

GROWTH, ANATOMY, QUALITY AND YIELD OF WILD GINGER
(*SIPHONCHILUS AETHIOPICUS*) IN RESPONSE TO NITROGEN
NUTRITION, FERTIGATION FREQUENCY AND GROWING
MEDIUM

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DECLARATION

I hereby declare that the work herein submitted as a dissertation for the Masters of Science in Agriculture (Agronomy) degree is the results of my own investigation. Work by other authors that served as sources of information have duly been acknowledged by references to the authors.

Thank you Prof. P.J. Robertse for making suggestions and providing me with valuable guidance throughout the entire period of this research.

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11/10/2004

Date

I would like to thank the University of Pretoria with Personal Development Programme (PDP) bursary and National Research Foundation (NRF) for financial assistance and the Council for Scientific and Industrial Research (CSIR) for providing with planting material, and provision of chemical compounds as well as the Department of Pharmacy and Pharmacology, University of the Witwatersrand for hydrodistillation of essential oil, without them this work would not have been accomplished.

I am also indebted to my parents as well as my brothers and sisters.

Without limit I would like to thank my wife, Mrs N.E Baloyi for being present and giving support throughout my studies.

Finally, I would like to say thanks to my colleagues, Miss K.W. Mpati & Mr M.R. Maseyhe and also technicians from the University of Pretoria's Hatfield Experimental Farm for always being there for me when I needed help. I gratefully acknowledge Prof. van Zyl and Dr van der Linde for assisting with statistical data analysis using a Statistical Analysis System Program (SAS).

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TABLE DEDICATION

To our Father who art in heaven and His only Son who saves our lives, You are my
 reason for living, thank You for immeasurable knowledge,
 understanding and wisdom that You gave me.

DECLARATION	iii
ACKNOWLEDGEMENTS	iv
DEDICATION	iii
LIST OF TABLES	viii
LIST OF FIGURES	ix
ABSTRACT	xii
GENERAL INTRODUCTION	1
CHAPTER	
1 LITERATURE REVIEW	1
1.1 Introduction	3
1.1.1 Effect of nitrogen on potato plant growth and yield	5
1.1.2 Nitrogen use efficiency	8
1.1.3 Nitrogen fertilizer application and crop production	9
1.1.4 Response of nitrogen application in crop production	10
1.1.4.1 Yield and rate of uptake	11
1.1.4.2 Effect of nitrogen fertilizer application rates	11
1.1.5 Crop nitrogen demand	12
1.1.6 Nitrogen nutrition and crop phenology	14
1.1.7 Soil and plant nitrogen dynamics for optimum crop yields	15
1.1.7.1 Soil N supply	15
1.1.7.2 Crop N uptake	16
1.1.8 Nitrogen availability for optimum crop production	17
1.1.8.1 Factors affecting nitrogen availability	18
1.1.8.1.1 Nitrogen losses in farming systems	18
1.1.9 Environmental factors affecting nitrogen availability	19
1.1.9.1 Temperature	19

TABLE OF CONTENTS

		PAGE
	DECLARATION	i
	ACKNOWLEDGEMENTS	ii
	DEDICATION	iii
	LIST OF TABLES	viii
	LIST OF FIGURES	ix
	ABSTRACT	xii
	 GENERAL INTRODUCTION	 1
CHAPTER		
1	LITERATURE REVIEW	3
1.1	Introduction	3
1.1.1	Effect of nitrogen on potato plant growth and yield	5
1.1.2	Nitrogen use efficiency	8
1.1.3	Nitrogen fertilizer application and crop production	9
1.1.4	Response of nitrogen application in crop production	10
1.1.4.1	Time and rate of uptake	11
1.1.4.2	Effect of nitrogen fertilizer application rates	11
1.1.5	Crop nitrogen demand	12
1.1.6	Nitrogen nutrition and crop phenology	14
1.1.7	Soil and plant nitrogen dynamics for optimum crop yields	15
1.1.7.1	Soil N supply	15
1.1.7.2	Crop N uptake	16
1.1.8	Nitrogen availability for optimum crop production	17
1.1.8.1	Factors affecting nitrogen availability	18
1.1.8.1.1	Nitrogen losses in farming systems	18
1.1.9	Environmental factors affecting nitrogen availability	19
1.1.9.1	Temperature	19

1.1.9.2	Precipitation	19
1.2	Effect of nitrogen on potato quality	20
1.2.1	Factors influencing quality on potato	20
1.2.1.1	Nitrogen source	20
1.2.1.2	Environmental factors	21
1.2.1.3	Plant genotype and age	22
1.3	Effect of drip fertigation on potato growth, quality and yield	22
2	EFFECT OF NITROGEN ON THE GROWTH AND YIELD OF WILD GINGER (Field experiment)	25
2.1	Introduction	25
2.2	Materials and Methods	26
2.3	Results and Discussion	27
2.3.1	Plant emergence	27
2.3.2	Yield	28
2.3.3	Soil analysis	33
2.4	Conclusions	36
2.5	Summary	36
3	EFFECT OF FERTIGATION FREQUENCY AND GROWING MEDIUM ON GROWTH, OIL QUALITY AND YIELD OF WILD GINGER	38
3.1	Introduction	38
3.2	Materials and Methods	39
3.3	Results and Discussion	42
3.3.1	Growth analysis	42
3.3.2	Yield	49
3.3.3	Leaf analysis	54
3.3.4	Growing media analysis	59

	3.4 Conclusions	60
	3.5 Summary	60
		PAGE
4	ANATOMY OF WILD GINGER ENLARGED ROOT IN RESPONSE TO NITROGEN, FERTIGATION FREQUENCY AND GROWING MEDIUM	63
3.1	Nutritional elements which were fertigated on wild ginger during	
	4.1 Introduction	63
	4.2 Materials and Methods	63
	4.3 Results	66
	4.4 Discussion and Conclusions	81
	4.5 Summary	82
	GENERAL DISCUSSION AND CONCLUSIONS	84
	GENERAL SUMMARY	87
	REFERENCES	90
	APPENDICES	102
3.5	Fresh and dry rhizome mass, number of rhizomes, fresh and dry rhizome mass, number of enlarged roots and the length of enlarged root as affected by growing medium at 112 and 224 DAE during 2002/03 seasons	
3.6	Wild ginger fresh rhizome and enlarged root oil yield as affected by fertigation frequency at 224 DAE	
3.7	Wild ginger fresh rhizome and enlarged root oil yield as influenced by growing medium at 224 DAE	
3.8	Leaf and rhizome analysis as affected by fertigation frequency at 112 and 224 DAE during 2002/2003 seasons	57

LIST OF TABLES

TABLE

PAGE

2.1	Effect of N fertilizer applications on the growth of wild ginger	29
3.1	Nutritional elements which were fertigated on wild ginger during 2002/2003 seasons	40
3.2	Wild ginger plant height, number of leaves and stems at 56, 112, 168 and 224 days after emergence (DAE), fresh and dry leaf mass and leaf area at 112 and 224 DAE as affected by fertigation frequency	44
3.3	Wild ginger plant height, number of leaves and stems at 56, 112, 168 and 224 DAE and fresh and dry leaf mass and leaf area at 112 and 224 DAE as affected by growing medium	45
3.4	Fresh and dry rhizome and enlarged root characteristics as affected by fertigation frequency at 112 and 224 DAE during 2002/03 seasons	51
3.5	Fresh and dry rhizome mass, number of rhizomes, fresh and dry enlarged root mass, number of enlarged roots and the length of enlarged root as affected by growing medium at 112 and 224 DAE during 2002/03 seasons	52
3.6	Wild ginger fresh rhizome and enlarged root oil yield as affected by fertigation frequency at 224 DAE	55
3.7	Wild ginger fresh rhizome and enlarged root oil yield as influenced by growing medium at 224 DAE	56
3.8	Leaf and rhizome analysis as affected by fertigation frequency at 112 and 224 DAE during 2002/2003 seasons	57

3.9	Leaf and rhizome analysis as affected by growing medium at 112 and 224 DAE during 2002/2003 seasons	58
-----	---	----

FIGURES PAGE

3.10	pH and electricity of conductivity analysis of pine bark s affected by fertigation frequency at 224 DAE during 2001/02 growing seasons	62
------	--	----

4.1	Anatomical structures of wild ginger enlarged root as affected by N nutrition during 2001/2002 seasons	60
-----	--	----

4.2	Anatomical structures of wild ginger enlarged root grown in pine bark as affected by fertigation frequency during 2002/2003 seasons	74
-----	---	----

4.3	Anatomical structures of wild ginger enlarged root grown in sand as affected by fertigation frequency during 2002/2003 seasons	78
-----	--	----

2.3	Length of enlarged roots as affected by six different N levels during 2001/02 seasons	30
-----	---	----

2.4	Regression between the number of enlarged roots and six N levels during 2001/02 seasons	33
-----	---	----

2.7	pH content in the soil as affected by six N levels	34
-----	--	----

2.8	Potassium content in the soil as affected by six levels of N	35
-----	--	----

2.9	Soil pH as affected by the application of six N levels	37
-----	--	----

3.1	Plant height as affected by fertigation frequency and growing media at 168 DAE	47
-----	--	----

3.2	Fresh leaf mass as affected by fertigation frequency and growing medium at 112 DAE	47
-----	--	----

LIST OF FIGURES

FIGURE	PAGE
2.1 Relationship between fresh rhizome mass and six N levels during 2001/02 growing seasons	30
2.2 Fresh rhizome circumference as affected by six nitrogen levels during 2001/02 seasons	30
2.3 Number of rhizomes as affected by six N levels during 2001/02 seasons	31
2.4 Relationship between fresh enlarged roots and six levels of nitrogen during 2001/02 seasons	32
2.5 Length of enlarged roots as affected by six different N levels during 2001/02 seasons	32
2.6 Regression between the number of enlarged roots and six N levels during 2001/02 seasons	33
2.7 P content in the soil as affected by six N levels	34
2.8 Potassium content in the soil as affected by six levels of N	35
2.9 Soil pH as affected by the application of six N levels	36
3.1 Plant height as affected by fertigation frequency and growing medium at 168 DAE	43
3.2 Fresh leaf mass as affected by fertigation frequency and growing medium at 112 DAE	47

3.3	Relationship between dry leaf mass, fertigation frequency and growing medium at 112 DAE	48
3.4	Leaf area as influenced by fertigation frequency and growing medium at 112 DAE	49
3.5	Number of enlarged roots as influenced by fertigation frequency and growing medium at 112 DAE	54
4.1	Cross section of wild ginger enlarged root that received no nitrogen	67
4.2	Cross section of wild ginger enlarged root fertilized with 50 kg ha ⁻¹ N	67
4.3	Cross section of wild ginger enlarged root fertilized with 100 kg ha ⁻¹ N	69
4.4	Cross section of wild ginger enlarged root fertilized with 150 kg·ha ⁻¹ N	70
4.5	Cross section of wild ginger enlarged root fertilized with 200 kg ha ⁻¹ N	71
4.6	Cross section of wild ginger enlarged root fertilized with 250 kg ha ⁻¹ N	71
4.7	Cross section of wild ginger enlarged root fertigated with 0.25L/day and grown in pine bark	72
4.8	Cross section of wild ginger enlarged root fertigated with 1L/day and grown in pine bark	73
4.9	Cross section of wild ginger enlarged root fertigated with 2L/day and grown in pine bark	75
4.10	Cross section of wild ginger enlarged root fertigated with 2L/2 nd day grown in pine bark	75

4.11	Cross section of wild ginger enlarged root fertigated with 2L/week and grown in pine bark	76
4.12	Cross section of wild ginger enlarged root fertigated with 0.25L/day every day and grown in sand	77
4.13	Cross section of wild ginger enlarged root fertigated with 1L/day and grown in sand	77
Abstract		
4.14	Cross section of wild ginger enlarged root fertigated with 2L/day and grown in sand	79
4.15	Cross section of wild ginger enlarged root fertigated with 2L/2 nd day and grown in sand	80
4.16	Cross section of wild ginger enlarged root fertigated with 2L/week and grown in sand	81

The response of wild ginger growth and yield to N nutrition was conducted. Treatments used were six levels of nitrogen (0, 50, 100, 150, 200 and 250). All N treatments were applied at planting in the form of limestone ammonium sulfate. Measurements of plant emergence, plant height, fresh rhizome and enlarged root mass, fresh rhizome circumference, length of enlarged root and the number of rhizomes and enlarged roots. There was a positive linear relationship in all yield parameters to nitrogen applied except for the number of rhizomes which had showed no relationship. This study revealed that N nutrition increased growth and yield of wild ginger.

The response of wild ginger growth, rhizomes and enlarged root yield as well as oil yield to fertigation frequency and growing medium were investigated in a mixed factorial experiment. Five fertigation frequencies (0.25L/day, 1L/day, 2L/day, 2L/2nd day and 2L/week) and two growing media (pine bark and sand). Measurements were made of plant height, number of leaves and stems at 56, 112, 168 and 224 days after emergence (DAE) and fresh and dry leaf mass and leaf area at 112 and 224 DAE. Yield was determined at 112 and 224 DAE. Fresh rhizomes and enlarged roots were hydrodistilled for essential oil at 224 DAE. During the initial sampling date (56 DAE), all fertigation frequencies improved wild ginger growth except a

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by

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Co-supervisor: Dr E.S. du Toit

Abstract

Wild ginger is an herbaceous perennial medicinal plant used for coughs, colds and flu as well as to treat malaria and also used for some other traditional and cultural practices. Due to its medicinal, wild ginger has become extinct from the wild due to over-harvesting. To improve its conservation, studies on wild ginger growth, rhizome and enlarged root yield, anatomy and oil yield were conducted in either an open field, tunnel or a laboratory.

The response of wild ginger growth and yield to N nutrition was conducted in the field. Treatments used were six levels of nitrogen (0, 50, 100, 150, 200 and 250). All N treatments were applied at planting in the form of limestone ammonium nitrate. Measurements were made of plant emergence, plant height, fresh rhizome and enlarged root mass, fresh rhizome circumference, length of enlarged root and the number of rhizomes and enlarged roots. There was a positive linear relationship in all yield parameters to nitrogen applied except for the number of rhizomes which had showed no relationship. This study revealed that N nutrition increased growth and yield of wild ginger.

The response of wild ginger growth, rhizomes and enlarged root yield as well as oil yield to fertigation frequency and growing medium were investigated in a tunnel. Treatments used were five fertigation frequencies (0.25L/day, 1L/day, 2L/day, 2L/2nd day and 2L/week) and two growing media (pine bark and sand). Measurements were made of plant height, number of leaves and stems at 56, 112, 168 and 224 days after emergence (DAE) and fresh and dry leaf mass and leaf area at 112 and 224 DAE. Yield was determined at 112 and 224 DAE. Fresh rhizomes and enlarged roots were hydrodistilled for essential oil at 224 DAE. During the initial sampling date (56 DAE), all fertigation frequencies improved wild ginger growth except a

fertigation frequency of 2L/day. However, during later sampling dates (112, 168 and 224 DAE), all fertigation frequencies were ideal except 2L/week which was inadequate to sustain wild ginger growth and development. Plants grown in pine bark had increased growth at initial growth stages (56 and 112 DAE), but at later growth stages (168 and 224 DAE), plants grown in sand had increased growth. Fertigation frequency and growing medium did not affect fresh rhizome and enlarged root oil yield.

An experiment was conducted in a laboratory to determine the effect of nitrogen nutrition as well as fertigation frequency and growing medium on the enlarged root anatomy of wild ginger. For the nitrogen study, wild ginger plants were grown in pine bark under a glasshouse with either 0, 50, 100, 150, 200 or 250 kg·ha⁻¹ N. Enlarged roots from the N study as well as the enlarged roots from the fertigation frequency and growing medium study (previously described) were harvested at 224 DAE for sectioning.

Anatomical structures observed were glandular cells, number of primary xylems and cells between them, size of pith, cortex, endodermis, pericycle layer and presence of starch grains. Glandular cells increased from two where no nitrogen was applied to eight where 250 kg·ha⁻¹ N was applied. This study demonstrated N nutrition for wild ginger is important for increasing glandular cells that are important for essential oil production. For plants grown in pine bark, glandular cells increased from one where where plants received 0.25L/day to three where plants received 1L/day. For plants grown in sand, there were no glandular where plants received the highest fertigation frequency (2L/day) and increased to sixteen where plants received the lowest fertigation frequency (2L/week). More glandular cells were, therefore, produced in plants grown in sand with the least fertigation frequency (2L/week).

Keywords: N nutrition, fertigation frequency, growing medium, growth, yield, anatomy, oil yield

GENERAL INTRODUCTION

Wild ginger (*Siphonochilus aethiopicus*) is a rhizomatous perennial plant and belongs to the Zingiberaceae family (Holzapfel, Marais, Wessels & van Wyk, 2002). It has been an important tropical horticultural plant valued all over Africa for its medicinal properties and in South Africa is known as the Natal ginger, *sherungulu* and *indungulu* (Van Wyk, van Oudshoorn & Gericke, 2000). Smith, Crouch & Condry (1997) reported it to occur in Limpopo Province, Mpumalanga Province, Swaziland and KwaZulu-Natal Province and it is the only member under Zingiberaceae family indigenous to South Africa. However, there is controversy about its occurrence in these regions. More experienced researchers emphasized that the species seemed to have never occurred naturally in the flora area, but it was introduced from tropical Africa and widely cultivated (Holzapfel *et al.*, 2002; Lock, 1985; Smith *et al.*, 1997). Earlier authors recorded its presence as *S. natalensis* at Ngoye and Inanda and there have been tentative suggestions of its possible occurrence in some of the river valleys south of Durban and into Transkei (Van Wyk, Makhuvha, van der Bank & van der Bank, 1997).

