 SS	P VALUE	, ag				
LOC 188	-~	3 02940802	0 52	0 4992				
DEPENDENT VAHIABLE	HGUPT	: !						
SOURCE	Ď	SUM OF SOUARES	MEAN S	SOUARE	F VALUE	F ~ ₹	R SQUARE	> U
MODEL	5.0	31 04856861	1 0706	06 40 30	2 04	0 0441	0 729125	96 9196
FRRUR	2.5	11 53473093	0 524	430595		ROUT MSE		MGUPT MEAN
CORRECTED TOTAL	6	42 58329954				0 72408974		0 81523640
SOURCE	DF	1YPE 1 55	F VALUE	P	ž	TYPE III SS	F VALUE	P •
	,	;			•			9
FOC PRF(LOC)	~ • • •	11 10/2/20 4 21511614 0 03298416 1 65874690	7 7 8 5 	0 0 0	4 4 – N	5 0366424 0 0366424 1 68110974	2000	0 1938
REPONENCE	1-0 1-1	553			4 04	9 9	-0	→ 47 (
LOC FER IRROFER LOC IMA FEN	₹ ₩ ₹	308			•~•	90	747	A UA
TESTS OF H POT 1 SES USING THE	USING THE TYPE	III MS FUN HEPIL	OC) AS AN ERHC	HOR TERM				
STAMCE	DF	1YPE 111 55	F VALUE	PA V F				
רסכ	•	11 342 33 7	6 75	1670 0				
11 575 1 P 1 E F	SIKILLIY	IY E III MS FOR NEP I	HRILIKI AS AN	EI HUR TERM				
SOURCE	ò	1YPE 111 S\$	F VALUE	u ^ 71				
; g	~∧	1 66110974	900	0 7434				

DEPENDENT VARIABLE	TODA							
STURKE	10	SUM OF SQUARES	A MARAN	Souther	42		;	
MODEL	5.5	353 05775377		05.30	,	. ;	-S00A	J
ENROR	20	A W VA C WAR A C C T		,	•	9150	0 741974	70 1110
COMMECTED TOTAL	; ;		8080	99.78		RODT MSE		KUP I NEAN
10101	ń	475 63563024				2 36237627		2 98612344
SOURCE	ò	TYPE 1 55	F VALUE		ŏ	TYPE 111 SS	7 VAL 17	9
	N.	115 94632105			a			
222	۰-	00000000000000000000000000000000000000	-	0 2200	10.	1589		3-
REPOTENT	~ <				- ~	0 23646 2 62330		0
) (*)				4 0 N	5 45660		: -
TARA-FER	• ~	37 03789846 27 71340046			•	0 11437		2-2
LUC • 1 RR• FER	•	••		6191 0	N 4	32 09720129	20 4	000
TESTS OF MYPOTIESES USING THE		TYPE III NS FOR REP (L	OC) AS AN ERROR	R TERM				•
SCHUCE	0	TYPE 111 55	F VALUE	ar v				
Luc	N	109 26545098	6	0 0448				
TESTS OF HYPOTHESES USING THE		TYPE ILL MS FOR REPOSI	RHILDC) AS AN E	ERRUA TERM				
SOURCE	ò	TYPE 111 5S	VALUE	ž				
188 LOC 188	-4	0 23646382	5 C	0 6762				
DEPENDENT VANTABLE	NA UP T			1				
SOURCE	å	SUM OF SQUARES	MEAN SC	AN SOUARE	FVALUE	¥ .	R SOUARE	>
MODEL	56	0 19097197	900 0	00658524	2 15	0 0342	0 736975	70 9967
Енелн	22	0 06745628	0 003	00306619		RUUT MSE		NAUPT MEAN
COMPECTED TUTAL	40	0 25842825				0 05537323		1004
SOUPCE	0.	TYPE 1 5S	FVALUE	¥ .	JO.	TYPE 111 SS	F VALUE	£
50 1	•	-			c			
REPLUCI		0 02462949	3:		٠ 4 -	0200225		
LOC• 1 4A	• ~				- (4	0159961		
AEP+1481100)	٥.	_			•	0350477		
LOCAFER	4 •				•	0262037		
LOCOLAROFER	N e			0 0871	N	0 01839146	00 - 00 -	0 0705
TESTS CF YPOT ESES	YPUT ESES UST G THE F		UC) AS AN EHRUR	4 E.H				
SUMPICE	å	1YPE 111 55	F VALUE	PR V R				
70 1	٧	0 03725716	3 85	0 0840				
TESTS OF OILESS	S USING HE	I PE III MS FON NEP II	RRILUCT AS AN	ENNUR TERM				
SIMRCE	90	1YPE 111 55	FVLUE	PH V F				
981 981	- 0	0 00203752	21 0 -	0 5763				
	•	:						