The plant is generally propagated from matured rhizomes. Rhizomes were reported to be used for coughs, colds and flu and also used to treat malaria, hysteria and for chest complaints (Smith *et al.*, 1997; Van Wyk *et al.*, 1997). The highly aromatic enlarged roots are used as a protection against lightning and snakes (Van Wyk *et al.*, 1997). In addition, the rhizomes are also used in the treatment of horse-sickness.

Cunningham (1988) reported that pressure on wild ginger populations has led to local extinction, notably in KwaZulu-Natal, due to the fact that wild ginger cures many illnesses. Hence, more people are resorting to using it and as such face the danger of being extinct (Van Wyk *et al.*, 1997). It was stressed that there is a problem of having limited supply of high quality rhizomes and that this is compounded by over-exploitation in some regions of South Africa, KwaZulu-Natal in particular (Van Wyk *et al.*, 1997).

Demand for sustained, high crop-yield has led to the application of increasingly larger amount of commercial fertilizers to agricultural soils. This is particularly the case for nitrogen, since it is still relatively inexpensive compared to the worth of increased crop production (Bergstrom & Brink, 1986). Wild ginger is an important crop as far as human health is concerned. Therefore,

as a result of its many medicinal purposes it was vital that research be conducted to increase its production. Therefore, protecting the crop from being over-exploited in the wild is of utmost importance.

LITERATURE REVIEW

The objectives of the study were to: (a) determine optimal nitrogen rates and their effects on the growth, enlarged root anatomy and yield of wild ginger, (b) determine the ideal fertigation frequency and growing medium on the growth, quantity and quality of rhizome and enlarged root oil, enlarged root anatomy and yield of wild ginger.

50% of all drugs in clinical use in the world (Kingdom & Salamir, 1993).

Wild ginger is scientifically known as *Siphonochloa verticillata* (Schweff.) B.L. Dart and is commonly known as Natal ginger or isiphephetho. It belongs to the Zingiberaceae family in the same family with true ginger (*Zingiber officinale*). Wild ginger is a rare African plant in the ginger family and is regarded as Africa's natural anti-inflammatory which is reported to have a number of spice plants such as turmeric and cardamom. The generic name *Siphonochloa* is derived from Greek *siphon* meaning tube, and *chloa* meaning lip in reference to the shape of the flower and the specific name *verticillata* means from southern Africa (Hanky & Reynolds, 2002).

Wild ginger is a deciduous plant with large, hairless leaves growing on a single, erect stem with distinctive, cone-shaped rhizome and they may reach the height of up to 100 cm and have an light green, lance shaped and borne at the end of stem-like leaf bases (Van Wyk *et al.*, 2000). It has very attractive flowers, which are borne at the ground level and are very short lived and appear in early summer, from the end of October to early December (Hanky & Reynolds, 2002). Flowers are broadly funnel-shaped, pink and white in colour with a small yellow blotch in the middle. Most of the flowers in the plants are bisexual, and they are usually more female flowers in plants than the male counterparts (Smith *et al.*, 1997). The small, berry-like fruits are borne below or above the ground and the leaves and rhizomes have a smell similar to that of true ginger, *Zingiber officinale* (Van Wyk *et al.*, 2000).

Rhizomes of wild ginger are used for colds, coughs, influenza and hysteria and to clear nasal passages and they may also be taken for pain. Several other traditional and cultural uses include the treatment of asthma and dysmenorrhoea (Pujol, 1993; Crouch, 1996; Hutchings, 1996).

CHAPTER 1

LITERATURE REVIEW

1.1 Introduction

Plants were once a primary source of all the medicines in the world and they still continue to provide mankind with new remedies. Natural products and their derivatives represent more than 50% of all drugs in clinical use in the world (Kinghorn & Balandrin, 1993).

Wild ginger is scientifically known as *Siphonochilus aethiopicus* (Schweif.) B.L. Burt and commonly known as Natal ginger or isiphephetho. It belongs to the *Zingiberaceae* family and the same family with true ginger (*Zingiberaceae officinale*). Wild ginger is a rare African plant in the ginger family and is regarded as Africa's natural anti-inflammatory which is reputed to have a number of spice plants such as turmeric and cardamom. The generic name *Siphonochilus* is derived from Greek siphono meaning tube, and *chilus* meaning lip in reference to the shape of the flower and the specific name *aethiopicus* means from southern Africa (Hankey & Reynolds, 2002).

Wild ginger is a deciduous plant with large, hairless leaves developing annually from a small, distinctive, cone-shaped rhizome and they may reach the height of up to 400 mm. Leaves are light green, lance shaped and borne at the end of stem-like leaf bases (Van Wyk *et al.*, 2000). It has very attractive flowers, which are borne at the ground level and are very short lived and appear in early summer, from the end of October to early December (Hankey & Reynolds, 2002). Flowers are broadly funnel-shaped, pink and white in colour with a small yellow blotch in the middle. Most of the flowers in the plants are bisexual, and they are usually more female flowers in plants than the male counterparts (Smith *et al.*, 1997). The small, berry-like fruits are borne below or above the ground and the leaves and rhizomes have a smell similar to that of real ginger, *Zingiber officinale* (Van Wyk *et al.*, 2000).

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Wild ginger contains volatile oil with α -terpineol which are generally used for their decongestant, antiseptic and diuretic effects and various other monoterpenoids, but the main compound is a highly characteristic sesquiterpenoid in the oil used for colds and influenza (Merck, 1989). However, the similarity between wild ginger and true ginger appears to be superficial only, as none of the terpenoids of ginger oil are present in the essential oil of *Siphonochilus*. The highly aromatic roots have a variety of medicinal and traditional purposes and they are used by Zulu people as a protection against lightning and to ward off snakes (Hankey & Reynolds, 2002).

The conical rhizome and roots contain a high percentage of a characteristic sesquiterpenoid, which is a key phytochemical active ingredient. Extracts of the rhizome have been demonstrated to be anti-inflammatory (prostaglandin-synthetase inhibition), bronchodilatory, smooth muscle relaxant, mildly sedative, and anti-candidal. The presence of antiseptic monoterpenoids contributes to the bioactivity (Hankey & Reynolds, 2002).

The plant is easily cultivated in the warm parts of South Africa and attempts have been made for the large-scale production of rhizomes through tissue culture in order to reduce the pressure of wild populations (Van Wyk *et al.*, 2000). Wild ginger is easy to propagate provided it is given a well-drained, compost rich soil and warm, but shady position in containers or in the garden. Watering should be reduced to a minimum during winter months while the plant is dormant and may be resumed with the onset of spring. During the growing season plants respond very well to high levels of feeding with organic matter (Hankey & Reynolds, 2002).

Fertilizer usage played a major role in the universal need to increase food production to meet the demand of the growing world population. Fertilizer application resulted in crop yield increases, which for most crops was more than 100 %. The extent to which fertilizers are used still differ considerably between various regions of the world (Stanford & Legg, 1984).

Nitrogen is a major essential nutrient element and required by plants in substantial quantities. It is the constituent of proteins and many metabolic intermediates involved in synthesis and energy transfer of nucleic acids (Goh & Haynes, 1986; Mengel & Kirkby, 1987). Olson & Kurtz (1982) reported that when the supplies of soil water is adequate, it is the most limiting factor for crop

production. On average, considerably more nitrogen than any other element is supplied to crops as fertilizer and is removed from agricultural lands in harvested crops.

It is commonly the most limiting plant nutrient for crop production in the majority of the world's agricultural areas and the only plant nutrient which can be added to the soil by biological nitrogen fixation (BNF). Therefore, adoption of good N management strategies often results in large economic benefits to farmers (Novoa & Loomis, 1981). It must be viewed as the control element because of its role in substances such as protein and nucleic acids that form the living material.

There was no literature available on the effect of nitrogen nutrition, fertigation frequency and growing medium on wild ginger as well as little is available on true ginger (*Zingiberaceae officinale Roscoe*). However, the literature in this study will be mainly of a potato crop. A potato crop was chosen on the basis that it is a tuberous crop and produces tubers underground similar to wild ginger. It is believed that a potato crop will respond to nitrogen levels, fertigation frequency and growing medium in a similar manner to wild ginger.

1.1.1 Effect of nitrogen on potato plant growth and yield

The most critical way in promoting extremely high yields, is supplying nutrients especially nitrogen, in sequence with crop demand without creating toxic conditions and affecting the quality of harvested products (Oagile, 1998).

The application of mineral fertilizer has to a large extent been responsible for increasing crop yields on a worldwide scale (Mengel, 1991). The author pointed out that of all plant nutrients, particularly nitrogen is applied at highest rates and has the greatest impact on crop yield. Under the economic conditions of the developed countries the price cost ratio is such that for most crops highest profit is obtained at the maximum yield. In numerous cases it is also only by the application of nitrogen fertilizer that a profit can be obtained (Mengel, 1991).

Nutrient content and assimilation by plants are the results of total plant growth and nutrient availability. Relatively high N applications can delay potato tuber growth 7 to 10 days, particularly for indeterminate potato varieties (Westermann, Kleinkopf & Porter, 1985). They

showed that N has a major role in the production and maintenance of an optimum plant canopy for continued tuber growth through long growing seasons. Therefore, during periods of high tuber growth rates, the demand for nutrients may exceed uptake rates and cause depletion of mobile nutrients from the tops to the tubers (Westermann *et al.*, 1985). If the depletion starts too early in the growing season, it may cause premature canopy senescence and reduce final tuber yields.

ABA/GA ratio controls tuber setting, a high ratio favouring and a low ratio restricting tuber initiation. Hence, ABA/GA ratios respond sensitively and rapidly to nitrogen nutrition (Mengel & Kirkby, 1987). They reported that a continuous N supply result in a relatively low ABA/GA ratio with 'regrowth' of tuber occurring. However, interrupting nitrogen supply increased ABA content dramatically and gave rise to tuber initiation. In practice this reversible cessation of tuber growth by a high level of nitrogen nutrition often occurred at late stages of tuber growth. In addition, nitrogen nutrition is important for root crops unlike cereals in that, enhanced nitrogen nutrition after flowering can stimulate vegetative growth and initiation of new leaves (Mengel & Kirkby, 1987).

During early stages the developing tuberous crops should be well supplied with nitrogen in order to develop the vegetative plant organs needed for photosynthesis. After flowering nitrogen supply to tuberous crops should decline, because continuous nitrogen supply affects quality of tubers, since later stages are characterized primarily by the synthesis of carbohydrates and their translocation to the tubers. Increasing the level of nitrogen nutrition may lead to excess of soluble amino acids, which cannot be used for growth processes (Mengel & Kirkby, 1987).

Crozier, Creamer & Cubeta (2000) stated that, although numerous studies document yield response to nitrogen application, yield and tuber quality could be reduced by excess fertilizer application. They reported that high nitrogen rate delay tuber initiation, which may be critical since the potato growing season is typically curtailed by high summer temperatures. However, the influence of residual nitrogen is important when evaluating crop response to N fertilization.

The increased crop yield resulting from the application of N fertilizer to N-deficient soils and increased protein percentage resulting from excessive N fertilizer applications have long been recognized (Deckard, Tsai & Tucker, 1984). The tuber protein percentage increased most

rapidly in response to N supply after the yield response leveled off. The response of yield and protein to N supply was strongly influenced by environmental conditions, especially the quantity and timing of water available to the crop (Westermann & Tindall, 1998).

Marshall & Vos (1997) showed that N influenced the productivity of potatoes by influencing the water use efficiency. Under low water availability conditions, a positive yield response to added N leveled off at low soil N level and an increased protein response was shown more readily (Deckard *et al.*, Tsai & Tucker, 1984). The relationship between yield and protein percentage across N level can vary from negative to positive, depending on the environmental conditions. They pointed out that the positive relationship occurred when both yield and protein percentage increased whereas negative relationships were less common and generally resulted from increasing low soil N levels at high available water or when soil N levels resulted in lodging of the crop.

Leaf area duration (LAD) is an important determinant of potato yield (Vos, 1995a). Nitrogen nutrition affects tuber yield of potato crops mainly by its effect on leaf area duration (Biemond & Vos, 1992; Vos & Biemond, 1992). Vos and Biemond (1992) observed that leaf area index (LAI) at a particular point in time, and its change in time were determined by the dynamics of leaf growth and branching. In a potato field experiment carried out by Vos (1995a), it was shown that stem density was variable and that modified response to nitrogen for a given rate of N supply per unit area and the amount of N that was available per stem was lower in dense population than in open stands. Similarly, Vos and van der Putten (1998) showed that N supply affected leaf expansion rate and size in potato and also the total number of leaves that emerge on the plant. It was found that the effect of N on leaf size and number of leaves per plant was affected by the seasonal pattern of light interception and crop production (Vos, 1995b).

Efficient use of N in plant production is an essential goal in crop management (Vos & Marshall, 1997). Application of N fertilizer to potato affected leaf growth, onset and duration of tuber growth and the composition of the progeny tuber (O'Brien & Allen, 1986). Variations in onset of tuber growth affected the chronological age of the tuber, which has been reported to influence its productivity. Many authors showed that in potato production, N is applied more frequently and in greater amounts than any other nutrient. It is also the nutrient that most often limits yield. Without added nitrogen, growing plants often shows N deficiency characterized by yellow leaves, stunted growth, and lower yields (Bowen, Cabrera, Barrera & Baigorria, 1998). Similarly, Baritelle, Hyde & Thornton (2000) stated that N is an important nutrient in potato production. Hence, tuber yields were greatly increased by increased N fertilizer treatments.

Westermann *et al.* (1985) found that increasing N fertilizer increased total tuber yield and reduced the yield of undersized tubers.

Marshall & Vos (1991) showed that N influenced the productivity of potatoes by influencing the size of leaves, maximum leaf area index, and leaf area duration, whereas the light conversion coefficient into dry mass was little affected by nitrogen. In a pot experiment with potato they observed a direct relation between photosynthetic capacity and the concentration of N in leaf dry matter, changes in both variables being associated with leaf aging. In the field, the N content of a leaf at a given level on the plant will depend on N supply. However, this dependency may be either direct, with a low rate of N supply resulting in an overall low concentration of N in the plant.

Millard & Marshall (1986) pointed out that as the amount of N applied to the potato crop is increased, a decreasing proportion was taken up, while the concentration of N in the dry matter in the plant tissues increased. Numerous studies showed that N in the canopy accumulates as both nitrate and reduced N. Thus, the rate of N uptake by the crop decreases towards the end of the season. In addition, accumulation of N in the canopy allowed redistribution of N between leaves to support new leaf growth late in the season (Millard & MacKerron, 1986). Hence, accumulation of N by the crop increased leaf dry matter production, LAI and LAD (Millard & Marshall, 1986).

1.1.2 Nitrogen use efficiency

Efficient use of N in plant production is an essential goal in crop management (Novoa & Loomis, 1981). They stated that a realistic approach to diminishing the environmental hazard of N fertilizers is to make a better use of N fertilizers or in other words to increase their efficiency of utilization and that crops take up less than 50% of N applied to soils.

Supplying the needed nitrogen just prior to the crop's greatest demand can maximize nitrogen efficiency. Vitosh (1990) reported for potatoes that this occurs during rapid growth and dry matter accumulation. Similarly, Waddell, Gupta, Moncrief, Rosen & Steele (1999) showed that a management option to increase N use efficiency is to split N applications over the growing

season. Westermann *et al.* (1985) showed that post-hilling N application increased yield by potentially limiting N leaching beyond the root zone.

Because of the high cost on nitrogen fertilizer, improving N efficiency of cultivars is an important goal. An important variation of N efficiency among genotypes has been reported in many commercial crops (Broadbent, Goh & Haynes, 1987). A higher efficiency would permit reducing N rate without reducing yield and profit and therefore, lead to a small proportion of N susceptible of being carried to surface and ground water (Clark, 1983). Similarly, Sinclair & Horrie (1989) stated efficient use of N is also important for minimizing environmental contamination.

The rate and kind of fertilizer used and the method of application, influence the recovery of N. The recovery could be improved when the fertilizer is concentrated in the root zone in band rather than broadcast on the surface. Improved efficiency of nitrogen use at the field and farm scale, both increased crop yield and quality and reduced losses was dependent upon dynamic optimization to match the N supply and the N requirements of the crop at a field scale. This optimization required measurement and prediction of soil N supply and their variability (Vos & Marshall, 1994).

1.1.3 Nitrogen fertilizer application and crop production

MacKerron, Young & Davies (1993) showed that requirements for N fertilizer differ greatly from field to field in ways that are difficult to predict. In a study by Neetson & Wadman (1987) they found economic optimum level of N application of potato to range between 0 and 450 kg ha⁻¹. In addition, they showed the best estimator of the available N from the soil to be the amount of mineral N in the top 60 cm of soil in spring. Traditionally, recommended fertilizer rates have been based on empirically derived relations between application rates and yield (MacKerron, Young & Davies, 1987).

The response curves of potato to applied N present an extending plateau over which commercial yields do not decline at higher applications. They affirmed that applications of N fertilizer should be adequate to ensure the potential growth of the crop, but low enough to minimize losses through leaching. They showed that relationships could be derived between the rate of N

uptake in plant tissues and final N level at the end of the growing season. Thus, suggesting that it might be possible to estimate the rate of uptake of N at an early stage in the season and comparing it with the required total uptake for anticipated yield.

for maximum yield

1.1.4 Response of nitrogen application in crop production

Fertilizer uptake efficiency is normally relatively constant with increasing rates of crop yield

Although crops usually respond to nitrogen fertilizer, this is not always the case. Response to nitrogen depends on soil conditions, the particular crop species and the plant nutrient supply (Mengel & Kirkby, 1987). They observed that when the soil N content is higher, the N response is poorer and in the absence of a response, residual N and/or the rate of N release by microbial decomposition of soil organic matter is probably adequate to meet the demand of a crop. Goh & Haynes (1986) outlined that yield response of plants to applied N fertilizer addition may occur as dry matter and protein yield and quality improvement or other plant features.

Molle & Jessen (1968), cited by Goh & Haynes (1986), considered the relationship between nitrogen fertilizer response and soil organic matter in their experiments. The authors found that on sandy soils under humid climatic condition, N application rates of 90 – 135 kg·ha⁻¹ for many crops resulted in optimum economic return and again on peat soils rich in organic nitrogen. Crops that are harvested before maturity such as forage grasses require high amounts of nitrogen (25 to 30 kg·ha⁻¹ of dry matter) (Stanford & Legg, 1984) in contrast to mature crops.

extremely rapid (2-3 kg ha⁻¹ N day⁻¹) during the rapid growth phase. A linear rate of application

Responses to nitrogen application is limited when water availability is restricted (Vitosh, 1990). The response of nitrogen also depends on how well the crop is supplied with other nutrients. Mengel & Kirkby (1987) observed that without phosphorus (P) and potassium (K) application, the yield response to increasing nitrogen levels was smaller than when adequate amounts of P and K were applied. The efficiency of nitrogen fertilizer usage is much dependent on factors such as water supply and the presence of other nutrients in the soil. Similarly, Lopez-Bellido & Lopez-Bellido (2000) pointed out that crop response to N is not influenced only by available water and N fertilizer management but also by factors such as soil type and tillage methods, crop sequence and N supply. Subsequently, increasing N fertilizer rates prompt to increase yield up to a point, beyond which there is no additional response, thus it prompts greater N loss.

soil organic matter is high, lower application rates need to be applied and for poor soils low in nitrogen,

In a study by Goh & Haynes (1986), they pointed out that the simple response of plants to applied N, is when N is the major growth-limiting factor, and that dry matter yield increase with

increasing rates of N up to a maximum and either stayed constant or declined with further rates of nitrogen. Hence, total N uptake by the crop increased up to the maximum yield so that maximum fertilizer uptake efficiency can be achieved at the same fertilizer rate as was required for maximum yield.

Fertilizer uptake efficiency is normally relatively constant with increasing rates of N up to a level at which maximum yield is first obtained and further fertilizer addition decrease uptake efficiency. The potential of excess NO_3^- in soil profile rises sharply above the fertilizer rate required for giving maximum yield. Several authors showed that the magnitude of the positive response to applied N is likely to be primarily dependent on the size of available and potentially available pool of N in the soil and the demand for N by crops as determined by its potential dry matter production (Greenwood, Cleaver, Turner, Hunt, Niedorg & Loquens, 1980; Olson & Kurtz, 1982).

1.1.4.1 Time and rate of uptake

Numerous researchers showed that N uptake by field crops involve a period of very slow accumulation followed by a rapid linear rate of accumulation that coincides with rapid plant growth (Neetson & Wadman, 1987). They found that for field crops, the rate of uptake can be extremely rapid ($3\text{-}5 \text{ kg}\cdot\text{ha}^{-1} \text{ N day}^{-1}$) during the rapid growth phase. A linear rate of uptake does not necessarily mean that N concentration in the plant is constant but in fact, N concentration in a young plant is initially high and characteristically decline as the plant age and accumulate dry matter.

1.1.4.2 Effect of nitrogen fertilizer application rates

The level of nitrogen that should be applied to a crop depends largely on the particular crop species and on the prevalent soil conditions (Mengel & Kirkby, 1987). Generally, the quantity of nitrogen taken up by a good crop over the growing period serves as a guideline in assessing the appropriate rate of nitrogen application. When the rate of inorganic N release from soil organic matter is high, lower application rates need to be applied and for poor soils low in nitrogen, application rate should be in excess of the total amount of N uptake (Mengel & Kirkby, 1987).

The distribution of N between component plant parts, such as leaves, stems, storage organs and harvest index, are relatively constant irrespective of the average N concentration of the plant (Biemond & Vos, 1992). Vos (1995a) stated that the pattern of N uptake in potato crop can be dramatically influenced by the dose and timing of split application. For most crops such as wild ginger (*Siphonochilus aethiopicus*), there is little information about which developmental stage N applications will continue to influence crop growth and quality of harvested components and in what form of N should be applied to achieve maximal effect.

Lee & Asher (1981) reported that substantial amount of nitrogen fertilizer are used to obtain higher yield in ginger (*Zingiber roscoe officinale*) (up to 830 kg·ha⁻¹) but little information is available concerning optimum rates and times of application or the most suitable form of nitrogen fertilizer. It was also reported that substantial leaching losses of N were likely because growers apply 50% of their total N at planting and a further 25% in the first sixteen weeks, a period during which only about 11% of the total growth of the crop occurs. In addition, higher yield of ginger was obtained with relatively low rates of N (less than 300 kg·ha⁻¹) in an experiment in which the total fertilizer N was divided into 10 applications.

Hegney & McPharlin (2000) pointed out that potato crops produced in the coastal plains used ranges from 356 to 1510 kg·ha⁻¹ N. However, these rates are high compared to the measured N requirement of potatoes grown on sandy soil elsewhere. They showed increased agronomic N efficiency in potatoes (i.e. tuber yield per unit N applied) to be achieved by reducing pre-plant application and frequently applying small amounts during the growing season. For example, yield of Russet Burbank potatoes was increased from 61.8 to 77.5 t·ha⁻¹ and rate of maximum yield decreased from 448 kg·ha⁻¹ (when all was applied pre-plant) to 336 kg·ha⁻¹ (when two-thirds of the N was applied post-planting). Similarly, Kolbe & Zhang (1995) reported that different rates of N fertilization lead to differences in tuber yield and chemical composition at harvest and affected the behaviour of stored tubers.

1.1.5 Crop nitrogen demand

Knowledge of the factors governing nitrogen demand is essential to predict the need of crops under a wide range of field conditions, so that growers can be given more reliable fertilizer recommendations (Greenwood, 1982; van Keulen, Goudriana & Seligman, 1989). Addiscot,

Whitmore & Powlson (1991) agreed with the statement by stating that this is important, not just for economic reasons, but because of the risk to the environment that can arise from over-application of nitrogen fertilizer, in particular the problem of nitrate leaching. They defined the nitrogen demand of plants as the N uptake over a set of period which would allow maximum (dry matter) growth rate under the given environmental conditions, i.e. when N supply just ceases to be a limiting factor for growth.

Several authors affirmed that there is good evidence that the growth of the shoot is the main determinant of the N demand and hence the potential of N uptake (Grindlay, Sylvestser-Bradley & Scott, 1993). Van Keulen *et al.* (1989) reported that N uptake by the plant will be less than the N demand if there is insufficient mineral N available for uptake by the roots, in this case N supply determine the amount of nitrogen taken up.

Numerous researchers concluded that the most widespread approach to N nutrition has been to express nitrogen content on a mass basis, usually as a percentage N in the dry matter (Greenwood, 1982; Grindlay, 1997). Grindlay (1997) suggested that the instantaneous rate on N uptake can be calculated by multiplying the concentration of nitrogen (which needs to be determined by chemical analysis), and is complicated by the fact that nitrogen content on a dry matter basis varies with N supply and the age of the plant.

Grindlay (1997) defined the crop N demand on the basis of concentrations of total N on a dry matter basis, but these values vary with the plant age and the N supply. In agreement with the above statement Stanford & Legg (1984) outlined that crop N demand is the product of expected yield and internal N requirement, which can be thought of as the minimum amount of plant N associated with maximum yield. According to Bowen *et al.* (1998), although a growing crop may take up more than minimum N needed, extra N (luxury consumption) does not usually result in any yield benefit. Therefore, to optimize N management and avoid its inefficient use, it is important to know the expected maximum yield and its associated internal N requirement (Stanford & Legg, 1984)

When N is non-limiting for growth, plants may restrict their N uptake to meet their immediate requirement for maximum dry matter growth rate (Greenwood *et al.*, 1980). According to Millard (1998) some species have or showed a tendency of luxury consumption, the

accumulation of NO_3^- and/or reduced form of nitrogen. According to Haremlink, Johnston, O'Sullivan & Poloma (2000) different rates of N fertilizer are used to derive functions for crop N demand, expansion of the green crop area and dry matter accumulation. Therefore, such functions can be used in simple crop growth models or for defining crop N demand in precision farming.

1.1.6 Nitrogen nutrition and crop phenology

Phenology refers to the changes in life stages of a biological material. Thies, Singleton & Bohlool (1995) defined phenology as the study of the timing of biological events and causes of timing with regard to biotic and abiotic factors. The information among phases of the same or different species e.g. the timing of a heading of a potato crop is a biological event, which depends on the temperature and photoperiod under which the crop is grown (i.e. the abiotic forces). They outlined the purpose of phenological studies as (i) to indicate whether a crop could be grown commercially in an area; (ii) to serve as a guide in developing varieties for a specific environment; (iii) to grow plants with varying maturity dates to facilitate harvesting at intervals suitable to commercial canning operation; (iv) in hybrid seed production involving inbreds of different maturity rating, adjusting planting dates so that inbreds will be at the appropriate stage of development for crossing; and (v) to facilitate planning of operations, such as irrigation, fertilization, and herbicides application, when such operations are made at the best stage of a crop development.

1.1.7 Soil N supply

Vegetative growth mainly consists of growth and formation of new leaves, stems, roots and meristematic tissues responsible for these organs that have a very active protein metabolism. Hence, photosynthates transported to these sites are used predominately during the vegetative growth stage in the synthesis of nucleic acids and proteins. It is for this reason that during the vegetative stage, the N nutrition of the plant controls the growth rate of the plant to a larger extent (Mengel & Kirkby, 1987). They showed that the concentration of N in the leaves might also determine the efficiency with which intercepted light is converted to dry matter accumulated. Numerous authors documented that the concentration declines as the plant develops (Greenwood *et al.*, 1980). Attempts have been made to relate the decline in total plant N concentration to either crop development using thermal time or to crop biomass (Greenwood, Lemaire, Gosse, Cruz, Draycott & Neetson, 1990)

Novoa & Loomis (1981) reported that leaves play an important role in N metabolism in crops because large amount of N is required for leaf growth. Finck (1982) also reported that three-quarters of total reduced N in the leaf might be connected with photosynthesis. He reported that tissues must have a certain N concentration and that decrease in N supply would limit the amount of tissue that can be produced. In agreement with this, Grindlay *et al.* (1993) found that there is a relationship between the amount of N in the shoot and dry matter. Vigorous leaf growth during early growth stage of a crop and the development of leaf area per plant is essential for voluminous roots. The more quickly in the growth period the leaves are able to form a complete canopy over the soil, the better are the chances of good yield. Leaf growth depends very much on a higher level of N nutrition during early stages of plant development. N translocation is an important process in plant life. However, young leaves are supplied with amino acids until they have reached maturity (Mengel & Kirkby, 1987).

1.1.7 Soil and plant nitrogen dynamics for optimum crop yields

Research on soil – plant nitrogen dynamics have been carried out from the very beginning of agricultural investigations. Soil – plant N dynamics lie at the heart of some questions being asked of researchers by farmers, environmentalists and policy makers. However, farmers seek to apply economic optimum rates of fertilizer, considering the costs of application and the effect on crop quality as well as yield (Neetson & Wadman, 1987; Vos, 1995b).

1.1.7.1 Soil N supply

Recently emphasis had been placed on the measurement of inorganic N (usually nitrate) in the soil before planting or at a specific time during the crop growing season to assess soil N supply (the N_{min} approach). The required soil N supply for any period depend not only on crop N demand, but also on the crop uptake efficiency. A measure of that efficiency is the apparent N fertilizer recovery, which is defined by $(N \text{ uptake of a fertilized crop} - N \text{ uptake of unfertilized crop}) \times \text{fertilizer applied}$ (Neetson & Wadman, 1987). According to Stockdale, Ganut & Seligman (1997) apparent N recovery usually range from 0.4 – 0.7 with 0.8 as the upper limit. These recoveries are low where conditions favour losses of nitrate from the soil by denitrification or leaching and where carbon is available for net microbial immobilization will occur at least temporarily. However, to minimize the effect of spatial variability in the N supply

from the soil, the distribution of fertilizer N applied would have to be negatively correlated with the soil.

N fertilizer is an expensive input and in many trials less than 60% of the applied N is recovered in the crop and soil with the remainder being lost. Numerous studies showed that increasing the amount of N applied at sowing does not increase the amount of N available to the potato crop because the fertilizer N is lost before the crop can assimilate it (Vos & Marshall, 1994). They reported that a minimum of six weeks is required before the crop has the capacity or potential to accumulate much of the N applied at sowing. Between 50 and 70% of the post sowing, N applications were recovered in the crops compared to less than 40% when N was applied at sowing (Vos & Biemond, 1992). Similarly, Biemond & Vos (1992) reported that the fertilizer N is used more efficiently when the supply of available N in the soil is matched with the demand for N by the crop.

Nitrogen supplied by soil comes mostly from two sources (1) mineralization of soil organic N during the growing season and (2) mineral N initially present in the soil profile at planting. Dahnke & Johnson (1990) pointed out that both sources should be considered when estimating the amount of supplemented N needed by a growing crop. Hence, the importance of initial mineral N needs an environment where significant leaching can occur.

The amount of mineral N present in the soil profile at planting often have a substantial impact on the need for supplemental N, particularly in less humid environments (Dahnke & Johnson, 1990). Initial mineral N usually varied across sites and years, with the amount largely determined by management and growth of the previous crop and the residual N left from earlier applications (Dahnke & Johnson, 1990). In addition, if rainfall is not excessive, much of the initial mineral N can remain available to a crop throughout the growing season.

1.1.7.2 Crop N uptake

Relationship between N uptake rate and growth rate is described by physiological efficiency of N use for a crop (Harper, 1994). Ingestad & Agren (1992) observed that during exponential growth, the relative growth rate is proportional to the relative N uptake rate of a crop when constant. The authors found that N concentration in the plant is then stable and controlled by the

relationship between the relative uptake and relative growth rate. This approach allowed the determination of physiological plant response to N application at a constant relative 'addition rate'. Although such approach is important for increasing understanding of the control of plant growth, it is difficult to apply in the field due to the fact that linear rather exponential growth occurs following canopy closure.

According to Greenwood *et al.* (1990) the crop critical N can be derived from the relationship between crop N uptake and dry matter production. Thus, a widely used relationship of critical crop N and crop dry mass for potato have been derived by Greenwood *et al.* (1990) and for the vegetative stage. For annual cultivated crops, the N uptake efficiency is less than 50% despite following good management practices (Gillian, Logan & Broadbent, 1985). Wang & Alva (1996) showed that in sandy soils receiving 100-120 mm annual rainfall, the efficiency of N uptake may exceed 20 to 30%. Therefore, the portion of the applied N which was not taken by a crop is either adsorbed by soil components, incorporated into organic matter, volatilized, denitrified, or leached below the effective root zone of a crop.

1.1.8 Nitrogen availability for optimum crop production

Nitrogen availability is the factor limiting primary production in most natural terrestrial ecosystem (Ohlson, Nordin & Nasholm, 1995). In boreal forests the availability of N determines species composition as well as the production and changes in the availability. Therefore, N can be predicted to have a great impact on the structure and function of these ecosystems. These limitations of N availability were not expected to integrate the numerous inter-related soil, plant environment and management factors which control N release and plant growth, but supply to provide extra information for assessment of soil N supply (Ohlson *et al.*, 1995). They stressed that, today chemical and biological indices are not thought to provide only a relative indication of N availability among soils differing in management, but used with other indicators to assess soil quality.

1.1.8.1. Factors affecting nitrogen availability

1.1.8.1.1 Nitrogen losses in farming systems

Intensive agriculture entails the risk of excessive fertilization. The magnitude of this excess can be measured as the difference between the amount of plant nutrients applied and those exported in the harvested crops (Kucke & Kleeberg, 1997). They showed that such fertilization surpluses have steadily increased the soil nutrient status, soil fertility and the nitrogen pools, which participate in the N turnover, and subsequently the leaching potentials.

It is widely argued that N leaching losses and ground water pollution can only be limited if the fertilization is reduced to an extent that the fertilization balance (= N fertilization – N removal by harvested crops) is nearly zero. Grandsedt (2000) concluded from the results of a long term field experiment that reduction of N fertilization will decrease yield and yield potentials (soil fertility), but may have little or even an adverse effect on nitrate leaching in a short period.

Environmental concerns are focused on nitrogen losses from soils, which may pollute the environment. Leaching is the major route by which nitrate enters ground and surface waters, while denitrification and nitrification are significant sources of nitrite, an important greenhouse gas (O'enenma, Boert, van Eerdt, Frakers, van der Meer, Roest, Schreder & Willems, 1998).

The main rate limiting process controlling the availability and loss of the mobile nitrate ion might be nitrification, rather than mineralization. Despite a reasonable knowledge of ecology of the bacteria, nitrification remains a poorly defined process in many soils. In temperate tilled agricultural soils, nitrification rates are usually limited by mineralisation. However, in grassland soils significant quantities of ammonium may accumulate where swards are grazed or farm wastes are applied (Kucke & Kleeberg, 1997).