DEPENDENT VARIABLE PL	JP T							
SOURCE	DF	SUM OF SQUARES	MEAN SQ	UARE	F VALUE	PI > F	R-SQUARE	C V
MODEL	29	1 29375298	0 0446	1217	3 43	0 0020	0 818795	65 8827
ERROR	22	0 28631707	0 0130	1441		ROOT MSE		PUPT HEAN
CORRECTED TOTAL	51	1 58007004				0 11408073		0 17315732
SERVICE	DF	TYPE 1 55	# VALUE	PR > F	DF	TYPE III SS	F VALUE	PR > F
LOC REP(LOC)	2 6	0 20721778 0 07107396	11 03	0 0005 0 5058	2	0 25816426 0 12479149	9 92 1 60	0 0009 0 1946
189	1	0 00000329	0 00	0 9875	1	0 00002691	0 00	0 9641
LOC+1RR REP+1RR(LUC)	2 6	0 04021994 0 13795367	1 55 1 77	0 2355 0 1528	2 6	0 04000701 0 18405811	1 54 2 36	0 2372 0 0655
FER LOCOFER	2	0 25352938 0 15025313	9 74 2 89	0 0009 0 0462	2	0 24634361 0 17999021	9 46 3 46	0 0011
IAR+FER	Ž	0 10722778	4 12	0 0302	2	0 13108931	5 07 4 73	0 0155
LOC+ I RR+FEH	•	0 24627365	4 73	0 0066	•	0 24627385	- 73	0 0066
TESTS OF HYPOTHESES U	-							
SOURCE	DF	14PE 1 55	F VALUE	PA > F				
LOC	2	0 25816426	6 21	0 0346				
TESTS OF HYPOTHESES U	SING THE TYP							
SOURC E	OF	TYPE III 55	F VALUE	PR > F				
FOC THE	1 2	0 00002691 0 04000701	0 00 0 65	0 9773 0 5543				
DEPENDENT VARIABLE S	16 T							_
	. If T DF	SUM OF SQUARES	MEAN SO	DUARE	F VALUE	PR > F	R SQUARE	c v
SOURCE		SUM OF SQUARES 4 28822994	MEAN SC U 1478		F VALUE 2 46	PR > F 0 0165	H SQUARE 0 764230	85 1767
SOURCE MODEL	DF		-	B 7000				
SOURCE	DF 29	4 26822994	U 1478	B 7000		0 0165		85 1767
SOURCE MODEL ERROR CORRECTED TOTAL	DF 29 22 51	4 26822994 1 32294690	U 1478	B 7000		0 0165 RUOT MSE		85 1767 SUPT MEAN
SOURCE MODEL ERROR CORRECTED TOTAL SOURCE	DF 29 22 51 DF	4 28822994 1 32294690 5 61117684 TYPE 1 55	u 1478	97000 13395 PR > F	2 46 DF 2	0 0165 RUUT MSE 0 24522225 Type 111 SS 1 34027178	0 764230 F VALUE 11 14	85 1767 SUPT MEAN 0 28789818 PR > F 0 0005
SOURCE MUDEL ERROR CORRECTED TOTAL SOURCE	DF 29 22 51 DF	4 28822994 1 32294690 5 61117684 TYPE 1 55 1 41148009 0 60779052	U 1478 O 0601 F VALUE 11 74 1 68	PR > F	2 46 DF 2	0 0165 RUUT MSE 0 24522225 TYPE 111 SS	0 764230 F VALUE 11 14 1 76 0 02	85 1767 SUPT MEAN 0 28789818 PR > F 0 0005 0 1543 0 8920
SOURCE MUDEL ERROR CORRECTED TOTAL SOURCE LOC REP(LOC) IRA	DF 29 22 51 DF 4	4 26822994 1 32294690 5 61117684 TYPE 1 55 1 41148009 0 60779052 0 00000001	U 1476 O 0601 F VALUE	PR > F 0 0003 0 1720 0 9997 0 2152	2 46 DF 2 6 1 2	0 0165 RGGT MSE 0 24522225 TYPE 111 SS 1 34027178 0 63492411 0 00112168 0 20263484	0 764230 F VALUE 11 14 1 76 0 02 1 68	85 1767 SUPT MEAN 0 28789818 PR > F 0 0005 0 1543 0 8920 0 2085
SOURCE MUDEL ERROR CORRECTED TOTAL SOURCE LOC REP(LUC) IRR LOC+IRR	DF 29 22 51 DF 4 6	4 28822994 1 32294690 5 61117684 TYPE 1 55 1 4146009 0 60779052 0 00000001 0 19830616 0 66449631	U 1476 O 0601 F VALUE 11 74 1 68 0 00 1 65 1 84	PR > F 0 0003 0 1720 0 9997 0 2152 0 1371	2 46 DF 2 6 1 2 6	0 0165 RGOT MSE 0 2452225 TYPE 111 SS 1 34027178 0 63492411 0 00112168 0 20263484 0 65254234 0 18286510	F VALUE 11 14 1 76 0 02 1 68 1 81 1 52	85 1767 SUPT MEAN 0 28789818 PR > F 0 0005 0 1543 0 1436 0 2407
SOURCE MUDEL ERROR CORRECTED TOTAL SOURCE LOC REP(LOC) IRR REP IRR(LOC) FER	DF 29 22 51 DF 4 6 1 2	4 26822994 1 32294690 5 61117684 TYPE 1 55 1 41148009 0 60779052 0 000001 0 19830616 0 66449631 0 20071887	U 1476 O 0601 F VALUE 11 74 1 68 0 00 1 65	PR > F 0 0003 0 1720 0 9997 0 2152 0 1371 0 2114 0 2454	2 46 DF 2 6 1 2 6	0 0165 RUOT MSE 0 2452225 TYPE 111 SS 1 34027178 0 63492411 0 00112168 0 20263484 0 65254234 0 10286510 0 42619208	F VALUE 11 14 1 76 0 02 1 68 1 81 1 52 1 78	85 1767 SUPT MEAN 0 28789818 PR > F 0 0005 0 1543 0 8920 0 2085 0 1436 0 2407
SOURCE MUDEL ERROR CORRECTED TOTAL SOURCE LOC REP(LUC) IRR REP BRR(LUC) FER LOC+FER	DF 29 22 51 DF 4 6	4 26822994 1 32294690 5 61117684 TYPE 1 55 1 41148009 0 6077902 0 0000001 0 19830616 0 66449631 0 20071887 0 35340750 0 42820744	F VALUE 11 74 1 68 0 00 1 65 1 64 1 67 1 47 3 56	PR > F 0 0003 0 1720 0 9997 0 2152 0 1371 0 2114 0 2454	2 46 DF 2 6 1 2 6 2 4 2	0 0165 RGOT MSE 0 2452225 TYPE 111 SS 1 34027178 0 63492411 0 00112168 0 20263484 0 65254234 0 18286510	F VALUE 11 14 1 76 0 02 1 68 1 81 1 52	85 1767 SUPT MEAN 0 28789818 PR > F 0 0005 0 1543 0 1436 0 2407
SOURCE MUDEL ERROR CORRECTED TOTAL SOURCE LOC REP(LUC) IRR LOC+IRR REP IRR(LUC) FER LOC+FER IRR+FER LOC+IRR FER	DF 29 22 51 DF 4 6 1 2 6 2 4 2	4 26822994 1 32294690 5 61117684 TYPE 1 55 1 41148009 0 60779052 0 0000001 0 19830616 0 66449631 0 20071887 0 35340750 0 42820744 0 42382304	U 1476 0 0601 F VALUE 11 74 1 66 0 00 1 65 1 64 1 67 1 47 3 56 1 76	PR > F 0 0003 0 1720 0 9997 0 2152 0 1371 0 2114 0 2454 0 0456 0 1726	2 46 DF 2 6 1 2 6	0 0165 RUOT MSE 0 2452225 TYPE 111 SS 1 34027176 0 63492411 0 00112168 0 20263484 0 65254234 0 16286510 0 42819208	0 764230 F VALUE 11 14 1 76 0 02 1 68 1 81 1 52 1 78 3 94	85 1767 SUPT MEAN 0 28789818 PR > F 0 0005 0 1543 0 8920 0 2085 0 1438 0 2407 0 1688 0 0344
SOURCE MUDEL ERROR CORRECTED TOTAL SOURCE LOC REP(LOC) IRA LOC+IRR REP IRRILUC) FER LOCOFER IRROFER	DF 29 22 51 DF 4 6 1 2 6 2 4 4 2 4 4 2 4 4 4 4 4 4 4 4 4 4 4	4 28822994 1 32294690 5 61117684 TYPE 1 55 1 41146009 0 60779052 0 00000001 0 19830616 0 66449631 0 20071867 0 35340750 0 42820744 0 42382304	U 1476 0 060) F VALUE 11 74 1 68 0 00 1 65 1 64 1 67 1 47 3 56 1 76	PR > F	2 46 DF 2 6 1 2 6 2 4 2	0 0165 RUOT MSE 0 2452225 TYPE 111 SS 1 34027176 0 63492411 0 00112168 0 20263484 0 65254234 0 16286510 0 42819208	0 764230 F VALUE 11 14 1 76 0 02 1 68 1 81 1 52 1 78 3 94	85 1767 SUPT MEAN 0 28789818 PR > F 0 0005 0 1543 0 8920 0 2085 0 1438 0 2407 0 1688 0 0344
SOURCE MUDEL ERROR CORRECTED TOTAL SOURCE LOC REP(LUC) IRR LOC+IRR REP IRR(LUC) FER LOC+FER IRR+FER LOC+IRR FER	DF 29 22 51 DF 4 6 1 2 6 2 4 2 4 2 4 USING THE 196	4 20822994 1 32294690 5 61117684 TYPE I 55 1 41146009 0 60779052 0 00000001 0 19830616 0 66449631 0 20071867 0 35340750 0 42820744 0 42302304 PE III MS FOH HEP4LE	F VALUE 11 74 1 68 0 00 1 65 1 64 1 67 1 47 3 56 1 76 0C) AS AN ERRO	PR > F 0 0003 0 1720 0 1720 0 1721 0 2152 0 1371 0 2154 0 2454 0 0456 0 1726 IR TERM PR > F	2 46 DF 2 6 1 2 6 2 4 2	0 0165 RUOT MSE 0 2452225 TYPE 111 SS 1 34027176 0 63492411 0 00112168 0 20263484 0 65254234 0 16286510 0 42819208	0 764230 F VALUE 11 14 1 76 0 02 1 68 1 81 1 52 1 78 3 94	85 1767 SUPT MEAN 0 28789818 PR > F 0 0005 0 1543 0 8920 0 2085 0 1438 0 2407 0 1688 0 0344
SOURCE MUDEL ERROR CORRECTED TOTAL SOURCE LOC REP(LUC) IRR REP IRR(LUC) FER LOC+FER IRR+FER LOC+FER	DF 29 22 51 DF 4 6 1 2 6 2 4 4 2 4 4 2 4 4 4 4 4 4 4 4 4 4 4	4 28822994 1 32294690 5 61117684 TYPE 1 55 1 41146009 0 60779052 0 00000001 0 19830616 0 66449631 0 20071867 0 35340750 0 42820744 0 42382304	U 1476 0 060) F VALUE 11 74 1 68 0 00 1 65 1 64 1 67 1 47 3 56 1 76	PR > F	2 46 DF 2 6 1 2 6 2 4 2	0 0165 RUOT MSE 0 2452225 TYPE 111 SS 1 34027176 0 63492411 0 00112168 0 20263484 0 65254234 0 16286510 0 42819208	0 764230 F VALUE 11 14 1 76 0 02 1 68 1 81 1 52 1 78 3 94	85 1767 SUPT MEAN 0 28789818 PR > F 0 0005 0 1543 0 8920 0 2085 0 1438 0 2407 0 1688 0 0344
SOURCE MUDEL ERROR CORRECTED TOTAL SOURCE LOC REP(LUC) IRA LOC+IRR REP HARILUC) FER LOC+FER LROFFER LOC+IRR FER LOC+IRR FER LOC+IRR FER LOC+IRR FER TESTS (P H F)E ESLS C	DF 29 22 51 DF 4 6 1 26 6 2 4 2 4 2 4 USING BR 146	4 28822994 1 32294690 5 61117684 TYPE I 55 1 41146009 0 60779052 0 0000001 0 19830616 0 66449631 0 20071867 0 35340750 0 42820744 0 42382304 PL III MS FOH HEPILE 1YPE III SS	F VALUE 11 74 1 68 0 00 1 05 1 67 1 67 1 47 3 56 1 76 1 76 1 76 1 76 1 76 1 76 1 76 1 7	PR > F 0 0003 0 1720 0 9997 0 2152 0 1371 0 2114 0 2454 0 0456 0 1726 IR TERM PR > F 0 0332	2 46 DF 2 6 1 2 6 2 4 2	0 0165 RUOT MSE 0 2452225 TYPE 111 SS 1 34027176 0 63492411 0 00112168 0 20263484 0 65254234 0 16286510 0 42819208	0 764230 F VALUE 11 14 1 76 0 02 1 68 1 81 1 52 1 78 3 94	85 1767 SUPT MEAN 0 28789818 PR > F 0 0005 0 1543 0 8920 0 2085 0 1438 0 2407 0 1688 0 0344
SOURCE MODEL ERROR CORRECTED TOTAL SOURCE LOC REP(LUC) IRR LOC+IRR REP HRRILUC) FER LOC+FER LOC-FER L	DF 29 22 51 DF 4 6 1 2 6 2 4 2 4 2 4 USING THE 196	4 28822994 1 32294690 5 61117684 TYPE I 55 1 41146009 0 60779052 0 0000001 0 19830616 0 66449631 0 20071867 0 35340750 0 42820744 0 42382304 PL III MS FOH HEPILE 1YPE III SS	F VALUE 11 74 1 68 0 00 1 05 1 67 1 67 1 47 3 56 1 76 1 76 1 76 1 76 1 76 1 76 1 76 1 7	PR > F 0 0003 0 1720 0 9997 0 2152 0 1371 0 2114 0 2454 0 0456 0 1726 IR TERM PR > F 0 0332	2 46 DF 2 6 1 2 6 2 4 2	0 0165 RUOT MSE 0 2452225 TYPE 111 SS 1 34027176 0 63492411 0 00112168 0 20263484 0 65254234 0 16286510 0 42819208	0 764230 F VALUE 11 14 1 76 0 02 1 68 1 81 1 52 1 78 3 94	85 1767 SUPT MEAN 0 28789818 PR > F 0 0005 0 1543 0 8920 0 2085 0 1438 0 2407 0 1688 0 0344
SOURCE MUDEL ERROR CORRECTED TOTAL SOURCE LOC REP(LUC) IRA LOC+IRR REP HARILUC) FER LOC+FER LROFFER LOC+IRR FER LOC+IRR FER LOC+IRR FER LOC+IRR FER TESTS (P H F)E ESLS C	DF 22 51 DF 2 4 4 USING THE 140 DF 2	4 28822994 1 32294690 5 61117684 TYPE 1 55 1 41146009 0 60779052 0 0000001 0 19830016 0 66449631 0 20071867 0 35340750 0 42820744 0 42382304 PL III MS FOR REP III	U 1476 0 0601 F VALUE 11 74 1 68 0 00 1 65 1 64 1 67 1 76 1 76 0C) AS AN ERRO F VALUE 6 33	PR > F 0 0003 0 1720 0 9997 0 2152 0 1371 0 2114 0 2454 0 0456 0 1726 IR TERM PR > F 0 0332	2 46 DF 2 6 1 2 6 2 4 2	0 0165 RUOT MSE 0 2452225 TYPE 111 SS 1 34027176 0 63492411 0 00112168 0 20263484 0 65254234 0 16286510 0 42819208	0 764230 F VALUE 11 14 1 76 0 02 1 68 1 81 1 52 1 78 3 94	85 1767 SUPT MEAN 0 28789818 PR > F 0 0005 0 1543 0 8920 0 2085 0 1438 0 2407 0 1688 0 0344

DEPENDENT VARTABLE	CNUBUPE						
SUUMCE	ğ	SUM OF SQUARES	MEAN SQUARE	F VALUE	£	R-SQUARE	> U
MODEL	50	58 95884869	6608<066 0	1 56	0 1362	0 675565	54 8423
ERRUR	22	13 90729935	0 63214997		ROOT MSE	3	CNUBLE T MEAN
CORMECTED TOTAL	5.1	42 86614804			0 79507859		0 63631624
SOURCE	Đ	TYPE I SS	F VALUE PR	7 T OF	TYPE III SS	F VALUE	7 × ×
LDC	N 4	,,,,	55		12 75627104		
) - - (0 0000000	200	2454	ORFOLENG O		0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
REP IRRILOCO	4 •0		•				
	~•	•					
TOTAL TERM	r (N) -	•	2				
LIK O I MWO PEW	TOAR AND THE STORY AND THE STO		O WE O				
•				4			
SOURCE	70	v	ξ.	L ^			
רסכ	N	12 75627104	12 57 0 0	0072			
TESTS UF MYPUTNESES	HYPUINESES USING THE TYPE III MS	PE III MS FOR REPOURALLOC!	AS AN EGRUA	1ЕВМ			
SOL RCE	90	1YPE 111 55	P VALLE PH	EL A			
188 100+188	- N	0 93470430	00 00 00 00 00 00 00 00 00 00 00 00 00	3911			
DEPENDENT VANTABLE	כרת					1	3
	į	SUM OF SOUARES	MEAN SOUANE	F VALUE	- A	1 1 2 5 5 5 F F	
SOUNCE	; é	-	91410140 0	2 30	0 0237	0 752275	63 1651
MODEL	~				BOOT MSE		CLUP I MEAN
ERROH	2.2	0 39167453	\$5509210 O				1106043911
CORRECTIO 131 L	83	1 56106518			C. 1.55 C. 1. 0		1
	Ì	1 907	F VALUE PR	7 T	17PE 111 55	F VALUE	F V E7
Sounce	Š		8		3063747	3	0
100	N 6	0 32355766	32:	1662	0 19803797	9 6 - 0	0000
IAR IAR	-	090000000	2.83		0594107		2
LOCe I RR	N -0	1926461	2		0766170	- (4)	-
FER	₩ €	0 07956004	00		1536793	N PA	-5
	N₹	0 11169402	77		1262468	-	-
1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	HADDE FOR USING 198 17	ITHE HIS WERFOLD	OC) AS AN ENHOR TERM	2			
5		1YPE 111 55	F VALUE PR	, F			
SOURCE	à		•	4000			
רחכ	N	U 30637478					
:	1 1 3	T FF LIEMS FOR REP LI	ARILUCI AS AN EHRUR	Te HM			
TESTS IF 1 71 CACA	,		F VALUE PH	, F			
SOURCE	5	0000	0 70	2000			
188	~ \	0 05041075	\$6.0	100			

Appendix B Analysis of variance of some dependent variables using data of amaranth experiment from Naipio site