The balance between mineralisation and nitrification is changed under environmental conditions and management. According to Vos (1992), leaching loss of N seems inevitable, however efficiently N is taken up by the crop, as plant processes have higher threshold temperatures for activity than mineralisation processes occurring in the soil. He pointed out that inefficient use of N fertilizer appear to be responsible for increased NO_3^- levels in ground waters. The increase

incidence of NO_3^- contamination of ground water was related to increased use of N fertilizer in intensive agricultural production (Wall & Magner, 1988). Leaching of N from soils has been demonstrated in several experiments carried out in areas with varying climate, soil type and cropping systems (O'enenma *et al.*, 1998).

Agriculture contributed substantially to an increase in nutrient leaching (Granstedt, 2000). Leaching of applied fertilizer N resulted in reduced uptake efficiency of applied N by a target crop and is an agricultural and an environmental problem (Wang & Alva, 1996). It is commonly perceived that increased reliance on industrial fertilizer has been the main cause of increased nitrate contamination of water resources. Based on this argument the reduction in sales of N fertilizers is important for improved water quality (Addiscott *et al.*, 1991). They showed that conservation tillage, particularly no-till can result in greater losses of fertilizer N by leaching following spring rains. Wall & Magner (1988) pointed out that the balance of N on the farm depended on the output, including losses to the environment, either in gaseous form by ammonium volatilization and denitrification or through leaching. For such systems a simplified N balance can be constructed. Additionally, any excess of N inputs over output of N in agricultural produce then represent a potential loss to the environment through leaching or in gaseous form.

1.1.9 Environmental factors affecting nitrogen availability

1.1.9.1 Temperature

O'brien & Allen (1986) pointed out that temperature influence microbial activity and the rate of mineralization, which is in general higher at higher temperatures. On the other hand higher temperatures may lead to greater losses of mineralised N by increased denitrification (a microbial process or by favouring volatilization or ammoniacal compounds).

1.1.9.2 Precipitation

Rainfall may have various effects on the N balance, it supplies N to the soil from atmospheric sources and through its effect on the moisture balance in the soil. It also influences the rate and duration of mineralisation as well as the magnitude of losses through denitrification and

leaching. Mineralization of organic N is the most important biological process that is involved in the availability of soil N under submerged conditions (O'Brien & Allen, 1986).

1.2 Effect of nitrogen on potato quality

Fertilizer nitrogen has been and is still used increasingly to supplement soil N for producing the needed quantity of food, feed and fibre for increasing world population, but unfortunately this is not always associated with improved quality (Deckard *et al.*, 1984). The level of nutrient supply in the soil played a major role in determining product quality. It is important as it forms an integral part of numerous plant compounds such as amino acids, proteins, nucleic acids and chlorophyll (Fink, 1982; Locascio, Wiltbank, Gull, & Maynard, 1984).

The increasing world population is confronted by a major shortage of plant products and there is a worldwide need to produce higher yielding quality crops (Mengel & Kirkby, 1987). Quality requirements are all influenced by plant nutrition. Therefore, fertilization should not only ensure high yield per unit area but also high quality produce by the improvement of either low initial quality caused by insufficient nutrient supplies, or the maintenance of high quality. Chemical composition controls the nutritional quality or value and important sensory attributes such as taste and texture of the product. Increased concentration of nitrogen in plants generally increase compounds such as amino acids, proteins and chlorophyll in some plants (Oagile, 1998).

Tuber dry matter percentage (TDM%) is an important component of tuber quality in potatoes (*Solanum tuberosum* L.) required for processing. Jenkins & Nelson (1992) reported that many factors are known to influence final TDM% and variation in N fertilizer showed significant effects.

1.2.1 Factors influencing quality on potato

1.2.1.1 Nitrogen source

Both nitrate (NO_3^-) and ammonium (NH_4^+) are dissolved in the soil solution from which they can be taken up by plant roots (Mengel, 1991). Several authors observed that NO_3^- may be transported to the roots by mass flow and /or diffusion (Novoa & Loomis, 1981; Stanford &

Legg, 1984; Neetson & Wadman, 1987), NH_4^+ mainly by diffusion (Mengel, 1991). They stated that since NO_3^- is virtually not absorbed to soil colloids, the total NO_3^- present in the rooting depth of a crop is in available form. Mengel (1991) reported that in most soil types NH_4^+ is negligible but in those soils in which illite and vermiculite make up the clay fraction, NH_4^+ bound at interlayer sites may amount to several thousand $\text{kg}\cdot\text{ha}^{-1}$ N at a soil depth of one meter. Therefore, this NH_4^+ is only partially available to crops.

Most studies showed that the concentration of NH_4^+ under a crop stand decreased during the period of highest N uptake (Vos & Marshall, 1994; Biemond & Vos, 1992). From these observations it may be concluded that plants are supplied from interlayer NH_4^+ (Mengel, 1991). This example showed that in soils with 'available' interlayer NH_4^+ this source may be more important for crop nutrition than NO_3^- .

1.2.1.2 Environmental factors

The speed of chemical reactions is very much dependent on temperature with an increase in temperature of 10°C usually increasing chemical reactions by a factor of two. However, NH_4^+ is absorbed more readily than NO_3^- when the ions are supplied together at the same concentration especially at a low temperature (Oagile, 1998). Any factor which increased the total N concentration of the tissue resulted in a corresponding increment in the N concentration of that tissue. When N fertilizer was applied, the increase in the total N due to light reduction was less than the increase in NO_3^- -N. This phenomenon attributed to the possible existence of critical total N levels above which any increase in total N showed up as predominately NO_3^- -N, and below which NO_3^- -N did not account regardless of the external factors applied (Oagile, 1998).

Several variations in nitrate contents are connected with temperature and especially with daylength and light intensity, which increase in spring during the development of a crop, but decrease in autumn (Oagile, 1998). Spring conditions are characterized by lower soil temperature than summer and together with above conditions are more favourable for both dry matter accumulation and nitrate reduction.

Water stress is one of the major factors limiting potato quality throughout the world (Mengel & Kirkby, 1987). It has been shown to inhibit incorporation of amino acids into proteins which in

turn have resulted in a decrease in protein content of the tissues. Another physiological aspect of stress is on the enzyme level in plants particularly the decrease in the level of nitrate reductase and this has been related to the suppression of protein synthesis (Oagile, 1998). CO₂ assimilation rate and reduction in the translocation rate of photosynthesis from leaves to other plant parts were also observed. Breimer (1982) have shown that water stress increased nitrate content in plants. They also attributed this phenomenon to a decrease in nitrate reductase activity prior to the moment at which uptake started to decline.

Crop production is not only dependent on light interception, but also on radiation use efficiency (RUE), the ratio between the amount of radiation intercepted and gain in total plant dry mass during particular time intervals (Vos & van der Putten, 1998). RUE, the photosynthetic properties of leaves and the aerial N concentration, N_a (gm⁻²) are interrelated (Sinclair & Horie, 1989; Hammer & Wright, 1994). However, N can affect RUE by an effect on average N_a in the canopy or on the pattern of decline of N_a with depth in the canopy.

1.2.1.3 Plant genotype and age

Published reports on the effects of physiological age are mainly restricted to tuberous crops in which large effects on tuber yield have been observed (O'brien, Jones, Allen, & Raouf, 1986). They found that increasing the age to reduce number of tubers has important implications for seed production where lower and upper size limit apply and changes in number of tubers may affect seed yield, where little or no effect on total yield can be detected. As physiological age of the seed tubers affect the timing of tuber initiation it may also affect the duration of dormancy of the progeny tuber (O'brein *et al.*, 1986). Possibilities exist that such effect may be cumulative over successive multiplication and thereby create changes in the growth pattern of a variety. However, the age of the seed tuber may affect both yield and physiological quality of the seed produced.

1.3 Effect of drip fertigation on potato growth, quality and yield

Although fertilization by irrigation, or "fertigation" as it is commonly known, is a relatively new concept to South Africa, it has been applied in various forms elsewhere since the beginning of the 20th century (FSSA, 2003)

Most researchers stressed that the combined application of irrigation and nitrogen through fertigation is now becoming a common practice in modern agriculture because of its advantages over conventional methods (Asadi, Clemente, Gupta, Loof & Hansen, 2002). They reported that some of these advantages include timely nitrogen application, excellent uniformity of nitrogen application, reduced environmental contamination, adequate movement of applied N into the rooting zone by irrigation water and reduced soil compaction and mechanical damage of the crop.

Irrigation and fertilization are the most important management factors through which farmers control plant development, crop yields and quality. Most studies showed that the introduction of simultaneous microirrigation and fertilization (fertigation) opened up new possibilities for controlling water and nutrient supplies to crops and maintaining the desired concentration and distribution of ions and water in the soil (Dasberg & Bresler, 1985).

In a study of Darwish & Nimah (1997) with potato where they had three levels of N fertilizers (73.3, 110, and 146.6 ppm), they found that the middle treatment of 110 ppm which was equivalent to 360 kg. ha⁻¹ N gave the highest yield. In comparison with conventional treatment, fertigation significantly increased the marketable tuber, although the yield was not significantly higher. Bar Yosef (1999) affirmed that fertigation management is aimed at maximizing grower income and minimizing environmental pollution. Some studies indicated that drip fertigation reduced fertilizer application cost by improving nutrient efficiencies by applying them close to where plants need them (Follett, 2002).

Mmolawa & Dani (2000) reported that drip irrigation has gained widespread popularity as an efficient and economically viable method for fertigation because of its highly localized application and the flexibility in scheduling irrigation. However, this method ensured that applied soluble plant nutrients become available to a substantial fraction of plant root system. They pointed out that drip irrigation has the potential to improve nutrient management and increase farm profit. Where management was not practiced properly, fertigation with drippers compounded salinity problems which affect crop growth, quality and yield because salts leached beyond the rooting zone and pollute the underlying ground water resources.

In most instances drip fertigation uses brackish water or recycled water of which is low quality water for irrigation. However, Mmolawa & Dani (2000) observed that in such cases fertigation with drippers using brackish or recycled water increased the amount of total dissolved solutes leading to salinization of the soil. Salinization induced unfavorable osmotic stresses and at high levels became toxic to plants. In addition, they emphasized that fertigation methods used to introduce salts and nutrients into the soil and salinity level of both the irrigation water and the resident soil water solution had some profound effect on root zone solute dynamics. Thus, fertigation had an impact on plant growth and development as well as quality (Mmolawa & Dani, 2000).

Several workers showed that drip irrigation is an effective method of supplying water and soluble nutrients to plants (Cooke, 1982). However, drip irrigation maximize profits through optimizing plant growth, resulting in higher yield and better quality by delivering precise amount of water in a uniform fashion directly to the root zone without runoff, wind drift, leaching below the rootzone or wetting the canopy. Furthermore, the dripperline apply water only to a portion of the surface thus maintaining high moisture within the root zone without water logging due to dry surroundings.

In a study of Marschner & Krauss with potato (1995), they reported that improperly managed fertigation led to the leaching of nitrate from the rootzone hence, tubers stopped growing when nitrate concentration was about $100 \text{ g}\cdot\text{m}^{-3} \text{ N}$. High nitrate concentration encouraged vegetative growth thereby delaying tuber initiation. This was due to the competition on carbohydrate consumption between the top and the underground storage organs in favour of the leaves at high N levels in the rootzone

In an experiment carried out by Steyn, Du Plessis, Fourie & Roos (1999) with potato, they found that the total yield of low frequency irrigation was higher than high irrigation frequency and this was attributed to the fact that the total amount of water applied to the pulse method (high irrigation frequency) was less than that applied to the non-pulse method (low frequency irrigation). They found no statistical significant relationships in frying chip colour. Significant differences were observed in the specific gravity between different irrigation frequencies, where the low frequency irrigation was significantly higher than that of the higher frequency irrigation.

EFFECT OF NITROGEN NUTRITION ON THE GROWTH AND YIELD OF WILD GINGER

The experiment was conducted at the University of Pretoria's Hatfield Experimental Farm, the summer (December) of 2011. The area is at an altitude of 1370 m with annual rainfall of 600-700 mm. The soil type of the experimental area was a sandy loam. Before

2.1 Introduction

Nitrogen is a major essential nutrient element required by plants in substantial quantities. It is the constituent of proteins and many metabolic intermediates involved in synthesis and energy transfer, and of nucleic acids (Goh & Haynes, 1986; Mengel & Kirkby, 1987). Numerous workers viewed it as the control element because of its role in the production of certain substances, such as protein and nucleic acids that form the living material (Novoa & Loomis, 1981). The authors stressed that it is also commonly the most limiting plant nutrient for crop production in the majority of the world's agricultural areas. Therefore, adoption of good N management strategies often results in large economic benefits to farmers.

Nitrogen is the only plant nutrient which can be added to the soil by biological nitrogen fixation (BNF), but for many cropping systems in the tropics, addition of nitrogen through BNF is insufficient to cover the loss of nitrogen with crop removal, leaching and denitrification (Olson & Kurtz, 1982). The authors reported that when the supply of soil water is adequate, N is the most limiting factor for crop production. Thus, on the average considerable more N than any other element is supplied to crops as fertilizer and is removed from agricultural lands in harvested crops.

No work was done on the effect of nitrogen nutrition on the growth and yield of wild ginger, however the study was undertaken to determine the effect of nitrogen nutrition on the growth and yield of wild ginger under field conditions.

rhizomes of 2-3 cm) were used and were obtained from the CSIR. Rhizome circumference was measured using vernier callipers (Switzerland) in order to obtain rhizomes of similar sizes and mass was determined using a measuring scale (Oxford, UK). Circumference details of each rhizome planted were recorded and each one kept in a separate netting bag. Before planting, rhizomes were dipped in a solution of copper oxy-chloride (5 g in 10 l water) to prevent soil borne diseases. Each rhizome was planted in a shallow hole of about 5 cm depth. Water was applied immediately after planting by sprinkler irrigation for 3

2.2 Materials and methods

2.2.1 Location

2.2.2 Records

The experiment was conducted at the University of Pretoria's Hatfield Experimental Farm, South Africa in the summer (December) of 2001. The area is at an altitude of 1370 m with annual rainfall of 600-700 mm. The soil type of the experimental area was a sandy loam. Before the experiment was established, the fertility status of the soil was determined (Appendix B). Samples were analysed at the Soil Science Laboratory in the University of Pretoria for P, K, Mg, Ca, Na and pH (soil analysis package) using ammonium acetate extractable method. Soil samples were taken from both topsoil (0-15 cm) and subsoil (15-30 cm) with an auger.

2.2.3 Data

3.2.2 Design

The data was analysed using the general linear model (GLM) procedure within the SAS

The experiment was designed according to a systematic, non-replicated design to establish response curves from mini plots for wild ginger. The gross plot size was 26 m². Plant spacing were 0.3m within a row and 0.5m between the rows. Plant population was 66 667 plants ha⁻¹.

2.2.3 Treatments

Six levels of nitrogen viz. 0, 50, 100, 150, 200, and 250 kg·ha⁻¹N designated (N1, N2, ...N6) were used and all was applied at planting. The source of nitrogen was limestone ammonium nitrate (28% N).

3.2.4 Planting material

Mature rhizomes of varying sizes (circumferences of 2-3 cm) were used and were obtained from the CSIR. Rhizome circumference was measured using vernier callipers (Switzerland) in order to obtain rhizomes of similar sizes and mass was determined using a measuring scale (Oerting, UK). Circumference details of each rhizome planted were recorded and each one kept in a separate netting bag. Before planting, rhizomes were dipped in a solution of copper oxy-chloride (5 g in 10 L water) to prevent soil borne diseases. Each rhizome was planted in a shallow hole of about 5 cm depth. Water was applied immediately after planting by sprinkler irrigation for 3

hours to aid in rhizome establishment. Thereafter, irrigation was applied twice every week (Tuesdays and Fridays). Weeds were eradicated by hand as soon as they were noticed.

2.2.5 Records

Emergence of the plants was recorded up to 140 days after planting (DAP) and plant height was recorded at 168 DAP. Number of leaves, fresh and dry leaf mass and leaf area was not recorded as the leaves wilted due to harsh winter environment before the final harvest. Yield in terms of fresh rhizome and enlarged root mass, fresh rhizome circumference, length of enlarged roots and the number of rhizomes and enlarged roots were recorded during the final harvest at 206 DAP.

2.2.6 Data

The data was analysed using the general linear model (GLM) procedure within the SAS computer software (SAS Institute, Inc., 1996).

2.3 Results and Discussion

2.3.1 Plant emergence

Wild ginger plants emerged very late. Emergence began 56 days after planting (DAP). Similarly, Wilson & Ovid (1993) detected that the germination of ginger began after 56 DAP. This was also in agreement with the results found by Lee, Edwards & Asher (1981), where good uniform emergence was obtained 50 DAP. Emergence at different N levels was slow during the initial growth stages and at later growth stages more plants emerged in different treatments. At 140 DAP, emergence of the plants stopped at all levels of nitrogen. Plants that received 250 kg·ha⁻¹ N had more plant emergence as compared to all other N levels followed by plants that received 50 kg·ha⁻¹ and the least was where plants received no nitrogen. In general, emergence of the plants was very poor due to the fact that plants were planted while the rhizomes had already sprouted and some of the sprouts dried off before planting. This brought the practice of timely planting of wild ginger rhizomes as an utmost important variable. Plants that received no nitrogen and 100 kg·ha⁻¹, through visual inspection, had stunted growth at 168 DAP with an average plant height of about 8.44 cm with reduced tillers (Table 2.1). However, plants that

received 50, 150, 200 and 250 kg·ha⁻¹ respectively showed good crop growth and had greater number of tillers (Table 2.1).

There was no data collected for the shoots since the plants suffered early senescence as the plants approached harsh winter environments due to delayed planting. With respect to true ginger (*Zingiber officinale*), planting occurs from late August to mid October, with the optimum period being mid to late August (Broadley, 2003). No flowering data was recorded. Broadley (2003) reported that wild ginger can produce 25-30 flowers during November and early December when planted during the appropriate planting period (August to early September).

2.3.2 Yield

2.3.2.1 Fresh rhizome mass

The results of this experiment are presented in Figures 2.1 to 2.6 and Appendix A. Yields were generally poor across nitrogen levels. However, this might be attributed to the fact that all nitrogen was applied during planting and this might have caused the sprouts of rhizomes beneath the soil to be burnt, leading to poor emergence which subsequently affected the final yield of wild ginger. Broadley (2003) stated that 20% of the nitrogen should be applied between germination and December and the remaining 80% should be applied in 7 to 10 applications between early January to April.

There was a positive linear relationship between fresh rhizome mass and applied nitrogen (Fig. 2.1). Where plants received no nitrogen, the yield was 0.65 g and 3.6 g at the highest N level (250 kg·ha⁻¹). Fresh rhizome mass increased by 0.01 g per unit increase in N application levels.

Table 2.1 Effect of N fertilizer applications on the growth of wild ginger

Nitrogen (kg·ha ⁻¹)	Days after planting (DAP)	Average number of tillers per treatment	Average root length (mm)	Average number of enlarged roots	Average number of rhizomes	Mean plant height (cm)
0	120	1	----	----	----	8.44
	150	2	----	----	----	----
	206	2	35	16	1.2	----
50	120	2	----	----	----	11.72
	150	3	----	----	----	----
	206	3	49	25	2.8	----
100	120	1	----	----	----	9.53
	150	2	----	----	----	----
	206	2	38	17	2.1	----
150	120	2	----	----	----	11.65
	150	3	----	----	----	----
	206	3	39	22	2.2	----
200	120	3	----	----	----	14.75
	150	4	----	----	----	----
	206	4	53	13	2.6	----
250	120	4	----	----	----	13.3
	150	4	----	----	----	----
	206	4	46	38	3.7	----

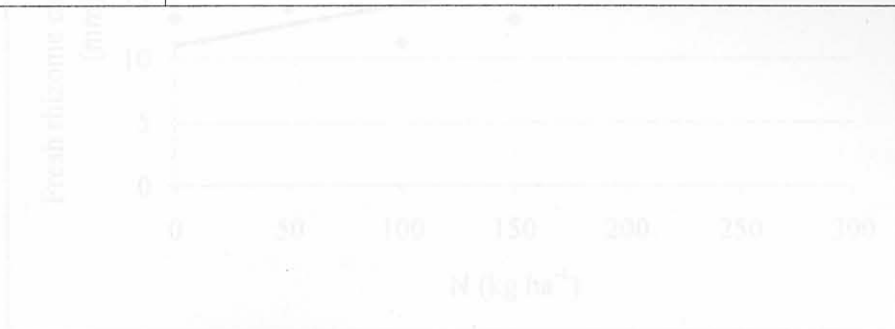


Fig. 2.2 Fresh rhizome circumference as affected by six nitrogen levels during 2001/02 seasons

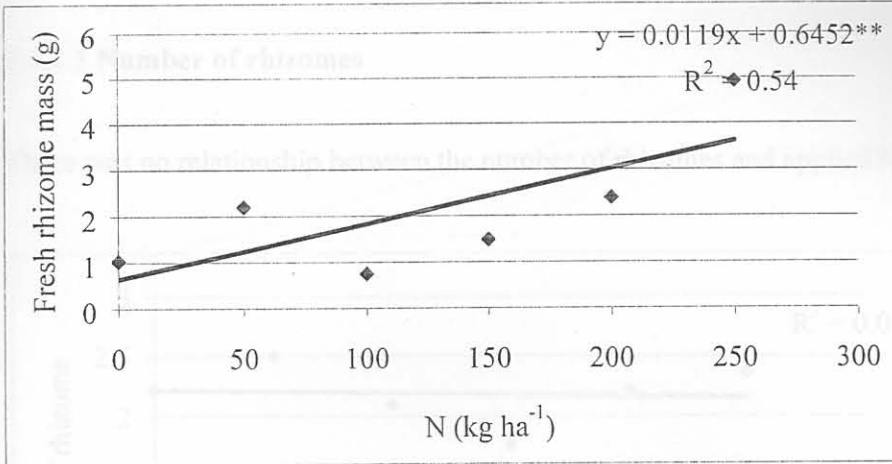


Fig. 2.1 Relationship between fresh rhizome mass and six N levels during 2001/02 growing seasons

2.3.2.2 Fresh rhizome circumference

There was a strong positive linear relationship between the fresh rhizome circumference and the applied nitrogen (Fig. 2.2). Plants that received the highest N level (250 kg·ha⁻¹ N) resulted in fresh rhizome circumference of 19.2 mm and there was rhizome circumference of 11.1 mm where no nitrogen was applied. For each increase in N application rate, fresh rhizome circumference increased with 0.03 mm.

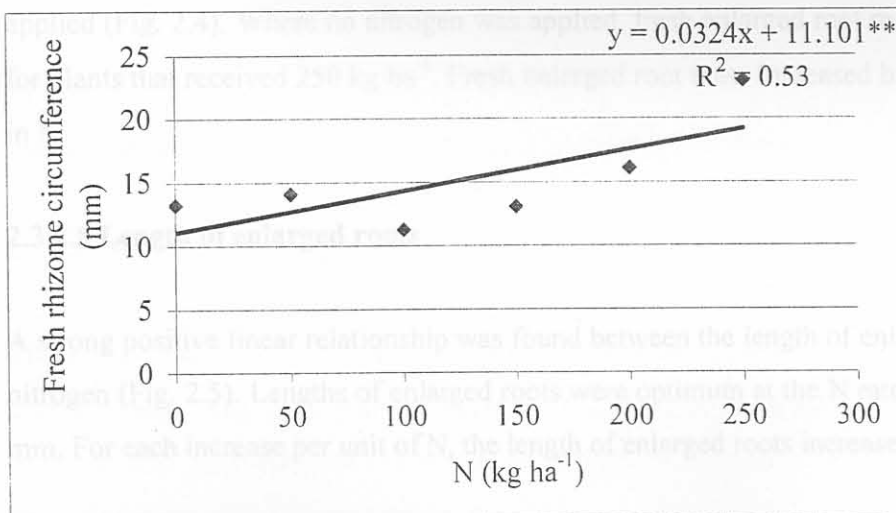


Fig. 2.2 Fresh rhizome circumference as affected by six nitrogen levels during 2001/02 seasons

2.3.2.3 Number of rhizomes

There was no relationship between the number of rhizomes and applied N (Fig. 2.3).

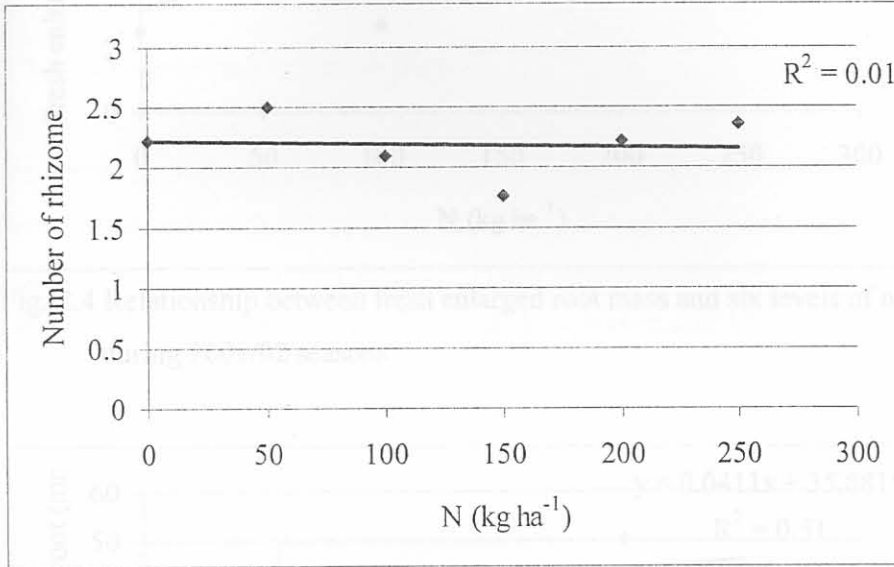


Fig. 2.3 Number of rhizomes as affected by six N levels during 2001/02 seasons

2.3.2.4 Fresh enlarged root mass

There was a strong positive linear relationship between fresh enlarged root mass and nitrogen applied (Fig. 2.4). Where no nitrogen was applied, fresh enlarged root mass was 3.3 g and 8.6 g for plants that received 250 kg·ha⁻¹. Fresh enlarged root mass increased by 0.02 per unit increase in N.

2.3.2.5 Length of enlarged roots

A strong positive linear relationship was found between the length of enlarged roots and applied nitrogen (Fig. 2.5). Lengths of enlarged roots were optimum at the N rate of 200 kg·ha⁻¹ with 44 mm. For each increase per unit of N, the length of enlarged roots increased with 0.04 mm.

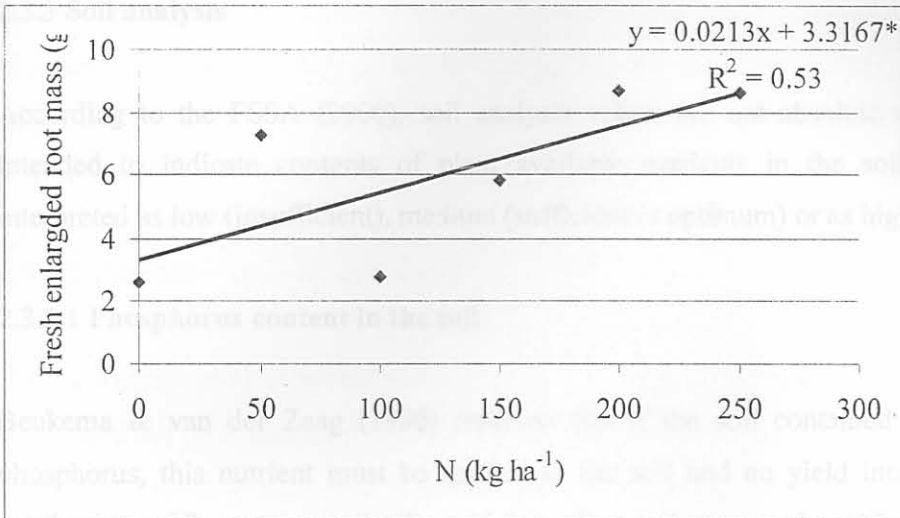


Fig. 2.4 Relationship between fresh enlarged root mass and six levels of nitrogen during 2001/02 seasons

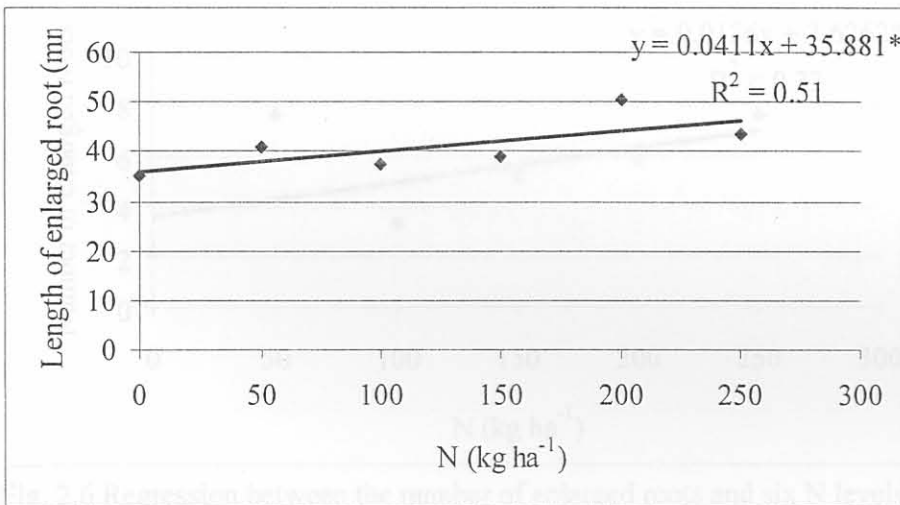


Fig. 2.5 Length of enlarged root as affected by six N levels during 2001/02 seasons.

2.3.2.6 Number of enlarged roots

There was weak positive linear relationship between applied nitrogen and the number of enlarged roots (Fig. 2.6). Plants that received 250 kg·ha⁻¹ had the highest number of enlarged roots (7) and an average of (4) where no nitrogen was applied. Average number of enlarged roots increased with 0.01 per unit increase in N.

2.3.3 Soil analysis

According to the FSSA (2000), soil analysis values are not absolute values. They are only intended to indicate contents of plant available nutrients in the soil. However, they are interpreted as low (insufficient), medium (sufficient or optimum) or as high (too high).

2.3.3.1 Phosphorus content in the soil

Beukema & van der Zaag (1990) reported that if the soil contained less than 15 ppm of phosphorus, this nutrient must be applied to the soil and no yield increase will result from application of P containing fertilizer if the soil contains more than 25 ppm and according to FSSA (2003), P content in the soil for potato is optimum at 30 to 60 ppm.

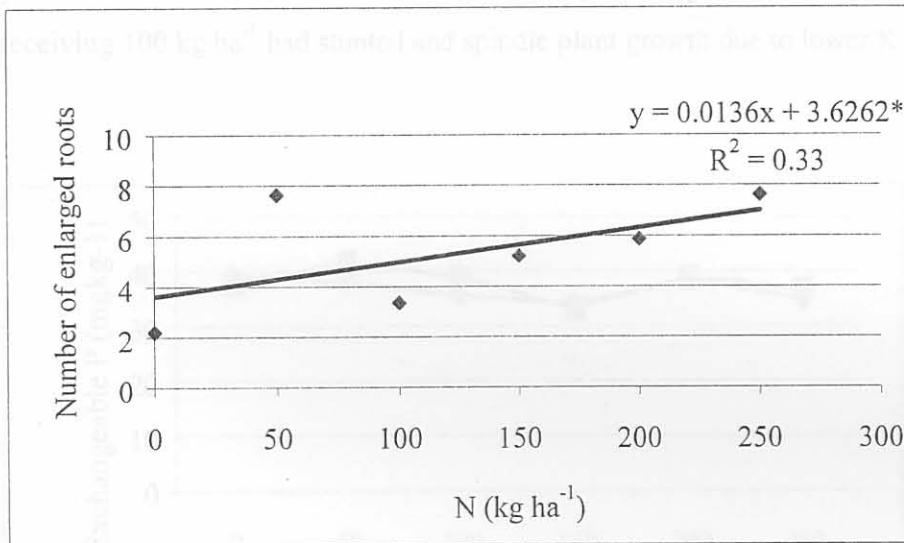


Fig. 2.6 Regression between the number of enlarged roots and six N levels during 2001/02 seasons

For both subsoil and topsoil, P content in the soil was higher than 25 ppm and P content was higher in the subsoil than in the topsoil (Fig. 2.7). P content was not limiting rather too much in the soil, therefore any application of P was not going to result in increased yield of wild ginger. For the topsoil and the subsoil, plants receiving no nitrogen resulted in maximum P and the least was observed for plots fertilized with 150 kg·ha⁻¹ whereas for the subsoil, plots fertilized with 50 kg·ha⁻¹ resulted in maximum P and the least was obtained in plots receiving 150 kg·ha⁻¹.

P has been reported to increase yield and the number of tubers. Plants fertilized with 50 kg·ha⁻¹ N resulted in superior yield and higher average number of rhizomes than plants receiving 0, 100, 150 and 200 kg·ha⁻¹. This might be attributed to the fact that they contained more P content which resulted in increased yield.

2.3.3.2 Potassium content in the soil

For the topsoil, plots fertilized with 250 kg·ha⁻¹ N resulted in the highest K content and the least was found for plots fertilized with 100 kg·ha⁻¹(Fig. 2.8). For the subsoil, plots fertilized with 50 kg·ha⁻¹ resulted in the superior K and the least was found at 100 kg·ha⁻¹. Plots fertilized with 50 and 250 kg·ha⁻¹ resulted in maximum yield whereas plots fertilized with 100 kg·ha⁻¹ was the least yielded treatment across N levels. The lower yield might be attributed to the fact that plants receiving 100 kg·ha⁻¹ had stunted and spindle plant growth due to lower K content.