DEPENDENT VARIABLE O	*					
5 CE	UF	SUM OF SOUARES	MEAN U WHP	F VALUE	PH > F	H SUUARE C V
MODEL	v	7704 87791111	856 09754568	5 24	0 0145	0 854960 7 6881
ERRUR	8	1307 10088889	163 40761111		ROUT MSE	OX MEAN
CORRECTED TOTAL	17	9011 97880000			12 78231634	166 <6000000
50URCE	DF	TYPE I SS	F VALUE PR > F	DF	TYPE 5\$	F VALUE PR > F
IHR REP(14HR) FEH 1AR+FER	1 4 2 2	130 68055556 831 39941111 3834 65343333 2908 14401111	0 80 0 3973 8 27 0 3566 11 73 0 0042 8 90 0 0092	1 4 2 2	130 68055556 631 37991111 3834 6534333 2908 14401111	0 80 U 3973 1 27 U 3566 11 73 U 0642 8 90 U 0092
TESTS OF YPT 1ESES U	SING TE T	TE 111 MS FOR REP(1R	R) AS AN ERHUH TEHM			
OURCE	D+	TYPE III SS	F VALUE PR > F			
		<u>ለ</u> ተበጓጓኝትዕ	0 63 0 4722			
CEPELLENT VALLABLE TI	•					
SOURCE	()	SUM UF SQUALES	MEAN SQUARE	F VALUE	141 > F	R-SULAKE C Y
MODEL	y	1 97024444	0 21891605	7 66	0 00 J	0 595299 5 1464
ERHO 4	U	Q 2279 5J6	U 02849444		HOLT M E	IN MEAN
CORNI CILD TOTAL	17	2 14850000			0 16480588	3 20000000
SUURC E	DF	14PE 1 55	F VALUE PR > F	DF	THE 111 55	F VALUE PR > F
INA Repulk)	1	U 283755Jb 0 9J224444	9 yo 0 0135 8 35 0 0059	<u>.</u>	0 28375006	à ão o 6132
FER IRR FER	2	CLE65349 0	11 62 0 00 3	2	0 95224444 0 66223333	8 35 6 6659 11 62 6 6043
SUM LEM	2	0 07201111	1 26 0 333	2	0 07201111	1 26 0 3335
TESTS OF MY OT ESTA US	541 G 1 R F	FI III MS FOR REPUR	P) AS AN EHIGH TERM			
SUURCE	t	1411 111 5	F VALUE P >			
IH .	•	0 2 37 5	1 1 0 3363			

DEFE DENT VARIALLE	I W					_	
SUURC E	UF	SUM UF SUVARES	MEAN SULAHL	F VALUE	PR > I	H-SUUARE	CV
MODEL	9	1H17 H2666667	209 7515 1852	5 02	0 0165	0 849641	21 3434
ERRUR	6	334 06444444	41 7605 356		RUQ1 MSE		DA MEW
CORRECTIO TOTAL	1.7	22 1 91111111			6 41 24075		30 2777/778
SOURCE	DF	TYPE I SS	F VALUE I R	> F UF	TYPC 111 S5	+ VALUE	PH > F
IRR	1	9 9755556 640 1888888 <i>)</i>		0361 1 0502 4	9 97535556 640 188 888	U 24 3 B3	0 6361 u 0502
REP(1HK) Fem	2	JO2 22111111		0700 4	1111115 200	3 4	0760
IRR FER	2	935 44111111	11 20 0	0045 2	935 44111111	11 20	U 0048
TESTS IF IN IT ESES	USING THE IN	IPL 111 MS FOR REPLIES	1) AS AN ERHUR TER	M			
SOURCE	L	171 L 111 S5	H A E I	> 1			
IRR	1	9 97555556	(up 0	1 1			
DE IN E I ATTACE	TNU T						
SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	F SOUARE	c v
MODEL	9	2 43737688	0 27081965	6 09	0 0091	0 672565	21 2959
ERRUR	8	0 35589310	0 04448664	•	ROOT MSE		THUPT PEAN
CORRECTED TOTAL	17	2 79326997			0 21091756		0 99041667
SOURCE	DF	TYPE 1 SS	F VALUE PR	I > F OF	TYPE III SS	F VALUE	PR > F
LAA	1	0 00396347	0 09 0	7729 1	0 00396 47	(66	0 7729
REPIINN) Fer	4 2	0 43330019 0 70529543		1322	0 43330¢19	2 44	0 1322
TAR FEH	Ž	1 29461776		0127 2	0 70529 43 1 29481778	14	0 0127 0 0022
TESTS OF HYPOTHESES	USING THE TO	YPE III MS FOR REPLIR	AS AN EDDAG TEG	2 44			
SUUNCE	DF	TYPE III SS		`~ ` > F			
LAR	1	0 00396347		#57A			
64	OKPHO	• 5037070		, in			
DE E VATABLE	1	SIM OF SQUARES	MEAN SOUAR	E F VALUE	PR > F	A SOUARE	C v
MO	٠,	1 60238747	1 7334986		0 0115	0 8640	24 0145
ERRJII	a	2 45478209	0 3068477	_	HOOT MSE		DXPRO MEAN
COR FLIFO TOL F	17	16 05716955		•	0 55393841		2 30668515
COR ECICO TOT C	••	111 03710133			5 53575044		2 30000013
SO CE	DF	TYPE 1 55	F VALUE P	P > F DF	TYPE III SS	F VALUF	PR > F
UFD IHH) Iku	1	0 96224345 2 51648640		6644 I 1798 4	0 06224 45 2 51648640	(0	0 6644 0 1796
FER	ž	3 33236742	5 43 0	0324 2	3 33236742	43	0 0324
1 1 111	2	9 691290 0			9 69129020	11 76	0 0017
JE T OF 1 POTIFSES	USING THE T	YPE III MS FOR REPLIF	THE AS AN ERROR TE	L.A.			
5 1 (DF	TYPE [II S	F VALUE P	R > F			

0 10

2 7188

0 002 4145

16

DEPENDENT VARIABLE	CXSPRO						
SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	F SQUARE	c v
MODEL	2	4 36409082	0 48489898	4 79	0 0190	0 643475	28 8#3
ERHUR	8	0 40982497	0 10125815		POOT MSF		UXSUED REWN
CORRECTED TOTAL	17	5 17391579			0 31816367		1 11291475
SOUNCE	DF	TYPF I SS	F VALUE PR >	F OF	TYPE []] \$\$	F VALUE	PR > F
IAR Rep(iam) Fem IBR _{Fe} m	5	0 0069 600 0 48465509 1 93274965 1 93956007	0 07 0 800 1 20 0 382 9 55 0 007 9 58 0 007	4 6 2	0 00692600 0 48485509 1 9377465 1 93976007	C 07 9 0 5 55 5 6	0 3824 0 0076
TESTS OF HYPOT CSCS	USING THE TY	PE III MS FOR REP(IR	R) AS AN ERPOR TERM				
SOURCE	DF	TYPE III SS	F VALUE PR >	F			
144		0 00697600	0 06 0 822	8			
DEPENDENT VARIABLE	CX3PRO						
OURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	A SQUARE	c v
MODEL	9	5 50206222	0 61134025	4 66	0 0205	0 839946	23 9774
ERROR	a	1 04843425	0 13105428		ROOT MSE		OX3PRO MEAN
CURRECTED TUTAL	17	6 55049646			0 36201420		1 50981450
SOUNCE	OF	TYPE SS	F VALUE PR >	F OF	TYPE III SS	F VALUE	P4 > F
IAH Repiiahi Fer Iar fer	2 2	0 01562576 1 34975279 1 16762775 2 96905592	0 12 0 736 2 57 0 118 4 45 0 050 11 33 0 004	8 4 1 2	0 01562 76 1 34975279 1 16762775 2 96905 92	C 1 7 4 45	
TESTS OF HYPOTHESES	USING THE TY	PE III NS FOR REPEIR	RI AS AN ERROR TERM				
SOUNCE	OF	TYPE 111 S5	F VALUE PA >	F			
IRR DEPENDENT VARIABLE	1 0×2 3PRO	0 01562576	0 05 O BAO	1			
SUUNCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PQ > F	R SQUARE	c v
MDOEL	9	IA 90977982	2 10108665	5 23	0 0146	0 0546	24 1771
EARDA		3 21667599	0 40208450		PODT MSE	•	X2_1PRO MEAN
CORNELILO FOTAL	1.7	22 12645581			0 63410133	_	2 6 227 31 25
SOURCE	OF	TYPE SS	F VALUE PR >	F OF	TYPE III SS	F V/LUE	P1 > F
IHR MEP(IHR) FER IAR FER	1 4 2 2	0 00174561 3 23770640 5 90306294 9 68726487	0 00 0 949 2 01 0 105 7 44 0 014 12 05 0 007	5 4	0 00174 61 3 23770640 5 98306294 9 AR A	6 CG 01 7 44	0 (055
TESTS OF EXPOTEESES	UST G THE TY	PE III MS FOR REPLIA	R) AS AN ERROR TERM				
SOURCE	OF	14PE 111 \$9	F VALUE PR >	F			

0 00

0 00174561

IAA

0 9652

DEPENDENT VAPIABLE	CAUPT						
SOUNCE	DF	SUM OF SQUARES	MEAN SQUARE	E F VALUE	PR > F	R SQUAFE	c v
MGDEL	9	0 65215347	0 0946837	2 4 45	0 0236	0 833394	23 2040
ERHUR	8	0 17035629	0 0212945		ROOT MSC		CAUPT PEAN
CORRECTED TOTAL	17	1 02250975			0 14592647		0 62888444
		• • • • • • • • • • • • • • • • • • • •			• • • • • • • • • • • • • • • • • • • •		0 020,0000
SOURCE	ÐΕ	TYPE I SS	F VALUE P	R > F DF	TYPE 111 SS	F VALUE	PR > F
IHH Rep(IRR)	1	0 04905756 0 26793979		1675 1 0786 4	0 04905756 0 26793979	15	0 1675 0 0786
FEH IRF+feh	5	0 10466341 0 43047272	2 46 0	1472 2 0065 2	0 10468341 0 43047272	10 11	0 1472 0 0065
_	-	• •••			• • • • • • • • • • • • • • • • • • • •		0 2001
TESTS OF HYPOTHESES	USING THE TY	PF 111 MS FCR REPLIR	R) AS AN ERROR TE	RM			
SUUNCE	DF	TYPE III SS	F VALUE P	R > F			
181	1	0 04905756	0 73 0	4404			
DEPENDENT VARIABLE	MGUPT						
SOURCE	DF	SUM OF SQUARES	HEAN SQUAR	E F VALUF	PP > F	F SQUAFE	C V
MODEL	9	0 37010666	0 0411229	6 4 72	0 0198	0 8414 1	23 1473
LHRUH	6	0 06973652	0 0057170	7	ROOT MSE		MCUPT HE N
CORRECTE > TOTAL	17	0 43984318			0 09336 23		0 40335267
SOUNCE	OF	TYPE I SS	F VALUE P	R > F DF	TYPE III SS	F VALUE	PR > f
IRR Rep(IRR)	1	0 01297841		2571 1 1240 4	0 01297841 0 08778566	1 49 2 52	0 2571 0 1240
FLR IAG FER	2	0 05571107	3 20 0	0955 2	0 05571107 0 21362752	02 E2 \$1	0 09 5
INN FEN	2	0 21362752	12 25 0	0037 2	0 21302192	15 52	0 0037
TEST OF HYPOT ESES	USING THE TY	PE III MS FOR RFP(IR	R) AS AN ERROR TE	7 M			
\$001CL	DF	TYPE III SS	F VALUE P	A > F			
IAA	1	0 01297841	0 59 0	4848			
DEPENDENT VARIABL	KUPT						
BUNCE	ΩF	SUM OF SOUARES	MEAN SQUAR	E F VALUE	PR > F	R SQUAFE	C V
MODEL	9	4 47957869	0 4977309	7 37	0 0049	0 89234	20 1070
E RR	A	0 54044568	0 0675557	1	ROOT MSE		KUPT #FAN
CONSECTED TOT L	17	5 02002437			0 25991481		1 2926 955
SQUACE	DE	TYPE 1 SS	F VALUE P	R > F UF	TYPE III SS	F VALUE	PF > F
IHR	•	0 000 4795		9305 L 0362 4	0 00054795 1 18383632	C 01 4 36	0 9305 0 0362
REPLIKKI Fer	2	1 16383632	9 10	0097 2	1 22692705 2 06625737	\$ 10 18 9	0 0007
ING FEH	2	2 06626737	15 29	0019 5	2 00020737	16 7	0 0013