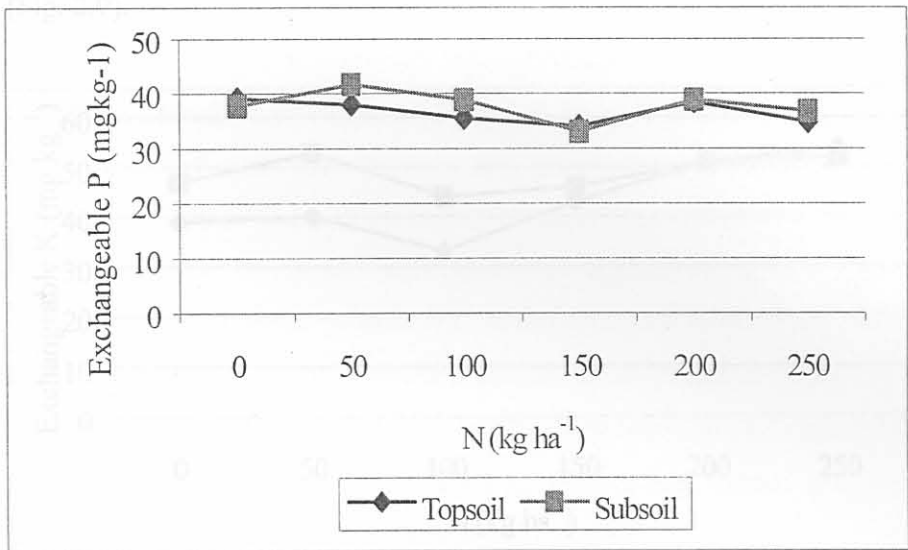


Fig. 2.7 P content in the soil as affected by six N levels

Fig. 2.8 Potassium content in the soil as affected by six levels of N

2.3.3.3 Soil pH

Application of N treatments affected the pH of the soil. For the topsoil, soil pH was neutral to slightly alkaline at plots that received 0, 50, 100 and 150 kg·ha⁻¹ and acidic at 200 and 250 kg·ha⁻¹ whereas for the subsoil, soil was acidic across the six N levels (Fig. 2.9). For the topsoil, plots fertilized with 100 kg·ha⁻¹ had a soil pH of slightly alkaline to neutral and resulted in poor yield. Broadley (2003) found that in normal soils at the pH of acidic to neutral (6.5 to 7.0), higher true ginger (*Zingiber officinale*) yields are obtained and if low, soil pH correction should be done approximately six weeks before planting.

Plants fertilized with 250 kg·ha⁻¹ N resulted in maximum yield and this was apparent as plants at that N application level was at medium acidity, which is the soil pH at which wild ginger crops are adapted. Similarly, for the subsoil, plants which performed better had their soil pH values of acidic to neutral, which are also best pH medium for wild ginger growth and significant yielding. Plants that received 200 and 250 kg·ha⁻¹ also resulted in superior yield at the pH of 6.4 (Fig. 2.9).

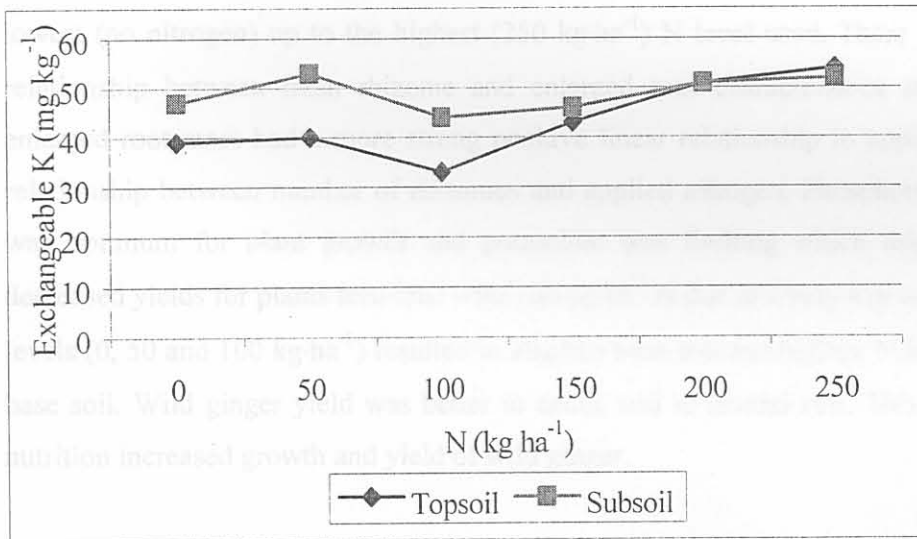


Fig. 2.8 Potassium content in the soil as affected by six levels of N

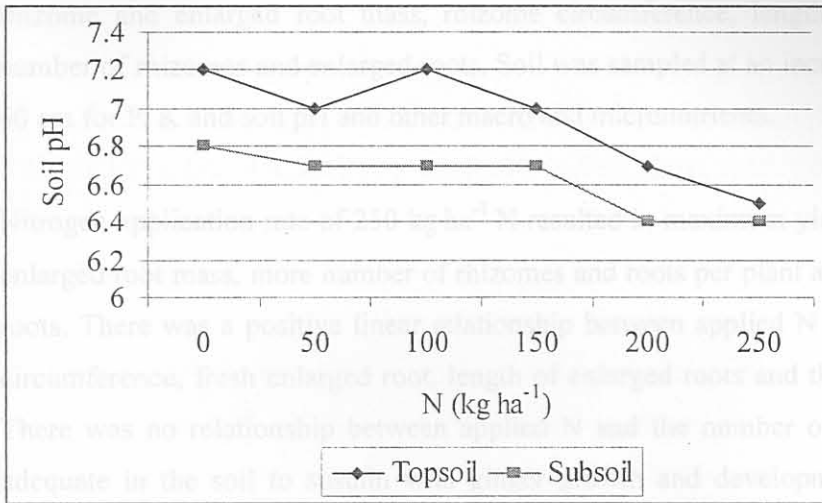


Fig. 2.9 Soil pH as affected by the application of six N levels

2.4 Conclusions

Plants that received 0 and 100 kg·ha⁻¹ N had poor stand establishments which subsequently reduced yield. A final conclusion was not reached as to which N level was optimal for fresh rhizome and enlarged root characteristics because the results showed a linear increase from the lowest (no nitrogen) up to the highest (250 kg·ha⁻¹) N level used. There was a positive linear relationship between fresh rhizome and enlarged root characteristics and applied N. Fresh enlarged root mass had a more strong positive linear relationship to applied N. There was no relationship between number of rhizomes and applied nitrogen. Phosphorus content in the soil was optimum for plant growth and potassium was limiting which might have resulted in decreased yields for plants fertilized with 100 kg·ha⁻¹ N due to a very low amount of K. Lower N levels (0, 50 and 100 kg·ha⁻¹) resulted in alkaline base soil and highest N levels exhibited acidic base soil. Wild ginger yield was better in acidic soil to neutral soil. This study reveals that N nutrition increased growth and yield of wild ginger.

2.5 Summary

A field trial was carried out on wild ginger on a sandy loam soil at the University of Pretoria's Hatfield Experimental Farm. Treatment used were six levels of nitrogen rates (0, 50, 100, 150, 200 and 250 kg·ha⁻¹) and it was all broadcasted at planting. Measurements were made of fresh

rhizome and enlarged root mass, rhizome circumference, length of enlarged roots and the number of rhizomes and enlarged roots. Soil was sampled at an increment of 0 - 30 cm and 30 - 60 cm for P, K and soil pH and other macro and micronutrients.

Nitrogen application rate of $250 \text{ kg}\cdot\text{ha}^{-1} \text{ N}$ resulted in maximum yield for the fresh rhizome and enlarged root mass, more number of rhizomes and roots per plant and greater length of enlarged roots. There was a positive linear relationship between applied N and fresh rhizome mass and circumference, fresh enlarged root, length of enlarged roots and the number of enlarged roots. There was no relationship between applied N and the number of rhizomes. Phosphorus was adequate in the soil to sustain wild ginger growth and development whereas potassium was limiting. Application of N affected soil pH. Nitrogen application levels of 0, 50 and 100 resulted in the acidity of soil and 150, 200 and 250 N levels resulted in the alkalinity of the soil. This study demonstrated N nutrition increased growth and yield of wild ginger.

Irrigation and fertilization are the most important management factors through which farmers control plant development, crop yields and quality. Most studies showed that the introduction of simultaneous microirrigation and fertilization (fertigation) opened up new possibilities for controlling water and nutrient supplies to crops and maintaining the desired concentration and distribution of ions and water in the soil (Dieberg & Reuter, 1985; Coelho & Dool, 1999).

Quality requirements are all influenced by plant nutrition. Therefore, fertilizer should not only ensure high yields per unit area but also high quality produce by the improvement of either low initial quality caused by insufficient nutrient supplies, or the maintenance of high quality. The chemical composition controls the nutritional quality or value, as well as important sensory attributes such as taste and texture of the product. Increased secondary metabolites in plants generally increases in some plants compounds such as amino acids, proteins and chlorophyll (Ongile, 1998).

CHAPTER 3

EFFECT OF FERTIGATION FREQUENCY AND GROWING MEDIUM ON GROWTH, OIL QUANTITY AND QUALITY AND THE YIELD OF WILD GINGER

3.1 Introduction

Fertigation refers to the application of nutrients through an irrigation system. The most common nutrient applied is N and elements less applied include P, K, S, Zn and Fe. It combines the two main factors in plant growth and development, water and nutrients. However, the right application of water and nutrients is the key for high yield and quality (Rollett, 2002).

The combined application of irrigation and nitrogen through fertigation is now becoming a common practice in modern agriculture because of its advantages over conventional methods (Asadi *et al.*, 2002). They reported that some of these advantages included timely nitrogen application, excellent uniformity of nitrogen application, reduced environmental contamination, adequate movement of applied N into the rooting zone by irrigation water and reduced soil compaction and mechanical damage to the crop.

Irrigation and fertilization are the most important management factors through which farmerS control plant development, crop yields and quality. Most studies showed that the introduction of simultaneous microirrigation and fertilization (fertigation) opened up new possibilities for controlling water and nutrient supplies to crops and maintaining the desired concentration and distribution of ions and water in the soil (Dasberg & Bresler, 1985; Coelho & Dani, 1999).

Quality requirements are all influenced by plant nutrition. Therefore, fertilization should not only ensure high yields per unit area but also high quality produce by the improvement of either low initial quality caused by insufficient nutrient supplies, or the maintenance of high quality. The chemical composition controls the nutritional quality or value, as well as important sensory attributes such as taste and texture of the product. Increased concentration of nitrogen in plants generally increases in some plants compounds such as amino acids, proteins and chlorophyll (Oagile, 1998).

There is no information about fertigation of wild ginger and the adaptability of the crop to growing medium as well as the influence of fertigation frequency and growing medium on the oil of wild ginger rhizomes and enlarged roots. This study was conducted to determine the influence of fertigation frequency on the growth, yield and the oil quantity and the quality of wild ginger grown in either pine bark or sand.

3.2 Materials and Methods

3.2.1 Location

The experiment was conducted in a tunnel at the University of Pretoria's Hatfield Experimental Farm. Wild ginger plants were planted together with other four plant species (viz. African potato, fever tea, bush tea and pineapple flower).

3.2.2 Treatments

The growth, oil quantity and quality and the yield of wild ginger were tested in a split plot design with five fertigation frequencies (0.25L/day, 1L/day, 2L/day, 2L /2nd day and 2L/week) and two growing media (pine bark and sand) replicated twice. Before the experiment was established, the fertility status of the growing media was determined (Appendix B).

Fertigation frequencies were applied through drip irrigation system. For every fertilization through the irrigation system, one (1) g of the fertilizer mixture (feed all) was dissolved in one (1) litre of water (Table 4.1). Each of the five fertigation frequencies consisted of twenty plants giving a total plant population of 200 plants for the experiment. Each planting material planted in a bag was regarded as a replicate which gave the order of 5 x 2 x 20 arrangement.

3.2.3 Planting materials

Planting materials used were young sprouted wild ginger rhizomes obtained from KwaZulu-Natal. Two hundred rhizomes were used with each planted in a 10 L plastic bag. One hundred rhizomes were planted in 10 L plastic bags filled with sand and the other one hundred planted in 10 L plastic bags filled with pine bark. The tunnel consisted of five rows in which plastic bags

were placed. Each row had two hundred plastic bags and planted in with either wild ginger plants, African potato, fever tea, bush tea or pineapple flower plants.

Table 3.1 Nutritional elements which were fertigated on wild ginger during 2002/2003 growing seasons

Element	Quantity
Nitrogen	160 g·kg ⁻¹
Phosphorus	50 g·kg ⁻¹
Potassium	220 g·kg ⁻¹
Calcium	11 g·kg ⁻¹
Magnesium	3 g·kg ⁻¹
Boron	335 mg·kg ⁻¹
Iron	356 mg·kg ⁻¹
Zinc	100 mg·kg ⁻¹
Manganese	125 mg·kg ⁻¹
Molybdenum	12.5 mg·kg ⁻¹
Copper	12.5 mg·kg ⁻¹

3.2.4 Records

Plant growth parameters such as plant height, number of leaves and stems at 56, 112, 168 and 224 days after emergence (DAE), fresh and dry leaf mass and leaf area at 112 and 224 DAE were measured. Yield parameters determined were fresh and dry rhizomes and enlarged roots, length of enlarged roots and the number of rhizomes and enlarged roots at 112 and 224 DAE. During the initial harvest (112 DAE) six plants were harvested per fertigation frequency in a replication and three plants were harvested from bags filled with pine bark and three from sand and the same was done with the other replication which gave a total of sixty plants. Ten plants were harvested before the termination of the experiment for the anatomy study of the enlarged roots of wild ginger. The remaining hundred and forty plants were harvested at the final harvest (224 DAE).

Fresh and dry rhizomes and enlarged root mass were recorded at 112 DAE whereas at 224 DAE, only the fresh rhizome and enlarged root mass was measured because samples were immediately taken to be hydrodistilled for essential oil determination.

Twenty samples of fresh rhizomes and enlarged roots were taken to the University of Witwatersrand at the Department of Pharmacy and Pharmacology for hydrodistillation of the essential oil. The hydrodistillation method used was similar to that described on the paper published by Viljoen, Demirci, Bayer and van Wyk (2002).

3.2.5 Sampling and plant analysis

Destructive analyses of plants were made on two occasions (112 and 224 DAE). Five randomly selected plants from each fertigation frequency were used at each sampling date. Measurements included fresh and dry leaf mass and leaf area. Leaf area was measured using a model 3100 leaf area meter (England). Dried and weighed samples were ground, bulked and mixed thoroughly from replicates to reduce the number of samples for chemical analysis. Sub-samples were taken from each fertigation frequency which were subsequently analysed for N, P and K. However, samples from replicates at both 112 and 224 DAE were bulked for chemical analysis in order to determine N, P and K fluctuations in the leaf during the entire growing season.

For the determination of N and P, 1 g of selenium and 300 g of K_2SO_4 was added to 800 cm³ of sulphuric acid in a 3 dm³ pyrex beaker. The beaker was covered with a watch glass and heated up to 400°C to dissolve the Se and K_2SO_4 . A dried sample of 0.5 g of leaves and rhizome samples from each fertigation frequency and either grown in pine or in sand was grounded to pass 0.5-1 mm mesh sieve into a digestive tubes. 5 cm³ of digestive mixture was added and swirled until the sample was moistened. The solution was cooled for 2 hours and three successively 1 cm³ of hydrogen peroxide were added. The tubes were placed into the digestive block and heated up at 330°C. The tube was removed from the block and cooled at room temperature.

For the determination of K, a 0.5 g of the leaf and rhizome samples were ground to pass 0.5-1 mm mesh sieve and placed into a digestive tubes. Thereafter, concentrated HNO_3 was added at 10 cm³. Plant samples were digested for the determination of total N, using sulphuric acid to

bind and reduce nitrate and HNO_3 . Tubes were placed in digestion blocks and boiled very gently until the production of red NO_2 fumes has ceased and dense white fumes appeared. The tubes were cooled and small amount ($2\text{-}4\text{ cm}^3$) of 70% HClO_4 was added. The tubes were heated again to allow small volume 70% (HClO_4) of to evaporate. Three glass beads were added into each tube and again 5 cm^3 of digestive mixture was added. The tubes were placed in the rack on a cold digestion block and the scrubber hood was placed on the rack. . The power was switched on at 230°C to heat up the digestion block. Samples were digested for 70 minutes, thereafter the rack was remove to let it cooled.

3.2:6 Data

The data was analysed using the general linear model (GLM) procedure within the SAS computer software (SAS Institute, Inc., 1996).

3.3 Results and Discussion

3.3.1 Growth analysis

3.3.1.1 Plant height as affected by fertigation frequency and growing medium

There were no interactions between fertigation frequency and the growing medium used at 56, 112 and 224 days after emergence (DAE). At 56 DAE, fertigation frequency resulted in differences in plant height (Table 3.2). Plants that received 2L/week resulted in the tallest plants that were significantly bigger than plants fertigated with 2L/day, but were not different from plants from any other fertigation frequency. At 112 DAE plants that received 2L/week were significantly shorter than plants from all other fertigation frequencies (Table 3.2). There was a significant interaction between fertigation frequency and growing medium used at 168 DAE (Fig. 3.1). Fertigation frequency did not affect plant height for plants grown in pine bark, but did for plants grown in sand. Plants that received 2L/day resulted in significantly shorter plant height as compared to plants grown in other fertigation frequencies.

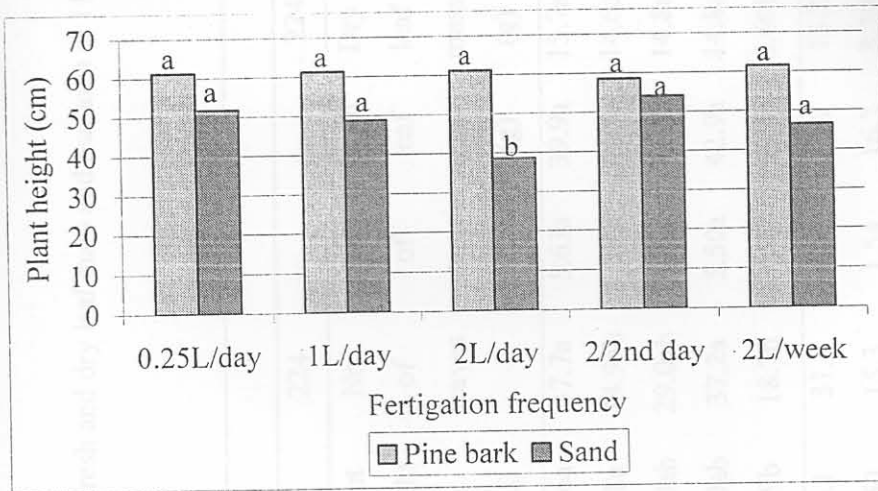


Fig. 3.1 Plant height as affected by fertigation frequency and growing medium at 168 DAE

At 224 DAE, plants that received 2L/week resulted in significantly shorter plants as compared to plants fertigated with 0.25L/day and 1L/day, but were not significantly different from plants that received 2L/day and 2L/2nd day (Table 3.2).

Growing medium affected plant height in all sampling dates. At 56 DAE, plants grown in pine bark were significantly taller than plants grown in sand (Table 3.3). Similar results were obtained at 112, 168 and 224 DAE sampling dates. However, the greatest difference in plant height (± 13 cm) between pine bark and sand was at 168 DAE.

3.3.1.2 Number of leaves as affected by fertigation frequency and growing medium

There were no interactions between fertigation frequency and growing medium used for the number of leaves at all sampling dates. However, at 56 DAE, plants that received the highest fertigation frequency (2L/day) had significantly less number of leaves compared to plants that received 0.25L/day, 1L/day, 2L/2nd second day and 2L/week (Table 3.2). However, at 112 DAE, plants fertigated with 2L/week resulted in a significantly fewer number of leaves compared to the other fertigation frequencies (Table 3.2).

Table 3.2 Wild ginger plant height, number of leaves and stems at 56, 112, 168 and 224 DAE and fresh and dry leaf mass and leaf area at 112 and 224 DAE as affected by fertigation frequency

Fertigation frequency	Time (days after emergence) ^z													
	56			112			168		224			224		
	Plant height (cm)	No. of leaves	No. of stems	Plant height (cm)	No. of leaves	No. of stems	No. of leaves	No. of stems	Plant height (cm)	No. of leaves	No. of stems	Fresh leaf mass (g)	Dry leaf mass (g)	Leaf area (cm ²)
0.25L/day	17.0ab	3.82a	1.25a	49.6ab	16.6a	4.05ab	35.8b	4.29a	58.6a	37.7a	5.63a	39.9a	15.7a	1259b
1L/day	17.0ab	3.52a	1.12ab	47.5a	14.6a	3.47bc	39.5a	4.75a	59.8a	34.9ab	4.88ab	42.5a	14.6a	791.5b
2L/day	15.4b	2.85b	1.07b	52.7a	14.6a	4.10ab	42.2a	5.07a	56.0ab	29.0ab	4.85ab	53.8a	14.8a	1044b
2L/2 nd day	17.0ab	3.47a	1.12ab	51.6a	16.4a	4.32a	39.7a	4.71a	55.0ab	37.2a	5.50a	42.9a	14.8a	2228a
2L/week	18.1a	3.87a	1.20ab	40.2b	11.3b	2.67c	43.2a	5.33a	49.9b	18.7b	3.68b	19.4a	6.60b	610.0b
Means	17.0	3.50	1.13	48.4	14.7	3.72	40.1	4.83	56.1	31.5	4.91	39.7	13.2	1186
LSD	2.29	0.58	0.15	4.67	2.69	0.82	3.91	1.97	8.60	15.3	1.54	16.2	8.30	605.4

^z Means followed by the same letter within the column are not significantly different at 5% level of probability

Table 3.2 Wild ginger plant height, number of leaves and stems at 56, 112, 168 and 224 DAE and fresh and dry leaf mass and leaf area at 112 and 224 DAE as affected by fertigation frequency

Fertigation frequency	Time (days after emergence) ^z													
	56			112			168		224			224		
	Plant height (cm)	No. of leaves	No. of stems	Plant height (cm)	No. of leaves	No. of stems	No. of leaves	No. of stems	Plant height (cm)	No. of leaves	No. of stems	Fresh leaf mass (g)	Dry leaf mass (g)	Leaf area (cm ²)
0.25L/day	17.0ab	3.82a	1.25a	49.6ab	16.6a	4.05ab	35.8b	4.29a	58.6a	37.7a	5.63a	39.9a	15.7a	1259b
1L/day	17.0ab	3.52a	1.12ab	47.5a	14.6a	3.47bc	39.5a	4.75a	59.8a	34.9ab	4.88ab	42.5a	14.6a	791.5b
2L/day	15.4b	2.85b	1.07b	52.7a	14.6a	4.10ab	42.2a	5.07a	56.0ab	29.0ab	4.85ab	53.8a	14.8a	1044b
2L/2 nd day	17.0ab	3.47a	1.12ab	51.6a	16.4a	4.32a	39.7a	4.71a	55.0ab	37.2a	5.50a	42.9a	14.8a	2228a
2L/week	18.1a	3.87a	1.20ab	40.2b	11.3b	2.67c	43.2a	5.33a	49.9b	18.7b	3.68b	19.4a	6.60b	610.0b
Means	17.0	3.50	1.13	48.4	14.7	3.72	40.1	4.83	56.1	31.5	4.91	39.7	13.2	1186
LSD	2.29	0.58	0.15	4.67	2.69	0.82	3.91	1.97	8.60	15.3	1.54	16.2	8.30	605.4

^z Means followed by the same letter within the column are not significantly different at 5% level of probability

A fertigation frequency of 0.25L/day resulted in significantly lower number of leaves compared to the other fertigation frequencies at 168 DAE (Table 3.2). However, at 224 DAE, plants that received 2L/week produced a significantly fewer number of leaves as compared to plants that received 0.25L/day and 2L/2nd day, but not significantly different from plants fertigated with 1L/day and 2L/day (Table 3.2).

At 56 DAE, there was significantly more number of leaves per plant in plants grown in pine bark compared to those grown in sand (Table 3.3). Similar results were found during the 112, 168 and 224 DAE sampling dates. In plants grown in pine bark, there was an average of four leaves per plant at 56 DAE, which increased to 49 leaves at 168 DAE, and thereafter declined to 35 leaves as these leaves started senescing. In plants grown in sand, on the other hand, there was an average of 3 leaves per plant at 56 DAE and increased to 11 leaves per plant at 112 DAE, and by 168 DAE, the number of leaves had increased to 31, and thereafter dropped to 29 at 224 DAE.

3.3.1.3 Number of stems as affected by fertigation frequency and growing medium

There were no interactions between fertigation frequency and growing medium found with number of stems at all sampling dates. At 56 DAE, plants fertigated with 0.25L/day had significantly more stems per plant than plants fertigated with 2L/day (Table 3.2). By 112 DAE, plants fertigated with 2L/2nd day had significantly more stems per plant than plants fertigated with 2L/week. However, at 168 DAE, there were no significant differences in number of stems amongst plants fertigated differently. By 224 DAE, plants that received 2L/week had significantly lower number of stems as compared to plants fertigated with 0.25L/day and 2L/2nd day, but were not different from plants receiving 1L/day and 2L/day.

At 56 and 112 DAE, plants grown in pine bark produced significantly more stems as compared to those grown in sand (Table 3.3). Growing medium did not significantly affect number of stems at 168 and 224 DAE.

3.3.1.4 Fresh and dry leaf mass as affected by fertigation frequency and growing medium

Significant interactions were found between fertigation frequency and growing medium with fresh leaf mass at 112 DAE (Fig. 3.2). For plants grown in pine bark, plants fertigated with 1L/day resulted in significantly higher fresh leaf mass as compared to plants grown in other fertigation frequencies, whereas for plants grown in sand, plants fertigated with 2L/day and 2L/2nd day produced significantly higher fresh leaf mass as compared to plants that received 1L/day.

There were no interactions between fertigation frequency and growing medium with fresh leaf mass at 224 DAE. As opposed to 112, at 224 DAE fresh leaf mass was not significantly affected by fertigation frequency and growing medium (Table 3.2 & 3.3).

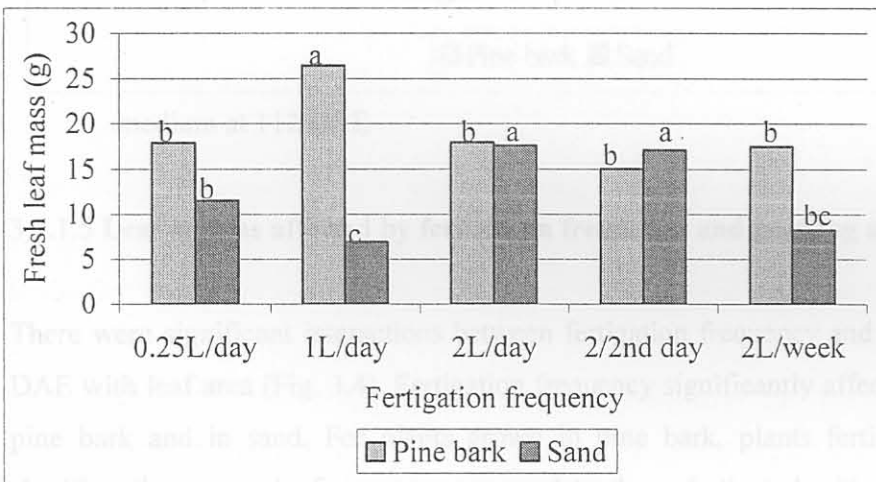
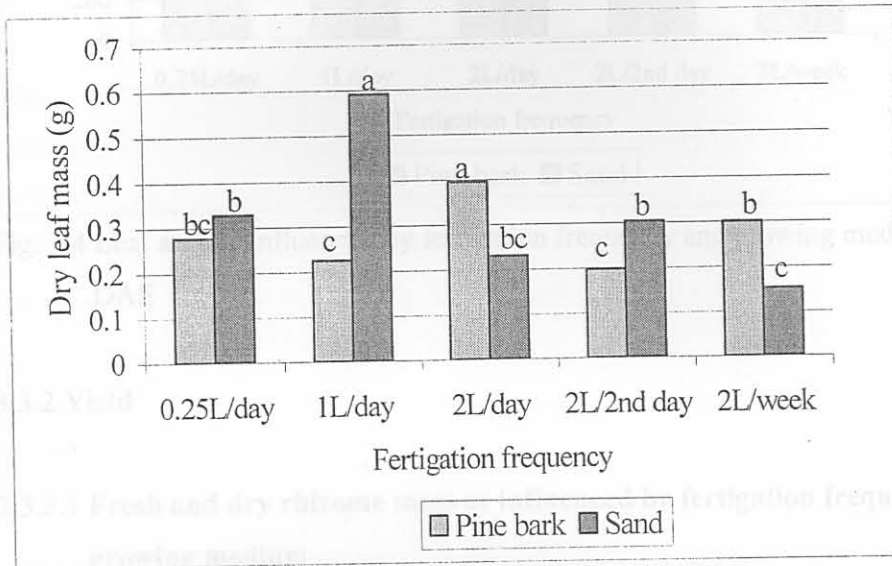


Fig. 3.2 Fresh leaf mass as affected by fertigation frequency and growing medium at 112 DAE

Significant interactions between fertigation frequency and growing medium were found with dry leaf mass at 112 DAE (Fig. 3.3). For plants grown in pine bark, plants fertigated with 1L/day resulted in significantly lower dry leaf mass as compared to plants fertigated with 2L/day. However, for plants grown in sand, a fertigation frequency of 1L/day had significantly more dry leaf mass as compared to plants grown in other fertigation frequencies.

There were no interactions between fertigation frequency and growing medium found with dry leaf mass at 224 DAE. However, plants fertigated with 2L/week resulted in significantly lower dry leaf mass as compared to the all other fertigation frequencies (Table 3.2). Dry leaf mass was not significantly affected by growing medium at 224 DAE (Table 3.3).

Fig. 3.3 Relationship between dry leaf mass, fertigation frequency and growing



medium at 112 DAE

3.3.1.5 Leaf area as affected by fertigation frequency and growing medium

There were significant interactions between fertigation frequency and growing medium at 112 DAE with leaf area (Fig. 3.4). Fertigation frequency significantly affected both plants grown in pine bark and in sand. For plants grown in pine bark, plants fertigated with 1L/day had significantly greater leaf areas as compared to those fertigated with 0.25L/day, 2L/week and 2L/2nd day. For plants grown in sand, plants that received 2L/day had significantly greater leaf areas as compared to plants that received 0.25L/day, 1L/day, 2L/2nd day and 2L/week.

There were no interactions between fertigation frequency and growing medium with leaf area at 224 DAE. However, fertigation frequency significantly affected leaf area at 224 DAE. Plants that received 2L/2nd day resulted in significantly greater leaf area as compared to all other fertigation frequencies (Table 3.2). Leaf area was not affected by growing medium at 224 DAE (Table 3.3).

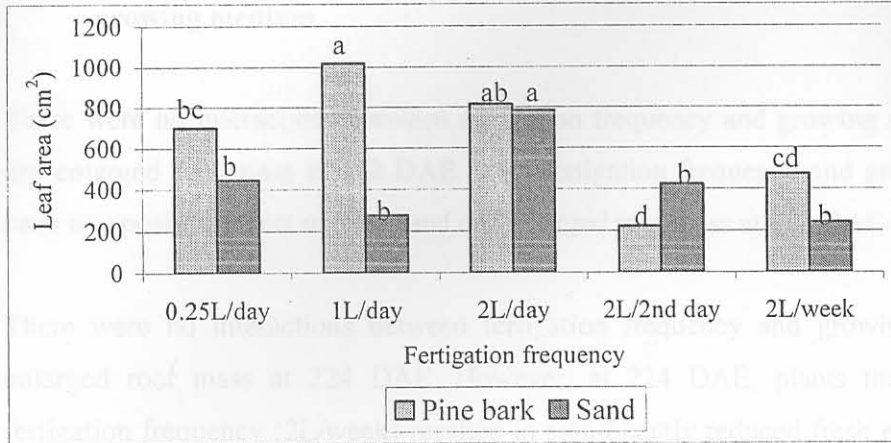


Fig. 3.4 Leaf area as influenced by fertigation frequency and growing medium at 112 DAE

3.3.2 Yield

3.3.2.1 Fresh and dry rhizome mass as influenced by fertigation frequency and growing medium

There were no interactions between fertigation frequency and growing medium with fresh and dry rhizome mass at 112 and 224 DAE. Fresh and dry rhizome mass were also not affected by fertigation frequency and growing medium at 112 DAE (Tables 3.4 and 3.5).

There were no interactions between fertigation frequency and growing medium with fresh rhizome mass at 224 DAE. However, fertigation frequency significantly affected fresh rhizome mass at 224 DAE (Table 3.4). Plants fertigated with 0.25L/day, 2L/day and 2L/2nd day had significantly greater fresh rhizome mass as compared to those of plants that received 1L/day and those that received 2L/week. These results showed that fresh rhizome mass required little fertigation at later stages of wild ginger growth and development.

3.3.2.2 Fresh and dry enlarged root mass as affected by fertigation frequency and growing medium

There were no interactions between fertigation frequency and growing medium with fresh and dry enlarged root mass at 112 DAE. Also fertigation frequency and growing medium did not have any positive effect on fresh and dry enlarged root mass at 112 DAE (Tables 3.4 and 3.5).

There were no interactions between fertigation frequency and growing medium with fresh enlarged root mass at 224 DAE. However, at 224 DAE, plants that received the lowest fertigation frequency (2L/week) resulted in significantly reduced fresh enlarged root mass than those of plants that received any other fertigation frequency (Table 3.4). Growing medium did not affect fresh enlarged root mass at 224 DAE (Table 3.5).

These results were in agreement with the results found by Avner (2003), who found that increasing daily fertigation frequency induced significant increase in yield of greenhouse crops. Yield improvement was primarily related to enhanced uptake of nutrients, especially phosphorus. Such results suggest that the reduced yield obtained at low frequency resulted from deficiency of nutrients rather than of water, and that high fertigation frequencies could compensate for nutrient deficiency. Furthermore, Avner (2003) reported that an increase in fertigation frequency enables the concentration in immobile elements such as P and K and trace metals in irrigation water to be reduced, thus reducing environmental pollution.

3.3.2.3 Length of enlarged roots as affected by fertigation frequency and growing medium

There were no interactions between fertigation frequency and growing medium with length of enlarged roots at 112 and 224 DAE. Fertigation frequency and growing medium also did not affect the length of enlarged roots at both 112 and 224 DAE (Tables 3.4 and 3.5).