PR > F

0 7677

F VALUE

0 00

TESTS I TESTS S USING THE TYPE ILL MS FOR REPLIRED AS AN ERICH TERM

SUUHCE

IRH

1 PE 111 55

0 00054795

DEPENDENT V RT BLF	NAUPT						
SCUHLE	DF	SUM OF SQUARES	MFAN SQUARE	F VALUE	PR > F	F SOUAFE	ζ ٧
MUDEL	9	0 02433814	0 00270424	A 24	0 0034	0 9026 (30 4688
FHHOK	•	0 00262527	0 00032816		HOUT MSE		NAUPT PEAN
CORRECTED TOTAL	17	0 02696341			0 01611514		0 05849468
SOURCE	DF	TYPE I SS	F VALUE PR > F	OF	TYPE III SS	F VALUE	PP > F
IRR	1	0 01176731	35 66 0 9903	•	0 01176731	t et	0 0003
REPLIARI Fer	4 2	0 00396216 0 00350351	3 02 0 0859 5 34 0 0337	\$	0 00396216 0 00350351	02 t 4	0 0859 0 0337
IRR FER	2	0 00510516	7 76 0 0133	ž	0 00510516	7 76	ō ō133
JESTS OF HYPOTHESES	USING THE TYP	PF 111 MS FOR REP(IRR	I AS AN ERROR TERM				
SOURCE	DF	14PF 111 55	F VALUE PR > F				
THE	1	0 01176731	11 88 0 0261				
***	•	0 01178731	11 0 0201				
DEPENDENT VARIABLE	CATURT						
S URCE	ÐF	SUM OF SQUARES	MEAN SQUARE	F VALUF	PA > F	F SQUAFE	C V
MLDEL	9	13 65301415	1 51 700157	5 92	0 0099	0 869504	21 2342
ERRUR	•	2 04905834	0 25613229		HOOT MSP		CATUPT PEAN
CUMRECTED TOTAL	17	15 70207249			0 50609514		2 30339154
SOURCE	DF	TYPE I SS	F VALUE PR > F	DF	TYPE III SS	F VALUE	PA > F
188	1	0 000 9142	0 00 0 9629	ļ	0 00059142	C 00	0 9679 0 0542
REP(IRH)	4 2	3 79943764 2 95894433	3 71 0 0542 5 78 0 0280	\$ •	3 79443764 2 95894433	3 71	0 0280
FER IAA FLA	2	6 89404076	13 46 0 0028	ž	6 89404076	1 46	0 0028
IESTS OF MYPOTHESE	S U ING THE TY	PF TIL MS FOR REPTIRE	I) AS AN ERROR TERM				
SOUNCE	DF	TYPE III SS	F VALUE IR > F				
-			0 00 0 9813				
THH	1	0 00059142	0.00 0.4413				

SOUNCE	DF	SUM OF SQUARES	MEAN SO	MARE	F VALUE	PA > F	A SQUAFE	C V
MODEL	9	0 03555146	0 0039	5016	8 41	0 0032	0 904416	24 6119
ERRUR	8	0 00375722	0 0004	6965		ROOT NSE		PUPT MEAN
CORNECTED FOTAL	17	0 03930868				0 02167146		0 08805281
SOURCE	DF	TYPE I SS	F VALUE	28 > F	OF	T YPF 1 55	F VALUE	PP > F
IRR		0 01758013	37 43	0 0003	-	0 01758013	7 43	0 0003
HEP(IHK) FER IAROFEH	2 2	0 00840367 0 00458399 0 00498367	4 47 4 88 5 31	0 0343 0 0412 0 0341	2 2	0 00840367 0 00458399 0 00498367	4 47 4 66 31	0 034
••••	•	• • • • • • • • • • • • • • • • • • • •	3 31	0 0344	•	0 00440307	3.	0 0341
TESTS OF HYPOTHESES	USING TIF T	YPE III MS FOR REPUT	IR) AS AN ERROR	TERY				
SOURCE	DF	TYPE III SS	F VALUE	PR > F				
IHR	1	0 01758013	8 37	0 0444				
DEPENDENT VA LABLE	SUPT							
SOURCE	DF	SUM OF SQUARES	MEAN SO	UARE	F VALUE	PR > F	F SQUAFE	c v
MGDE L	9	0 04147030	0 0046	0761	4 24	0 0270	0 82675	24 2335
ERROR	8	0 00868763	0 0010	8595		ROOT MSF		SUPT PEAN
CORRECTED TOT t	17	0 05015793				0 03295301		0 135984 3
SQUACE	OF	TYPE I SS	F VALUE	PP > F	OF	TYPE	F VALUE	PR > F
IHA REPLINA)	1	0 00010905	0 10 3 58	0 7594 0 0557	į.	0 00010905 0 01557094	C 10 3 58	0 7594 0 0 87
FER BRH FEH	2 2	0 00532060 0 02046971	2 45 9 42	0 1479 0 0079	2 2	0 00532060 0 02046971	5 42	0 1479 0 0079
ILSIS 6 TYPTHESES	USING THE T	YPE ILI MS FOR REPÇIA	DRI AS AN FRROS) TEGY				
SOURCE	pr	TYPE 111 SS	F VALUE	PR > F				
188	1	0 00010905	0 03	0 8752				
ULP NUENE AREABLE	·=							
SOURCE	0 F	SUM OF SQUARES	MEAN SO	UAPE	F VALUE	PR > F	F SOUAFE	c v
MODEL	9	0 17787183	0 0197	6354	1 67	0 1956	0 67760	55 e447
EMPOH		0 08462983	0 0105	7873		ROOT MSE	c	HOSUPT PEAN
COPIECTED TOTAL	17	0 26250166				0 10285294		0 18463855
SOUNCE	DF	TYPE 1 \$5	F VALUE	PR > F	OF	TYPE III SS	F VALUE	PR > F
IRA	!	0 00000510	0 00	0 9830	1	0 00000 10	0 00 0 39	0 9630 0 8076
KEP(IHK) Fea	4 2	0 01668548 0 0782327?	0 39 3 70	0 8076 0 0729	4 2	0 0166654A 0 07823272	0 39	0 0729

F VALUE

g 00

PR > F

0 9738

TYPE III SS

0 00000510

SOURCE

IAR

DF

DEPENDENT VARIABLE	CLUPT							
SOURCE	0F	SUM OF SQUARES	MEAN S	QUARF	F VALUE	PR > F	F SQUAFE	c v
MULL L	9	0 01379198	0 001	53244	4 01	9 0316	0 81860	24 0615
FHHUH	6	0 00305500	0 000	38187		ROOT MSE		CLUPT PEAN
CURRECTED TOTAL	17	0 01684698				0 01954161		0 05121512
SOURCE	D₹	TYPE SS	F VALUE	PR > F	DF	TYPE III JS	F VALUE	PR > F
IRH REP(IMH) FER IRK FER	1 4 2 2	0 000 7824 0 00632485 0 00177535 0 00531354	0 99 4 14 2 32 6 96	0 3448 0 0416 0 1600 0 0178	1 4 2 2	0 00037624 0 00632465 0 00177 5 0 00531354	(55 4 14 6 56	0 3468 0 0416 0 1600 0 0178
TESTS OF EXECUTESE	USING THE TY	PE III MS FOR REPEIRE) AS AN ERAD	A TERM				
SUURCE	DF	TYPE 111 SS	F VALUE	PR > F				
188	1	0 00037824	0 24	0 6504				
DEPENDE IT VARIABLE	CANUPT							
SOURCE	DF	SUM OF SQUARES	MEAN S	QUARE	F VALUE	PR > F	F SQUARE	c v
MODEL	9	0 59527955	0 066	14217	2 83	0 0710	0 76085¢	31 2010
FHHUH	8	0 18706022	0 023	38253		ROOT PSE		CANUPT REAN
CORRECTED TOT L	17	0 78231977				0 15291346		0 49009104
SOURCE	OF	TYPE I SS	F VALUE	PR > F	OF	TYPE III SS	F VALUE	PR > F
IAH AEP(IMM) FER IMA FEM	1 4 2 2	0 02567099 0 06109292 0 19208678 0 31642886	1 10 0 65 4 11 6 77	0 3254 0 6406 0 0593 0 0191	1 4 2 7	0 02567099 0 06109292 0 19208679 0 31642686	1 10 0 65 4 11 6 77	0 3254 0 6408 0 6593 0 0191
TESTS OF HYPOTIESE	USING THE T	YPE III MS FOR REPCER	R) AS AN ERRO	OR TERM				
50uH(E	OF	TYPE 111 \$5	F VALUE	PR > F				
188	1	0 02567099	1 68	0 2646				

Correlation matrix of oxalates and nitrate-N concentrations and various soil and climatic 0 00174 0 77611 0 14113 # CT E T O 1681 0 24219 1000° d 0 0001 -0 97049 = F (2 (0 0 6162 0 67801 0 41763 0 3224 1961 0 37607 4) 74 variables using data from non-irrigated plots of amaranth experiments at three sites 0 53960 0 99278 00000 0 0 0035 0 60779 0 53915 0 4711 0 0001 0 77165 0 37607 0 0731 0 0 000 1 96424 97772 0 000 1 0 0470 06285 0 0020 0 0001 0 0001 0 93974 0 0301 00000 0 0 99772 0 0010 0 65994 0 0002 0 81461 0 7554 91169 0 0224 0 05296 0 02259 0 9113 0 0104 0 0001 1 00000 0 50813 0 91936 0 0001 0 0001 98233 0 0226 0 1244 0 06536 06666 0 26219 9 000 1 0 000 1 1964 . . 0 54935 0 00 10 3811 2317 0 09763 0 31333 000000 0 69205 0 77365 0 87804 0 0001 0 95171 0 0001 0 0001 0 70241 0 14987 0 56481 0 0001 0 0001 0 95789 -0 22195 0 29420 0 05242 0 09674 0 31799 0 99410 0 97368 1 03000 0 0000 0 56481 0 98656 0 36059 0 0646 0 11443 0 5698 0 93674 95984 0 10099 0 000 1 0 616 0 19927 0 03110 0 6369 0 04539 0 99179 0 20110 0 3145 0 2364 000000 0 91836 0 14113 0 12579 0 5319 0 066 16 0 74 10 0 77310 0 91254 0 34987 0 92936 97069 0 0001 0 0001 0 3097, 0 05640 0 04745 0 491 6 000000 0 70241 0 99990 0 98503 0 52953 0 46947 0 44507 A 1 P 1 00310 0 6 9369 0 98410 0 0001 0 93441 0 1633 66644 0 0 0185 0 91254 0 0001 0 0001 0 39041 0 24407 0 27339 0 1677 1 00000 0 49156 0 25112 0 43453 -0 56744 0 0020 0 0037 0 0010 0 65233 0 21282 0 2865 102 0 53980 60899 0 -0 68891 0 65681 0 0002 -0 39799 0 0338 0001 0 02259 0 63733 0 0004 0 87035 0 00000 0 27339 0 9369 0 20110 0 31333 -0 06286 0 7554 0 43386 -0 40003 0 0387 0 38103 0 39537 0 41526 09821 0 4617 0 09674 0 03225 0 8731 0 6312 c 0 7421 0 06536 0 03449 19791 0 0 41374 00000 0 0 6617 03130 0 09763 0 6281 0 6415 = 0 26407 0 1832 0 07776 0 6999 0 6395 0 07360 09718 0 05262 0 7943 0 09573 0 7152 0 6654 6348 0 67794 00000 0 0 9319 1 117335 0 0503 0 04745 0 R142 0 05296 0 9250 0 35343 0 1046 9 3 1 4 6 9 11443 0 569B 0 23911 0 3190 0 2317 0 00691 7276 0 0 30092 15981 0 0 1272 6373H 0 0000 0 1159 0 (1794 0 / 1908 0 6499 0 27195 U 2659 0 2030 0 14 63 0 35736 0 1244 55983 53419 55627 J 0026 55753 0 0025 1 000 0 Appendix C 10 Ju - -74 7 7 T T V SUL 1 PTOS Sin: sole 2013 8105 3 JUL 5 3 7 70 3

11.1	0 0.1773	0 32613	0 10797	0 63104	0 64715	0 76926 0 0001	0 40214	0 30111	0 14402 0 4736	611911 0 812c 0	0 0001	0 27754	0 45484	0 39290	0 99595	0 97825	0 9 915	0 97394
=	0 , 77 ,	0 9 JH 5 6 0 000 1	0 96175	0 48145	0 94977	0 66784	0 96623	0 99683	0 86277 0 0001	0 96481	0 78127	0 99456 0 0001	0 99625	0 99983	0 29130	0 56007	0 62285	0 57642
Ĉ	0 67719	0 39272	0 94110	0 42126 0 0286	0 0001	0 91684	0 98140	0 98920	0 82672 0 0001	0 94489	0 02157	0 98528	0 99982	0 99879	0 35513	0 61965	0 67418	0 63020
1 10	0 15139 0 0001	0 99776	0 98767	0 58312	0 90537	0 82622 0 0001	0 92033	0 99918 0 0001	0 91720	0 98919	0 7007 0	0 99987	0 97867	0 99042	0 17443	0 45664	0 52449	0 47423
h: 14	0 43608	0 73981	0 57050 0 0019	0 19787	0 93308 0 0001	0 97844	0 91080	0 72076	0 34714 0 0761	0 57982 0 0015	0 99993	0 70347	0 R2558 0 0001	0 78508	0 63149	0 95821	0 97757	0 96371
4 I B 3	0 94393	0 97341	0 9999H 0 0001	0 70402	U 62382 0 0001	0 72308	0 85512 0 0001	0 97912	0 96997	0 99983	0 57476	0 98383	0 93196	0 95456	0 01120 0 9558	0 30514	0 37833	0 32401
A 1 R 2	0 9956J	0 88981 0 0001	0 96899	0 85702 0 0001	0 66340	0 53582 0 0040	0 70542	0 90154 0 0001	000000	0 96611	0 35 119	0 91193	0 61747	0 85493	0 22950	0 06727 0 7388	0 19472	0 0870U 0 6658
1181	1000 0	0 99862	0 98530 0 0001	0 57131	0 91141	0 B3428 0 0001	0 91361	0 99966 0	0 91134	0 98718	0 71098	0 00000	0 98154	0 99232	0 16664	0 46945	0 53675	0 48690
FLON	0 11011	0 51623 0 0058	0 40187	0 11539	0 64669	0 67628	0 63190 0 9004	0 50401	0 24925	0 40822	0 68899 0 0001	0 49228	0 57483	0 54752	0 56689	0 65704	0 67119	0 66103
015]	0 15185	0 00796 0 9685	0 09366 0 6422	0 38339	0 17306	0 23806 0 2318	0 14845	0 00407	0 20229 0 3116	0 06857	0 30929	0.01532	0 07151	0 04054	0 44480	0 39512 0 0414	0 37616 0 0 0 5 3 1	0 39051
£ IO	0 03283 0 8436	0 07007	0 05319 0 7922	0 02085	0 09000 0 6551	0 6378	0 08766 0 6637	0 06825	0 03103	0 05412 0 7886	0 09759 0 6282	0 06651	0 07888	0 07476	0 08287 0 6611	0 6394	0 09608	0 09490 0
017	0 16761	0 02736	0 11189	0 14010 0 0826	0 11368 0 5724	0 17067	0 09234	0 03747	0 20023	0 10770 0 5929	0 23444 0 2392	0 04688	0 02637	9666 0	0 36556 0 0608	0 31444	0 29630	0 10999
D.	0 4763	U 31387 U ORBA	0 22 50 0 7581	U 20612 0 1023	0 0124	0 51592 0 0059	0 45647	0 32169	0 09017	0 2457	0 54851	0 1150	0 39335	0 36516	0 51685	0 55759	0 56014	U 55956 0 0025
	ŗ,	H11.2	E 11 3	7 4	4	25 1	40	A1 61 54	41825U	ALKINU	7054	\$0F120	n 7100	Solisa	rs+10s	Or TOS	Soresu	\$ 1105

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uLisy	u 14853 u 4597	N 31873 O 1051	0 01040 98cp 0	0 35437 0 0697	0 04127	0 656 79 0 0002	0 90775 0 0001	0 74029 0 0011	0 U7539 0 7096	0 66762	3 51695 0 00 1	0 7148 0 0118	0 4741
มมีใจมู	0 14095 U 4932	0 16902 0 3994	0 03747 0 8450	0 16358 0 4149	0 30761 0 11P5	0 94552 0 0001	0 99399	0 99931	0 43242	0 95013 0 0001	0 P7523 0 0001	0 90585 0 0001	0 05193 9 7974
BSZ	0 1215 <i>t</i> 0 0997	0 03606 0 0503	0 06851 0 7342		0 5) 74 0 0071	0 99956 0 0001	0 99994 0 0001	0 97836 0 0001	0 72333 0 0001	0 99902 9 0001	0 78973 0 0001	0 99712 0 0001	0 31465 0 1223
PRIZE	U 21880 O 2729	0 11668 0 5622	0 05212 0 7963	0 09947 0 6216	0 39459 0 0917	-0 98299 0 0001	0 97213 0 0001	0 9999H 0 0001	0 55975 0 0024	0 98559 0 0001	0 93661 0 0001	0 310 0 0001	0 09491
8184 7	0 19386 U 3326	0 33581 0 0868	0 01861 0 9266	0 37749 0 0522	0 09944 0 6217	0 59039 0 0012	0 06886 0 0001	0 72437	0 15978 0 4260	0 60200 0 0009	0 44249 0 0209	0 50186 0 0077	0 61273
MPSa	0 45435 0 0173	0 08990 0 6556	0 08738 0 6647		0 63010 0 0004	0 93593 0 0001	0 71002 0 0001	0 85848 0 0001	0 90809 0 0001	-0 93074 0 0001	U 98263 O 0001	0 96789 0 0001	0 59693 0 0010
zr 1	U 52043 U 0054	0 17809 0 3741	0 09537 0 6361	0 24645 0 2153	0 67897 0 0001	0 82214 0 0001	0 51740 0 0057	0 70794 0 0001	0 98268 0 0001	0 01382 0 0001	0 90798 0 0001	0 87766 0 0001	-0 77415 0 0001
MSP w	0 47533 0 0122	0 11479 0 5686	0 09011 0 6549	0 17433 0 3045	0 64739 0 0001	0 91017 0 0001	0 66114 0 0002	0 82211 0 0001	0 93416 0 0001	0 90408 0 0001	0 96796 0 0001	0 94882 0 0001	0 64944 0 0002
SOLTN	0 55197 0 0028	0 26362 0 1840	0 05607 0 7812	0 31876 0 1051	0 70125 0 0001	0 62687 0 0005	0 25597 0 1975	0 47962 0 0114	0 99038 0 0001	0 61563 0 0006	0 75072 0 0001	0 70497 0 0001	0 91535 0 0001
SULCA	0 59073 0 0012	0 45051 0 0184	0 03514 0 8618	0 47391 0 0125	0 67100 0 9001	0 41147	0 03161 0 8756	0 25550 0 1982	0 88748 0 0001	0 39928 0 0391	0 55027 0 0030	0 49796 0 0082	0 9217) 0 9011
JULAG	0 58684 0 0013	0 28573 0 1985	0 06871 0 7335	-0 34979 0 0737	0 71647 0 0001	0 65603 0 0002	0 30281 0 1247	0 51711 0 0058	0 97567 0 0001	0 64551 0 0003	0 77092 0 0001	0 72875 0 0001	0 97637
POTV	U 45687 Q 0166	0 12517 0 5339	-0 19391 0 3325		0 63469 0 0004	0 69738 0 0001	0 36512 0 0611	0 56847 0 0020	0 96193 0 0001	0 68772 0 0001	0 80129 0 0001	0 76352 0 0001	0 93032 0 0001
SULVA	0 11486 0 5683	0 00227 0 9910	0 11349 0 5730	0 06387 0 7516	0 10900 0 5884	0 00036 0 9986	0 11320 0 5740	0 04937 0 8068	0 19618 0 3267	0 00434 0 9828	0 04723 0 9150	0 02879 0 8866	0 26515 9 1811
SOLP	0 21127 0 2901	0 25243 0 2040	0 12131 0 5467	0 21058 0 2917	0 20243 0 3112	0 11141 0 5801	0 00220 0 6836	0 10118 0 6156	-0 11213 0 5776	0 11071 0 5825	0 11795 0 5579	0 11581 0 5651	0 07651 0 7045
	S01.5	SOL6	SOL7	SOLe	881	882	803	884	RT	S R	WSP	021814	&IR2S2
01	0 55627 0 0026	0 55983 0 0024	0 55753 0 0025	0 27959 0 1576	0 14315 0 4761	0 33187 0 0060	0 22550 0 2581	0 20612 0 3023	0 47443 0 0124	0 51582 0 0059	-0 45647 0 0167	0 32189 0 1016	0 09017 0 6547
013	0 31921 0 1046	0 30092 0 1212	0 31469 0 1099	0 36248 0 0632	-0 16761 0 4033	0 02736 0 8922	0 11189 0 5784	0 34010 0 0826	0 11368 0 5724	0 17067 0 3947	-0 09234 0 6469	0 03747 0 0528	0 20023 0 3167
013	U 09385 U 6415	0 09573 0 6348	0 09440 0 6395	0 03449 0 8644	0 03983 0 8436	0 07007 0 7284	0 05319 0 7922	0 02085 0 9178	0 09000 0 6553	0 09489 0 6378	0 08767 0 6637	0 06825 0 7352	0 01103

^	202 J	1925 093	91134	03033	16997	16711	91720	82672 0001	86277 5001	10002	04247	12 R9 5414	05325	77495	99533	88847 0001	6827	A5852 0001
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	0 00407	0 0401	94666 0	0 90154 0 0001	0 97912	0 72078 0 0001	0 99918 0 0001	0 96920	0 99663	0 30114	0 47317 0 0127	0 54267	0 49140	-0 42252 0 0281	0 93810	0 99965	0 94050 0 0001	-0 54967 0 0030
2	n 14 145 U 4599	0 63190	0 93361	0 70547	0 85512 0 0001	0 91080	0 92833	0 98140	0 96623 0 0001	0 60214 0 0009	0 74009	0 79189 0 0001	0 75391 0 0001	0 0 1578 0 6 340	-0 76871 0 00001	0 95113	-0 85868 0 0001	-0 23933 0 2292
Is	0 23406 U 2318	0 6762A U 0001	U 834 B	0 53582 0 0040	0 72308	0 97844	0 82627	0 91694 0 0001	0 86784 0 0001	0 76026	0 86778 0 0001	0 90502	0 67793	0 12130 0 5467	0 61255 0 0007	0 86208	0 72794	0 02414
h.	0 1730° 0 3880	1000 U	0 91141	0 66340	0 92392	0 93308 0 0301	0 90537	0 96871	0 94977	0 64715	0 77760	0 82577 0 0001	0 79052 0 0001	0 03826 0 8497	0 73057	0 93176 0 0001	0 82772	0 16297
B ==	0 34119	0 11539	0 0019	0 8570°	0 70802 0 0001	0 18287	0 54312	0 92126 0 0246	0 48145	9 6310A 0 0004	0 47586	0 40339	0 45745	0 98940	0 80499 0 0001	0 52744	0 70312	1 00000 0 0000
FII.)	0 09360	0 40187	0 485 10	0 16899	0 99998	0 57050	0 98767	0 94110	0 96175	0 10787	-0 29082 0 1411	0 3670)	0 31067	0 59240	0 98788 0 0001	0 97497	00000	0 70312
L 11 2	0 00795 0 9585	0 51621 0 005A	0 49962 0 0901	0 86961	0 47341	0 73861	n 99776 0 0001	0 99272	99958 0 0001	0 32619	0 49625	0 56464	0 51421	0 19846	0 92864 0 0001	1 00000	0 97497	0 52744 0 0047
ī	0 16185	0 31011	0 34683	0 44560	0 94891 0 0001	0 43609	0 95119	0 0001	9 30757 0 00001	0 04778	-0 13876 0 4900	0 21816 0 2743	0 15933	0 71029	1 00000	0 92864	0 98789	0 40499 0 0001
910	0 41526 0 0312	0 21242 0 2865	0 64607	0 11110	0 59795	0 32371	0 45896	-0 28508	0 34906 0 0 0 143	0 7370%	0 59854	0 53200	0 58174 0 0015	0 00000	0 71029	0 39646	0 59240	0 98940
100	0 19517	0 65681	0 46947	0 06616	0 10409	0 95789	0 45565 0 0169	0 61378	0 55915	0 97848	0 99978 0 0001	0 99821 0 0001	0 0000	0 58174	0 15933 0 4273	0 51421	0 31067	0 45745
)To	0 14103	0 56809 0 0001	0 52053	0 1 579 0 5319	0 36059 0 0646	0 97337	0 50813 0 0068	0 65994 0 0002	0 60779	0 00001	0 99674 97674	0 00000	0 99821	0 53200	0 21816 0 2743	0 56464	0 16702	6910 0
\$10	1910 0	0 65333	0 44493	U 04539 0 A 21	0 24420 0 1508	U 95171 U 0001	0 43703 J 0226	0 54722 0 0010	0 54178 0 0035	0 98257 0 0001	0 00000	0 99674 0 0001	0 99978 0 0001	0 59954	0 13876	0 49625 0 0085	0 1411	0 0121
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N	0 92185 0 0301	0 04941	0 94760	000000	0 90027	0 98267	0 72312	0 99970	0 99620	0 99521	0 2 14 24 0 293	0 49237	0 55861	0 50957	0 63686	0 93669	0 99999	0 97786 -
7	1 94934	0 9714)	000000 1	0 94266	0 70335	0 86446 0 0001	0 91573 0 0001	n 93414 0 0001	0 98490	0 97077 0 00001	0 \$2797	0 75464	0 80342	0 76753	0 34301 0 079A	-0 76610 0 0001	0 94388	0 85193 0 0001
	0 3872	0 0000 0	0 97643	0 84841	0 03336 0 0042	0 73360	0 98087 0 0001	0 81510	0 92413	80968 O	0 69882	0 87846 0 0001	0 91299	0 68778 0 0001	0 13220 0 5110	0 60934 0 0008	0 85015 0 0001	0 71894 0 0001
<u>-</u>	000000	0 1872,	0 99834	0 12185	0 66121	0 83406	0 93736	0 91202 0 0001	0 97329	0 95532	0 57604	0 79120 0 0001	0 83639	0 80319	0 28631	0 72779 0 0001	0 92328	0 82034
	n 11297 0 3610	0 02414	0 21933 0 2292	0 54967	0 62852	0 69497	0 17095	0 57010	0 40379	0 45538	0 69822	0 45647	0 34584 0 0463	0 43870 0 0221	0 99413	0 80739	0 54659	0 71232 0 0001
8 11 3	0 42777	0 72784	0 85869 0 0101	-0 98052 0 0001	0 96827	1000 0	0 58040 0 0015	0 98505	0 93444	0 95650	0 01811	0 31172 0 1135	0 38473	0 11054	0 77595	0 98724	0 97977 0 00001	1000 0 76666 0
~ III 7	0 93176 0 0 0001	0 R6204 0 0001	0 15113	n 99965 n 0001	n H8847 0 0001	0 97744	0 74692	0 99870 0 0001	0 99023	0 99744	0 23994 0 2290	0 51516 0 0060	0 58030 0 0015	0 53209	0 61629 0 0006	0 92713 0 0001	0 99974 0 0001	0 97199
=	0 0001	0 61255 0 1007	0 76871	0 93810 0 0001	0 93533	0 38604	0 44694	0 94636	0 85782 0 0001	94646 0	0 13733 0 4946	0 16043	0 23676 0 2364	0 16001	0 86447	0 99999	0 93682	0 98981
OF B	0 03426	0 12130	0 09578	0 42252 0 0281	0 77495	0 58318	0 31223 0 1128	0 44474	0 26665	0 33191	0 79478	0 58084 0 0015	0 51573 0 0059	0 56455	0 96767	0 71314	0 41918	0 60284
170	0 79052	0 0001	0 7 391 0 0001	0 49140	0 06325	0 32147	0 95435	0 45978	0 62880	0 57418 0 0017	0 95599	0 00000	0 0001	0 99978	0 35852	0 15533	0 49461	0 29825 0 130A
970	0 12577	0 0001	0 00001	0 54267	0 12289	0 17759 0	0 97052	0 52179	0 67423	0 62217	0 93671	0 99427	0 99982 0 0001	0 9992 6 0 0001	0 30199	0 21419	0 54576	0 15467
ነ ነ	0 7 1760	0 96778	0 0001	0 47317	0 04247 U N334	0 30170	0 0001	0 45131	0 61249	0 55702	0 96189	0 99976	0 99502	0 99913	U 17787 O 0520	0 13474	0 47642	U 27833 O 1598
	•	ر ت	<u>ت</u> ت	O LHIA	\$187°	AIBJSG	PIRe C	0 170	SOLZEJ	201354	50F475	0.6104	Dr 970S	501752	Ps 810s	us 1su	7 CZ 118	Der III

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0 00015	0 91403	0 88810	0 14279	0 57528	0 95089 0 0001	0 71280 0 0001	10 00 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	0 98157 0 0001	0 99977	0 77153	0 41473	0 51473
0 0001	0 0001	0 79236	-0 03525 0 8614	0 72851	0 93066	0 82603	0 18001	00000 0	0 98772	0 99816 0 0001	0 92368	0 65895
0 17222 0 0001	0 98616	0 97642	0 41204	0 34491	0 66657	0 48564	0 27613	0 84934	0 94936	0 86214 0 0001	0 0000	0 25315 0 2026
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90612	0 92932	0 91267	0 28259	0 44692	0 73124 0 0001	0 57383	0 14593	0 90652	0 94749	0 68672	0 71447	0 36 52 0 0631
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09264 Q 6458	0 09863	0 09424	0 00709	0 09017	0 11375 0	0 10164 0 6119	0 9031	0 12113	0 11919 0 5538	0 12107 0 5475	0 11261 0 5760	0 0 6P4S
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	0 74692	0 99870	0 99023	0 99744	0 23994	-0 51516 0 0060	0 58030	0 53209	0 61629	0 92713	0 99974 0 0001	0 97193 0 0091
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Appendix D Correlation matrix of oxalates, nitrate-N concentrations and various soil and climatic variables using data from irrigated plots of amaranth experiments at three sites

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013	U 46623 O 0216 24	1 00000 2 0000	-0 44709 0 0285 24	0 56075 0 0029 24	0 37898 0 0745 23	0 30759 0 1437 24	0 60698 0 0017 24	0 50699 0 0115 29			0 20264 0 3423 24	0 33:17 0 1094 24	0 47493 0 0193	t
013	0 23006 0 2795 24	0 94709 0 9285 24		0 46854 0 0209 24	0 00706 0 9745 23	0 79426 0 0001 24	-0 52931 0 0010 24	0 87908 0 0001 24			-0 71529 0 0001 24	0 80168 0 0001 24	0 19347 0 3645 4	
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1142	0 94259 0 0267 25	0 60648 0 0017 24	-0 62931 0 0010 24		0 55698 0 0031 25	0 54316 0 0034 27	1 00000 0 0000 27	0 92571 0 0001 27	-0 17407 0 3852 27	0 74721 0 0001 27	0 39176 0 0433 27	0 36 39 0 0014 27	0 7911 0 0001 7	t t
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3	U 58944 0 0020 25	0 0387 24	0 09065 0 6716 24	0 33680 0 1075	0 64696 0 300 2	0 21453 0 2712 27	0 59171 0 0001 27	0 17478 7 397 72	0 12 30 0 0001 21	0 04326 0 0119	1 34317 0 0485 27	0 113 4 0 1175	0 00011 7 75
7105	0 00 30 0 00 30 52	0 43356 n 0316 24	0 12017 0 5760 24	0 32476 0 1215 24	0 5173 0 0004 25	0 17984 0 3194 27	0 17809 0 0001 21	0 1451 0 2426 27	0 90175 0 0001 27	0 09847 0 6678 27	0 34545 0 077f 22	0 111 0 7713 0 75	0 994 0 3001
o Fa	U 54083 0 0053 25	0 56548 0 0040 24	0 42758 0 0371 24	0 16935 0 4289 24	0 0005 0 0005 25	0 2 14 0 0 2058 27	0 9491 0 0001 27	0 60614 0 0008 27	0 47522 0 0122 27	0 50000 0 0079 72	0 08220 0 6836 27	0 29687 0 1127	0 94431
- - -	0 01602 0 194 25	0 42987 0 0365	0 64063 0 0001 24	0 34149 0 1024	0 13475 0 5208 25	0 97570 0 00001 27	0 71409 0 0001	0 99453 0 0001 27	0 56506 0 0021 27	0 99692) 0001 27	0 92385 n n001 27	0 99196 0 0001 27	0 14113 0 4825 27
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s 77 78 78 78 78 78 78 78 78 78 78 78 78	0 117 8 0 5757 25	0 31385 0 1353 24	0 79192 0 0001 24	0 41075 0 0462 24	0 01541 0 9417 25	0 99994 0 0001 27	0 55225 0 0028 27	0 92628 0 0001 27	0 72482 0 0001 27	0 96670 0 0001 27	0 98339 0 0001 27	0 99933 0 0001 27	0 06941 0 7339 23
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7	0 1988 0 0001	n 1 385 0 9041 27	0 + 7 > 20 0 001	0 68359 0 0001 27	0 7 304 0 0001 27	0 86403 0 0091 75	0 01089 0 9570 75	\$ 1 Hz	0 01794 0 9320 25	0 43038 0 0358 24	0 84098 0 0001 24	0 34032 0 1037 24	0 1369U 0 5141 25	0 97503 0 0001 72
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-	0 724 J 0 0011 72	n 16766 0 0031 27	0 47359 0 0001 72	0 91461 0 3001 27	0 63316 0 0004 27	0 94148 0 0001 27	0 12281 0 1005 72	801186	0 58875 0 0020 25	0 43297 0 0346 0	0 10711 0 0184 24	0 33014 0 1151 24	0 64973 0 0000 25	0 19745 0 3235 27
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11.3	0 13940 0 4869 27	0 13610 0 3259 27	0 15714 0 4339 27	0 23088 0 1910 27	0 99331 0 00001 27	n 98705 n 2001 27	0 99995 0 0001 27	0 51452 0 0060 27	0 97485 0 0001 27	0 71039 0 0001 27	0 35158 0 0721 27	0 56729 0 0020 27	0 62180 0 0005 27
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	0 0990	0 0001	0 0 00	0 00001	0 0001	0 0001	0 0001	0 0101	0 0042	0 0001	0 0001	0 0001	0 0001
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OLCA	0 44746	0 75999	0 61721	0 74388	0 79087	0 82044	0 80023	0 50855	0 48281	0 75390	0 64337	0 93483	0 87358
	0 0208	0 0001	0 0006	0 0001	0 0001	0 0001	0 0001	0 0068	0 0107	0 0001	0 0003	0 0001	0 0001
	27	27	27	27	27	27	27	27	27	11	27	23	72
11.1	0 1 643	0 72764	0 55215	0 04079	0 98256	0 90812	0 89072	0 61919	0 41145	0 72040	0 59189	0 99343	0 96766
	6 0 0	0 0001	0 0021	0 0001	0 9001	0 0001	0 0001	0 0006	0 0330	0 0001	0 0012	0 0001	0 0001
	27	27	27	27	27	27	72	27	27	11	27	27	27
05454	0 13043	0 54141 0 0036 27	0 34571 0 0773 75	0 94394 0 0001 27	0 97197 0 0001 72	0 98404 0 0001 27	0 97603 0 0001 27	0 79341 0 0001	0 17837 0 3734 72	0 53250 0 0043 27	0 37981 0 0507	0 98663 0 0001 27	-0 72423 0 0001 27
C as	17	0 84304	0 70599	0 73050	0 78499	0 61963	0 79596	0 46673	0 57298	0 83732	0 73142	0 96455	0 94602
	1800 0	0 0001	0 0001	0 0001	0 0401	0 0001	0 0001	0 0141	0 0018	0 0001	0 0001	0 0001	0 0001
	1800 0	27	27	27	72	27	27	27	27	27	27	27	27
9 4 0	0 49905	0 99993	0 97343	0 26006	0 33980	0 39414	0 35660	0 07033	0 91973	0 99975	0 98116	0 67999	0 97459
	0 0001	0 0001	0 0001	0 1902	0 0829	0 0419	0 0679	0 7279	0 0001	0 6001	0 0001	0 0001	0 0001
	27	27	27	27	27	27	27	75	27	27	27	27	27
ያ th n	0 30696 0 1194 27	0 68405 U 0001 27	0 0914 0 0067 27	3 97e56 0 0001 27	0 91370 0 0001 27	0 93587 0 0001 27	0 92093	0 67074 0 0001 27	0 35276 0 0711 27	0 67631 0 0001 27	0 54029 0 0036 27	0 99985 0 0001 27	0 83664 0 0001 27
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10	0 4109 0 0001 7	0 86903 0 0001 27	0 75400 0 0001	0 16646 0 4066 27 0 42481	0 0272 27 0 98858 0 0001	0 54216 0 0035 27	0 94881 0 0001 27	0 71543 0 0001 27	0 63490 0 0004 27	0 77630 0 0001 27	0 81002 0 0001 27	0 78692 0 0001 27
ĭĭ	0 72440 0 0101	0 72304 0 9301 7 5	0 43375 0 0 39 27	3019 126 2 1462	0 466 5 27 0 72936 0 0001	0 24434 0 2193 27	0 52764 0 0047 27	0 19777 0 0 199 72	0 60442 0 0009 27	0 68108 0 0001 27	0 69741 0 0001 27	0 68634 0 0001 27
OLM	0 97780 0 0001 77	0 8835 t 0 0001 7 t	0 71194 0 00001 1	0 21,32 0 908 27 0 17337	0 97681 0 97681 0 0001	0 49280 0 0690 21	0 81255 0 0001 27	0 67158 0 0001 27	0 66707 0 0001 27	0 79925 0 0001 27	0 83022 0 0001 27	0 80904 0 0001 21
4 10	0 9476)	0 H7620 0 0001 21	0 66273 0 0002 27	0 24996 0 2006 27 0 32193	0 04940 0 94340 0 0001	0 44246 0 0208 27	0 76583 0 0001 27	0 62180 0 0005 27	0 67852 0 0001 27	0 8004) 0 0001 27	0 82853 0 00001 27	0 80934 0 0001 27
ī.	0 97683 0 0001 27	0 45385 0 0001	0 61407 0 00007	37.	0 4 5 2 7 2 7 0 9 5 2 2 7 0 0 0 0 0 1	0 16693 0 0597 15	0 73959 0 0001 27	0 56729 0 0020 77	0 79093 0 0001 27	0 89090 0 0001 27	0 91497 0 0001 27	0 89860 0 0001 27
-	0 90149 0 0001	0 99882 0 0001	0 40552 0 0159 27	0 57976 0 001 27 0 00541	0 91423 0 91423 0 0001	0 13043 0 5167 27		0 35158 0 0721 27	0 91125 0 0001 27	0 97612 0 0001 72	0 98693 0 0001 27	0 97978 0 0001 27
6450	0 99989 0 0001 23	0 84731 0 0001 27	0 75019 0 0001 27	0 18653 0 3516 27 0 41268	0 94991 0 00001	0 53255 0 0043 27		0 71039 0 0001 27	0 65600 0 0002 27	0 79620 0 0001 72	0 82944 0 0001 27	0 80669 0 0001 27
6 6 8 9	0 45753 0 0001 27	0 1029 0 0065 27	0 99618 0 0001 27	0 36007 0 0651 27 0 83126	~	0 9990J 0 0001		0 97485 0 0001 27	0 15886 0 1287 27	0 35697 0 0676 27	0 40971 0 0338 27	0 37329 0 0551 27
7	1000 0 1000 0	871179 0 0 00001 12	0 56360 0 0072 12	0 42344 0 0277 27 0 17498	60	U 30696 0 1194 27	691	0 51452 0 0060 27	U 82212 0 0001 27	0 92099 0 0001	0 94173 0 0001 27	0 92768 0 0001 27
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301	U 26492 0 1751 27	0 45122 0 0142 23	0 40153 0 0 179	0 4295A 0 0253 27	0 09331 0 6439 27	0 38690 0 0462 27	0 20079 0 3153	0 01126 0 9555 75	0 11658 0 5675 27	0 27985 0 1574 27	0 27913 0 1505 75	0 0930R 0 6442 27	0 25514 0 1990 27	1 10000 U 00000 Z Z
Y ~ 10	0 44535 0 0199 27	0 57922 0 0916 12	0 41569 0 0001	0 72242 0 0001 27	0 95375 0 0001 27	0 84965 0 0001 27	0 98693 0 0001 27	0 89091 0 0001	0 96741 0 0001 27	0 95996 0 0001 27	0 96468 0 0001 27	0 74604 0 0001	1 00000 0 0000 27	0 25514 0 1990 27
=	0 4 11168 0 9 19 7 2 7	0 27616 0 1632 7	0 433HB J 008B	0 49421 2 0165 72	0 74630 0 0001 23	0 52351 0 0046 27	0 72713 0 0001 27	0 73119 0 0001 27	0 74189 0 0001 27	0 80096 0 0001 27	0 76010 0 0001 27	1 00000 0 0000 27	0 74604 0 0001	U U9374 O 044 27
10	0 486.20 0 0101 27	0 5307b 0 0044 21	0 77712 0 00001 15	0 67487 0 0001 27	0 95637 0 0001 27	-0 81345 0 0001 27	0 97845 0 0001 27	0 90299 0 0001 27	0 96464 0 0001 27	0 98192 0 0001 27	1 00000 0 0000 77	0 76010 0 0001 27	0 98468 0 0001 27	0 1585
OI CA	0 50853 0 0068	0 49011 0 0112 27	0 74731 0 0001	0 62918 0 0004 27	0 91781 0 0001 27	0 76676 0 0001 27	0 944947 0 0001 77	0 89313 0 0001 27	0 94397 0 0001 21	1 00000 0 0000 27	0 98192 0 0001 27	0 80096 0 0001 27	0 95996 0 0001 27	0 27985 0 1574 27
) LTH	0 01919 0 0000 21	0 40469 0 0343	0 69134 0 0001 27	0 57569 0 0017 27	0 99471 0 0001 27	0 73570 0 0001 75	0 97987 0 0001 27	0 96676 0 0001 27	1 00000 0 0000 27	0 94397 0 0001 27	0 96464 0 0001 27	0 74189 0 0001 27	0 96741 0 0001 27	0 11659 0 5625 27
5 C	0 79341 0 0001 7	0 17538 0 3916 7	0 41715 0 00H3 77	0 36119 0 0642 27	0 98363 0 0001 27	0 55138 0 0029 27	0 90863 0 0001 27	1 00000 0 0000 27	0 96676 0 0001 27	0 89319 0 0001	0 90299 0 0001 27	0 73119 0 0001 27	0 89091	0 01126 0 9555 27
C Sur	0 4(67) 0 7141 27	0 57048 0 0019 75	0 81406 0 0001 27	0 71760 0 0001 27	0 96901 0 0001 27	0 84939 0 0001 27	1 00000 0 0000 27	0 90863 0 0001 27	0 91987 0 0001 27	0 94887 0 0001 27	0 97845 0 0001 27	0 12711 0 0001 75	0 98893	0 20079 0 3153
9880	0 07033 0 7274 27	0 91802 0 0001 27	74747 0 00001 72	0 97709 0 0001 27	0 69269 0 0001 27	1 00000 0 0000 27	0 94939 0 0001 27	0 55138 0 0029 27	0 13570 0 0001 72	0 76676 0 0001 27	0 81345 0 0001 27	0 52851 0 0046 27	0 94965 0 0001 27	0 186 10 0 0 0 6 2 2 7
9 22 6	0 0001 27	0 0736 10 0736	0 64536 0 0003 27	0 52331 0 0051 27	1 00000 0 00000 27	0 69269 0 0001 27	0 95901 0 0001 27	0 98363 0 0001 27	0 0001	0 93781 0 0001 27	0 95637 0 00001 27	0 74630 0 0001 27	0 95175 0 00001 72	0 0 1311 0 04 30 14
	Le alor	Dr. Ha	7 7 18	OSF AS	als 4Su	? *a	70,40	SP L	SOLFE	SULCA	ULBG	0.LK	SOLWA	

Appendix E Oxalate content of cassava leaves from plants grown in irrigated plots

	Oxalate	(cmol kg ^{-l} dry wei	ght)	
Site	Total	Insoluble	Soluble*	
Molokan '	32 6 19 3 28 9 21 6	25 1 13 3 21 2 19 1	7 5 6 0 7 4 2 5	
Waipio " "	34 3 29 8 23 3 24 4	38 4 32 0 24 7 22 5	- 1 9 1 9	
Iole " " "	35 9 18 9 19 9 23 1 21 8 18 2	32 8 13 2 17 2 21 0 18 5 12 6	2 1 5 7 2 7 2 1 3 3 5 6	
Kuka 1au '' '' '' ''	25 9 17 8 26 1 15 6 18 3 16 1	28 0 14 3 20 0 26 4 19 7 23 0	3 5 6 1	

^{*}Calculated from the difference between total and insoluble oxalate

Appendix F Correlation matrix of various plant compositions and soil and climatic factors in taro leaves from three experimental sites

	013	OI 3	01573	C_A	TOTALS	CA	ЭR	ĸ	WA	sc	₽	s	#03
011	1 00000	0 34731	0 42104	0 19704	0 09385	0 78908	0 28956	0 69308	-0 56753	0 25365	0 75798	0 27868	0 14715
	0 0000	0 0303	0 0076	0 2292	0 5699	0 0001	0 0738	0 0001	0 0002	0 1192	0 0001	0 0858	0 3713
	39	39	39	39	39	39	39	39	19	19	39	39	39
013	0 34731	1 00000	0 70435	0 38540	0 10987	0 43819	0 12856	0 18969	0 35255	0 40520	0 10937	0 17983	0 07447
	0 0303	0 0000	0 0001	0 0154	0 5055	0 0053	0 4354	0 2479	0 0277	0 0105	0 2482	0 2733	0 6505
	39	39	39	39	39	39	39	39	39	39	39	39	39
017 3	0 42104	0 70435	1 00000	0 22362	0 17731	0 17272	0 34359	0 34117	0 08861	0 19992	0 39061	0 03701 -	0 18382
	0 0076	0 0001	0 0000	0 1712	0 2802	0 2930	0 0323	0 0335	0 5917	0 2224	0 0139	0 8230	0 2626
	39	39	39	39	39	39	39	39	39	19	39	39	19
. .	0 19704	0 38540	0 22362	1 00000	0 19320	0 53635	0 63420	0 1651B	0 45080	0 96785	0 01419	0 15206 -	-0 03377
	0 2292	0 0154	0 1712	0 0000	0 2323	0 0004	0 0001	0 3084	0 0035	0 0001	0 9107	0 3489	0 8361
	39	39	39	40	40	40	40	40	40	40	40	40	40
TOTALS	y 09385	0 10987	0 17731	0 19320	1 00000	0 01608	0 16829	0 33655	0 20624	0 06914	0 01902	0 04382	0 17915
	g 5699	0 5055	0 2802	0 2323	0 0000	0 9216	0 2993	0 0337	0 2017	0 6716	0 9121	0 9813	0 2687
	39	39	39	40	40	40	40	40	40	40	40	90	40
CA	0 78808	0 43819	0 17272	0 53635	0 01608	1 00000	0 04170	0 66308	0 62699	0 61434	0 68465	0 51313	0 09643
	0 0001	0 0053	0 2930	0 0004	0 9216	0 0000	0 7983	0 0001	0 0001	0 0001	0 0001	0 0007	0 5539
	39	39	39	40	90	40	40	40	90	40	40	4u	41
ĦĠ	0 28956	0 12856	0 34354	0 63420	0 16829	0 04170	1 00000	0 30344	0 12293	0 67199	0 45701	0 16383	0 15108
	0 013H	0 4354	0 0323	0 0001	0 2991	0 7983	0 0000	0 0570	0 4198	0 0001	0 0030	0 3124	0 3521
	39	39	39	40	40	40	40	40	40	40	40	40	4)
	0 6930a	0 18969	0 34117	0 16510	0 33655	0 66 108	0 30344	1 00000	0 23197	0 0410A	0 73651	0 51634	0 0093
	0 0001	0 2474	0 0335	0 3084	0 0337	0 0001	0 057J	0000	0 1499	0 8013	0 0001	0 0007	0 2117
	39	39	19	90	47	40	40	40	40	40	40	40	11

<u>3</u>	0 32475 0 0412 40	0 07021 0 6669 40	0 02946 0 8569 0	-0 37114 0 0184 40	000000	0 28944 0 0701 40	0 14662 0 3666 40	0 16145 0 3196 40	-0 15800 0 3302 40	-0 16450 0 3104	0 14230 0 3811	0 15079 0 3532	0 16324 0 3142
	0 02940 0 8571	0 32319 0 0419 40	0 42328 0 0065 40	00000 0	0 37116 0 0184 40	0 0001	0 68808	0 10528 0 5179 40	0 00747 0 9635 0 40	-0 07869 0 6293	-0 04265 0 7938 40	0 14140 0 3841	0 09340 0 5665 40
	0 4038/ 0 0094 40	0 03453 0 8325 40	1 00000 0 0000 40	0 42328 0 0065 40	0 02946 0 8568 40	0 25245 0 1161 90	-0 19239 0 2343 40	0 64363	0 33282 0 0359	0 56695	0 14626 0 3678	-0 73490 0 0001	0 61021 0 0001 40
<u>ر</u> ۲	0 43290 0 0053 40	1 00000 0 0000 40	0 03453 0 8325 40	0 32319 0 0419	0 07021 0 6668 40	0 17465	0 18630 0 2497	-0 09614 0 5551	0 14583 0 3659	0 11373	0 15949 0 3256	0 06597	0 1044 I 0 5214
*	1 00000 0 0000 42	0 43290 0 0053 40	01 9600 0 9600 0	0 02940 0 8571	0 32426 0 0412 90	0 06256 0 7014 40	-0 04361 0 7893	0 52391 0 0009 42	0 44164 0 0039 42	-0 51193 0 0005	0 36476 0 0175 42	0 52172 0 0004 42	0 51973 0 0004 42
¥	0 23197	0 04108 0 6013	0 73651 0 0001 40	-0 51634 0 0007 40	0 20098 0 2137	0 54791 0 0003 40	0 48221 0 0016 40	0 29649 0 0614	0 04530 0 7613 40	0 20323 0 2085 40	0 21514 0 1825 40	0 43086	0 25573 0 1112 40
E	0 12243 8644 0	0 67199 0 0001 90	0 45701 0 0030 40	0 16383 0 3124 40	-0 15108 0 3521	0 15465 0 3407 40	0 18838 0 2444 40	0 41734 0 0018 40	0 24726 0 1240 40	0 42060 0 0069 40	-0 10907 0 5029	0 0003	-0 45261 0 0034 40
5	0 62699 0 0001	0 61434	-0 68466 0 0001	0 51313 0 0007 40	0 09643 0 5539	0 38444 0 0143 40	0 34192 0 0308 40	-0 52379 0 0005 40	0 26240 0 1019 40	-0 45887 0 0029 40	0 10704 0 5109	0 60189 0 0001	0 49545 0 0012
LIATCT	0 20624 0 2017	0 06914 0 6716 40	0 01802 0 9121 40	0 00382 0 9813 40	0 17915 0 2687 40	-0 50879 0 0008	0 48035	0 62805	0 82693 0 0001	0 70339 0 0001 40	0 85443 0 0001	0 49064 0 0013 40	0 66401
T-0	0 45080	0 96785 0 0001	0 9307	0 15206 0 3489 40	0 03377 0 8361 40	0 07487 0 6461	0 06662 0 6821	0 15862 0 3277 40	-0 25199 0 1167 40	-0 19070 0 2385 40	0 27684 0 0837	0 5201	0 17377 0 2836
012_J	0 08861 0 5917 39	0 19992 0 2224 39	0 39061 0 0139 39	0 03701 0 8230 39	0 18382 0 2626 39	0 08200 0 6197 39	0 07839 0 6352 39	0 17216 0 2946 39	-0 05869 0 7227 39	0 10820 0 5121 39	0 17221 0 2945 39	0 26131 0 1081 39	0 14345 0 3836 39
OX 3	0 35255 0 0277 39	0 40520 0 0105 39	-0 18937 0 2462 39	0 17983 0 2733 39	0 07487 0 6505 39	0 12287 0 4562 39	0 10590 0 5211 39	-0.29020 0.0731	0 27336 0 0922 39	-0 29260 0 0707 39	0 23960 0 1418 39	0 27589 0 0691 39	0 29201 0 0713 39
012	U 56753 Q 0002 39	0 25165 0 1192 19	0 75798 0 0001 39	0 27668 0 0858 39	0 14715 0 3713 39	0 26512 0 1026 39	0 23888 0 1430 39	0 54625 0 0001 39	0 27176 0 0942 39	0 51682 0 0008 39	0 07667 0 6340 98	0 69774 0 00001 39	0 56263
	1	Ŋ	<u>م</u>	or.	E 0 3	ಕ	₹5	4	A 1 88 1 B	AIBATE	M 4 6 8	8 H 8 1 D	## ## ##

013 012,3	0 1448 0 2817 0 39 39	-0 27748 0 03992 -0 0 0872 0 8140 0 39 39	0 25771 0 11877 0 0 1132 0 4714 0 39 39	0 26977 0 28397 -0 0 0967 0 0798 0 39	-0 27505 0 26472 -0 0 0901 0 1034 0 39	-0 27233 0 27500 -0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	0 26910 0 28620 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	0 28813 0 19270 -0 0 0753 0 2399 0 39 39	0 29251 -0 12673 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	0 05770 0 47560 0 0 7272 0 0022 0 39 39	0 26255 0 30618 -0 0 1064 0 0580 0 39	0 27093 0 27996 -0 0 0953 0 0843 0 19 39	0 28082 0 23914 0 0 0831 0 1426 0 39 19
C_A TUTALN	27753 0 85451 0 0829 0 0001 40 40	24626 0 81742 1 1256 0 0001 40 40	26690 0 84740 0959 0 0001 40 40	08679 0 44829 1 5859 0 0037 40 40	10240 0 48449 1 5295 0 0015 40 40	09523 0 46549 5589 0 0025 40 40	08716 0 44394 1 5928 0 0041 40 40	14745 0 60004 3 3639 0 0001 40 40	18199 0 68332 2611 0 0001 40 40	18049 0 36959 2651 0 0189 40 40	07209 -0 40319 6584 0 0099 40 40	09168 0 45602 5737 0 0031 40 40	11939 0 52888 4631 0 0005 40
•	0 10037 0 5377	0 28759 0 0 0720 40	0 18255 0 0 2596	0 61880 0 0 0001	0 60452 0	0 61227 0 0 0001 40	0 62038 -0 0 0001 40	-0 54319 0 0 0003	0 47834 0 0 0018	0 22300 0	0 63388	0 61592 0	0 58406
F	0 10312 0 0 5266 (0 26958 0 0 0925 (0 17633 0 0 2764 (0 55925 0 0 0002 (0 54709 0	0 55370 0 0 0002 (0 56059 -0 0 0002 40	0 49420 0	0 43765 -0	0 49291 0 0 0012 (0 57200 0 0 6661 1	0 55681 0 0 0002 (0 52954 0 0 0004 0
¥	22201 0 36 0 1686 0 0 40	01585 0 4520 0 9227 0 00.	13501 0 404 0 4062 0 000	46432 -0 5166 0 0025 0 000 40 4	43591 0 52 0 0049 0 0	45108 0 51 0 0035 0 0	46759 0 515 0 0024 0 00 40	32904 0 52 0 0382 0 0 40	23084 0 51 0 1518 0 0	71402 -0 1 0 0001 0	49694 0 5 0 0011 0	45842 0 51 0 0029 0 0	39804 -0 52 0 0110 0 0
KA C	113 0 15983 188 0 3246 42 40	260 0 14391 326 0 3760 42 4 9	101 0 15456 180 0 3410 42 40	560 0 05701 005 0 7268 42 80	2109 D 06466 0004 D 6918 42 RO	1889 0 06063 1004 0 7101	197 0 05610 305 0 7310 42 40	558 0 08982 00% 0 5815 42 %0	638 0 10994 005 0 5034 42 40	7423 U 09768 2698 N 5487 42 40	0928 -0 04761 0006 0 7705 42 40	1766 0 05864 1004 0 7193 42 40	473 0 07418 004 0 6492 42 40
2	0 13822 0 1950 00	0 36297 0 0213 40	0 2370h 0 1406 40	0 75441	0 73795 0 0001 40	0 74689 0 0001	-0 75622 0 0001 40	0 66642 0 0001 40	0 59000 0 0001	0 66580 0 0001 40	0 77166 0 0001	0 75110 0 0001 40	0 0001
	0 08471 0 7841	0 01504 9 9218 10	0 01835 - 0 9081 40	-0 1.03J 0 3545	0 14275 0 3796 40	0 14680 0 36£0 40	0 15120 0 J517 90	0 11371 0 4648 40	0 096 44 0 5953 0 40	0 20425 - 0 2061 40	0 15895 - 0 3273 40	0 14876 0 3596 40	-0 13254 0 4149 40
NOT	0 14146 0 1819 40	0 1 979 0 3247 90	0 15089 0 3577	0 14652 0 3670 40	0 15015 0 1551 40	0 14828 0 3612 40	0 14607 0 3685	0 15971 0 1249 40	0 16397 0 3120 40	0 01576 0 9231	0 14166 0 3832 40	0 14732 0 3643 40	0 15422 0 3420

(O)	0 04577 0 7792 40	1 1 E S	0 70116 0 0001	0 27505 0 0901 19	0 26472 0 1034 39	-0 10243 0 5295 40	0 48449 0 0015	0 60452 0 0001	0 54709 0 0003	0 43591	-0 52109 0 0004 42	-0 06466 0 6918	0 73795 0 00001	0 14275 0 1796 40
	0 249 0 0 1945	SUPA	0 71994 0 0001 39	0 26977 0 0967 39	0 28397 0 0798 39	0 08879 0 5859 40	0 44929 0 0037	0 61880 0 0001	0 55925 0 0002 40	0 46432 0 0025 40	-0 51660 0 0005 42	0 05701 0 7268 40	0 75441 0 0001	0 15033 0 3545 40
-	0 740A9 0 0001 0	TSAV	0 17239 0 2940 39	0 25771 0 1132 39	0 11877 0 4719	-0 26690 0 0959 40	0 84740 0 0001	0 18255 0 2596	0 17633 0 2769 90	-0 13501 0 4062 90	-0 40401 0 0060	-0 15456 0 3410	0 23706 0 1408 40	0 01885 0 9081
•	0 07191 0 6592 40	TSATH	0 30314 0 0607 39	-0 27748 0 0872	0 03892 0 8140 39	0 24626 0 1256 40	0 81742 0 0001 90	0 28759 0 0720 40	0 26958 0 0925 90	0 01585 0 9227 40	-0 45260 0 0026 42	-0 14381 0 3760	0 36297 0 0213	-0 01604 0 9218 40
4 >	0 26502 0 0899	TSHAT	0 070+1 0 6702 39	0 23786 0 1448 39	-0 17677 0 2817 39	-0 27753 0 0629	0 85451 0 0001	0 10037 0 5377	0 10312 0 5266	0 22201 0 1686	0 36113	0 15983	0 13822 0 3950 40	0 7841
¥	0 71892 0 0001	BHATE	0 56263 0 0002 39	0 23201 0 0713 39	-0 14345 · 0 3636 39	0 17377	0 66401	0 49545 0 0012	0 45261 0 0034 40	-0 25573 0 1112 60	0 51973 0 0004 42	0 10441	-0 61021 0 0001	0 09340 0 5665 0 5665
ř	0 54067 0 0002	71 ER G	0 69774 0 0001 9	0 27589 0 0891 39	n 26131 0 1081 39	0 10474	0 19064 0 0013	0 60169 0 0001	0 54464 0 0003 40	0 41086	0 52172 0 000%	0 06597 0 6859	0 73490	0 14140 0 3841
4 0	0 0001 0 0001	RITAX	0 07867 0 6340 39	0 23960 0 1418 39	0 17221 0 2945 39	0 27684 0 0837 40	0 85443 0 0001 \$0	0 10704 0 5109	0 10907 0 \$029 40	0 21514 0 1825 40	0 36476 0 0175	0 15949 0 3256 40	-0 14626 0 3679 40	0 04265
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