Table 3.4 Fresh and dry rhizome and enlarged root characteristics as affected fertigation frequency at 112 and 224 DAE during 2002/2003 seasons

Fertigation frequency	Yield ^z										
	112 DAE						224 DAE				
	Fresh rhizome mass (g)	Dry rhizome mass (g)	Number of rhizomes	Fresh enlarged root mass (g)	Dry enlarged root mass (g)	Length of enlarged roots (cm)	Fresh rhizomes mass (g)	Number of rhizomes	Fresh enlarged root mass (g)	Number of enlarged roots	Length of enlarged roots (cm)
0.25L/day	32.0a	3.04a	4.83a	15.7a	0.74a	5.40a	161.5a	7.40a	151.5a	54.7a	7.80a
1L/day	35.0a	3.38a	5.33a	16.7a	0.90a	5.35a	121.1b	6.10a	159.2a	47.4a	8.10a
2L/day	32.7a	3.65a	5.25a	13.5a	1.01a	5.12a	163.8a	6.60a	175.8a	57.6a	8.40a
2L/2 nd day	41.0a	4.18a	5.66a	16.0a	1.03a	5.70a	178.0a	6.80a	158.7a	52.8a	8.30a
2L/week	40.7a	4.05a	5.00a	16.3a	1.08a	5.89a	76.50b	5.60a	66.10b	23.6b	7.80a
Means	36.3	3.66	5.21	15.6	0.95	5.49	410.2	6.50	142.3	47.2	8.10
LSD	18.4	1.64	1.54	6.90	0.40	1.39	60.2	2.30	60.9	16.6	0.80

^zMeans followed by the same letter within the column are not significantly different at 5% level of probability

Table 3.5 Fresh and dry rhizome mass, number of rhizomes, fresh and dry enlarged root mass, number of enlarged roots and length of enlarged root as affected by growing medium at 112 and 224 DAE during 2002/03 seasons

Growing medium	Yield ^z										
	112						224				
	Fresh rhizome mass (g)	Dry rhizome mass (g)	Number of rhizomes	Fresh enlarged root mass (g)	Dry enlarged root mass (g)	Length of enlarged root (cm)	Fresh rhizome mass (g)	Number of rhizomes	Fresh enlarged root mass (g)	Number of roots	Length of enlarged root (cm)
Pine bark	41.9a	3.70a	5.20a	14.2a	1.10a	5.80a	151.4a	6.60a	149.1a	48.2a	7.90a
Sand	30.6a	3.50a	5.80a	12.8a	0.80a	5.18a	193.8a	6.50a	173.2a	58.7a	8.40a
Means	36.3	3.70	5.50	13.5	1.00	5.49	172.6	6.50	161.2	53.5	8.10
LSD	11.7	1.04	0.90	3.60	0.30	0.80	49.6	0.95	39.1	10.6	0.59

^z Means followed by the same letter within the column are not significantly different at 5% level of probability

3.3.2.4 Number of rhizomes as affected by fertigation frequency and growing medium

There were no interactions between fertigation frequency and growing medium with number of rhizomes at 112 and 224 DAE. Number of rhizomes was also not affected by fertigation frequency and growing medium at 112 and 224 DAE (Table 3.4).

3.3.2.5 Number of enlarged roots as influenced by fertigation frequency and growing medium

There were highly significant interactions between fertigation frequencies and growing medium used at 112 DAE with number of enlarged roots (Fig. 3.5). For plants grown in pine bark, plants that received 2L/week resulted in significantly lower number of enlarged roots as compared to plants fertigated with 1L/day. For plants grown in sand, plants fertigated with 2L/2nd day resulted in significantly more number of enlarged roots as compared to plants that received 1L/day.

There were no interactions between fertigation frequency and growing medium with number of enlarged roots at 224 DAE. However, plants fertigated with 0.25L/day, 1L/day, 2L/day and 2L/2nd day produced significantly more number of enlarged roots as compared to plants that received 2L/week (Table 3.4). Growing medium did not affect the number of enlarged roots at 224 DAE (Table 3.5).

3.3.2.6 Fresh rhizome and enlarged root oil yield as influenced by fertigation frequency and growing medium

There were no interactions between fertigation frequency and growing medium with fresh rhizome and enlarged root oil yield at 224 DAE. Fertigation frequency as well as growing medium also did not significantly affect fresh rhizome oil yield (Tables 3.6 and 3.7).

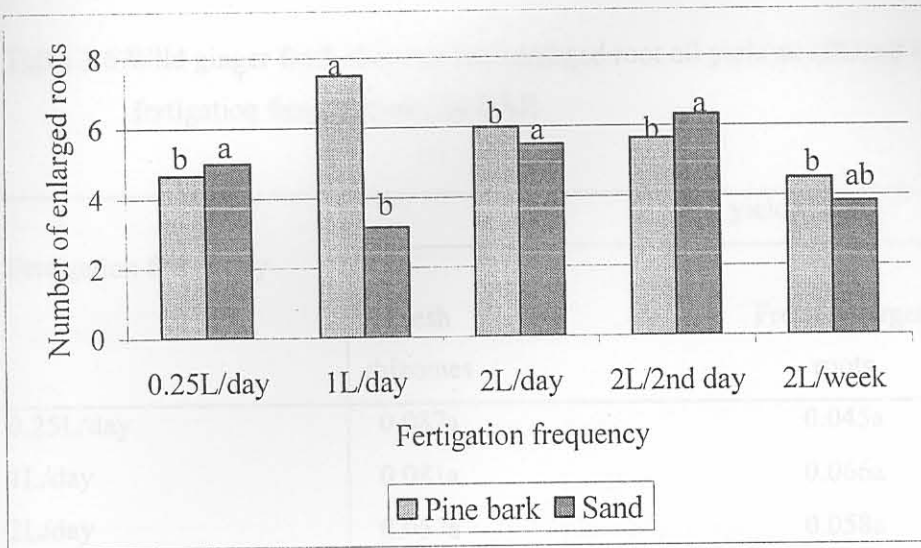


Fig.3.5 Number of enlarged roots affected by fertigation frequency and growing medium at 112 DAE

3.3.3 Leaf nutrient analysis

The purpose of leaf analysis is to determine the nutrient status of the crop and it is used in conjunction with soil analysis as a useful tool in determining nutrient deficiencies and making fertilizer recommendations (FSSA, 2003).

3.3.3.1 N, P and K concentrations in leaves as affected by fertigation frequency and growing medium at 112 DAE

There were no interactions between fertigation frequency and growing medium with concentrations of N, P and K in leaves at 112 DAE. Fertigation frequency also did not affect N and P concentrations at 112 DAE, but fertigation frequencies of 2L/day and 2L/week increased concentration of K with 59% (Table 3.8). Although plants that received 2L/day at initial and 2L/week at later growth stages resulted in reduced plant growth and a reduction in yield, they had resulted in the maximum K concentration in leaves (Tables 3.2 and 3.4).

Concentration of N and P were not affected by growing medium, but plants grown in sand had significantly lower K concentration as compared to that of plants grown in pine bark at 112 DAE (Table 3.9).

Table 3.6 Wild ginger fresh rhizome and enlarged root oil yield as affected by fertigation frequency at 224 DAE

Fertigation frequency	Oil yield (%) ^z	
	Fresh rhizomes	Fresh enlarged roots
0.25L/day	0.087a	0.045a
1L/day	0.081a	0.066a
2L/day	0.057a	0.058a
2/2 nd day	0.052a	0.053a
2L/week	0.071a	0.089a
Means	0.069	0.062
LSD	0.054	0.044

^z Means with the same letter within a column are not significant different at 5% level of probability

3.3.3.2 N, P and K concentrations in leaves as affected by fertigation frequency and growing medium at 224 DAE

There were no interactions between fertigation frequency and growing medium with N, P and K concentrations in leaves at 224 DAE. However, fertigation frequency did not affected concentrations of N and P in leaves, but fertigation frequencies of 2L/day and 2L/2nd day significantly increased the concentration of K in leaves, but these were not significantly different from plants fertigated with 0.25L/day and 2L/week (Table 3.6).

Growing medium did not have any significant effect on the concentration of P and K in leaves, but plants grown in sand had shown an increased in higher N concentration as compared to that of plants grown in pine bark (Table 3.7).

Table 3.7 Wild ginger fresh rhizomes and enlarged roots oil yield as affected by growing medium at 224 DAE

Growing medium	Oil yield ^z	
	Fresh rhizomes	Fresh enlarged roots
Pine bark	0.073a	0.055a
Sand	0.069a	0.069a
Means	0.070	0.062
LSD	0.038	0.042

^zMeans with the same letter within a column are not significant different at 5% level of probability

3.3.3.3 N, P and K concentrations in rhizomes as affected by fertigation frequency and growing medium at 224 DAE

There were no interactions between fertigation frequency and growing medium found with concentrations of N, P and K in rhizomes. The concentrations of N and K in rhizomes were also not affected by fertigation frequency at 224 DAE (Table 3.6). Plants that received 1L/day resulted in significantly more P concentration as compared to plants fertigated with 0.25L/day, 2L/day, 2L/2nd day and 2L/week. Interestingly, plants fertigated with 1L/day resulted in poor plant growth and a reduction in yield at 224 DAE, despite more concentration of P in the rhizomes than any other fertigation frequency (Table 3.2).

Growing medium did not have any significant effect on the concentration of N and P, but plants grown in sand had shown a reduction in K concentration as compared to that of plants grown in pine bark at 224 DAE (Table 3.9).

Table 3.8 Leaf and rhizome nutrient analysis as affected by fertigation frequency at 112 and 224 DAE during 2002/2003 seasons

Fertigation frequency	Leaf nutrient analysis ^z						Rhizome nutrient analysis ^z		
	112 DAE			224			224 DAE		
	N %	P %	K %	N %	P %	K %	N %	P %	K %
0.25L/day	2.79a	0.49a	2.88b	0.89a	0.33a	3.22ab	3.29a	0.47a	1.09a
1L/day	3.78a	0.56a	3.34b	0.97a	0.35a	3.05b	4.73a	0.64b	1.41a
2L/day	3.34a	0.58a	4.47a	1.22a	0.34a	4.36a	3.53a	0.54a	1.25a
2L/2 nd day	3.11a	0.57a	3.86ab	1.35a	0.35a	4.37a	3.32a	0.52a	1.31a
2L/week	2.73a	0.49a	4.25a	0.99a	0.33a	4.01ab	2.55a	0.50a	1.80a
Means	3.05	0.53	3.56	1.08	0.34	3.78	3.48	0.53	1.37
LSD	1.12	0.10	0.36	0.52	0.03	1.21	2.20	0.08	0.76

^z Means followed by the same letter within the column are not significantly different at 5% level of probability

Table 3.8 Leaf and rhizome nutrient analysis as affected by fertigation frequency at 112 and 224 DAE during 2002/2003 seasons

Fertigation frequency	Leaf nutrient analysis ^z						Rhizome nutrient analysis ^z		
	112 DAE			224			224 DAE		
	N %	P %	K %	N %	P %	K %	N %	P %	K %
0.25L/day	2.79a	0.49a	2.88b	0.89a	0.33a	3.22ab	3.29a	0.47a	1.09a
1L/day	3.78a	0.56a	3.34b	0.97a	0.35a	3.05b	4.73a	0.64b	1.41a
2L/day	3.34a	0.58a	4.47a	1.22a	0.34a	4.36a	3.53a	0.54a	1.25a
2L/2 nd day	3.11a	0.57a	3.86ab	1.35a	0.35a	4.37a	3.32a	0.52a	1.31a
2L/week	2.73a	0.49a	4.25a	0.99a	0.33a	4.01ab	2.55a	0.50a	1.80a
Means	3.05	0.53	3.56	1.08	0.34	3.78	3.48	0.53	1.37
LSD	1.12	0.10	0.36	0.52	0.03	1.21	2.20	0.08	0.76

^z Means followed by the same letter within the column are not significantly different at 5% level of probability

3.3.4 Growing media analysis

Nutritional status of pine bark was analysed before the trial started. It was observed that nitrates (NO_3^-) and ammonium (NH_4^+) in pine bark were very high prior to the application of fertigation frequency treatments. NO_3^- content was 45 mg/L and NH_4^+ was 198 mg/L. This was apparent as fresh rhizome mass for plants grown in pine bark at the initial harvest (112 DAE) were higher and at the final harvest, (224 DAE) there was no difference in yield realized with plants grown in sand (Appendix B4).

During the final harvest at 224 DAE, fertility status of pine bark for N, P and K was not analyzed for the growing media. Only the pH status and electrical conductivity of pine bark was analyzed, as it was apparent from the initial analysis that pine bark contained adequate nutrients for plant growth. However, it was found that fertigation frequency did not affect the pH of pine bark as it remained acidic, but electrical conductivity (EC) was affected (Table 3.10). EC estimates the amount of dissolved salts in the water. The EC for plants that received more frequent fertigation (2L/day) was low as compared to plants that received medium frequent fertigation (1L /day and 2L/2nd day) and very low for plants that received less frequent fertigation (0.25L/day and 2L/week) (Table 3.10).

Plants fertigated with 0.25L/day had the lowest EC as compared to the other fertigation frequencies, but had better plant growth compared to plants that received more frequent fertigation (2L/day), but not different from plants fertigated with 1L/day, 2L/2nd day and 2L/week at 112 DAE (Table 3.10). As opposed to 112 DAE, at 224, plants fertigated with 2L/day resulted in significantly better plant growth as compared to the other fertigation frequencies.

4.5 Summary

The study was undertaken to determine the response of wild yinger growth, till quantity and quality and the yield to five fertigation frequencies (0.25L/day, 1L/day, 2L/day, 2L/2nd day and 2L/week) and two growing media (pine bark and sand). An experiment was conducted in a tunnel at the University of Pretoria's Hatfield Experimental Farm.

Table 3.10 pH and electrical conductivity analysis of pine bark as affected by fertigation frequency at 224 DAE

Fertigation frequency	PH (Kcl)	Electrical conductivity (µS/cm)
0.25 L/day	4.590	0.970
1L/day	4.900	2.420
2L/day	4.680	1.910
2L/2 nd day	4.770	3.00
2L/week	4.810	1.310

4.4 Conclusions

Response of wild ginger growth, oil quantity and yield to fertigation frequency and growing medium is depended on the sampling date. During the initial sampling date (56 DAE), a fertigation frequency of 2L/day is not recommended to improve wild ginger growth and at later sampling dates (112, 168 and 224 DAE) as well, a fertigation frequency of 2L/week is not recommended to improve wild ginger growth Wild ginger plants should be grown in pine bark during initial growth stages (56 and 112 DAE) and at later stages of growth (168 and 224 DAE) should be produced in sand. For the production of fresh rhizome yield, wild ginger plants should be fertigated with 2L/2nd day and for the fresh enlarged root yield, plants should be fertigated with 2L/day at later stages of growth. Either pine bark or sand is recommended to produce yield of wild ginger at initial as well as later stages of development.

4.5 Summary

The study was undertaken to determine the response of wild ginger growth, oil quantity and quality and the yield to five fertigation frequencies (0.25L/day, 1L/day, 2L/day, 2L/2nd day and 2L/week) and two growing media (pine bark and sand). An experiment was conducted in a tunnel at the University of Pretoria's Hatfield Experimental Farm.

Measurements were made of plant growth such as plant height and the number of leaves and stems at 56, 112, 168 and 224 DAE, fresh and dry leaf mass and leaf area and yield parameters such as fresh and dry rhizome mass, fresh and dry enlarged root mass, length of enlarged root and the number of rhizomes and enlarged roots at 112 and 224 DAE. Fresh rhizome and enlarged root was not oven dried at 224 DAE, because samples were taken for hydrodistillation of essential oil immediately after harvest. Records made were for fresh rhizomes and enlarged root oil yield.

At 56, 112 and 168 DAE, plants grown in pine bark had better growth and increased fresh rhizome mass and the number of rhizomes as compared to plants grown in sand, but at 224 DAE, there were no differences in growth for both media. Plants grown in pine bark had better fresh rhizome yield and more number of rhizomes while those grown in sand had better fresh enlarged roots and more number of enlarged roots. At 112 DAE, fertigation frequency and growing medium did not affect yield of fresh rhizomes and enlarged roots.

Wild ginger plants that received highest fertigation frequency (2L/day) had significantly higher yield as compared to plants that received the lowest fertigation frequency (2L/week). Also growing media did not affect fresh rhizomes and enlarged roots yield. Fertigation frequency and growing media did not affected fresh rhizome and enlarged root oil yield at 224 DAE.

Fertigation frequency and growing medium did not affect the concentration of N and P in leaves at 112 and 224 DAE, but affected the concentration of K. Wild ginger plants that received 2L/day have shown a 55% increase in K concentration in leaves. At 112 DAE, plants grown in pine bark produced more K concentration than for those grown in sand and at 224 DAE, more N concentration was realized for plants grown in pine bark than in sand. The concentrations of N and K in rhizomes were not affected by fertigation frequency, but P concentration was affected. Wild ginger plants fertigated with 1L/day have shown an increase by 36% in the concentration of P. Wild ginger plants grown in pine bark had more K concentration in rhizomes higher than those grown in sand.

During the initial sampling date (56 DAE), exceptionally a fertigation frequency of 2L/day is not recommended to improve wild ginger growth in that plants are still developing roots and unable to utilized too much fertigation supplied to them and at later sampling dates (112, 168 and 224

DAE) as well, a fertigation frequency of 2L/week is not recommended to improve wild ginger growth in that plants were big, therefore required more rather adequate fertigation to improve their growth. Wild ginger plants should be grown in pine bark during initial growth stages (56 and 112 DAE) and at later stages of growth (168 and 224 DAE) should be produced in sand. For the production of fresh rhizome yield, wild ginger plants should be fertigated with 2L/2nd day and for the fresh enlarged root yield, plants should be fertigated with 2L/day at later stages of growth. Either pine bark or sand is recommended to produce yield of wild ginger at initial as well as later stages of development.

Wild ginger is an indigenous forest floor plant of southern Africa - scientifically known as *Siphonochilus aethiopicus* (Thunberg) D. L. Don, and belongs to the family Zingiberaceae. The generic name *Siphonochilus* is derived from the Greek name *siphon* meaning tube and *chilos* meaning lip in reference to the shape of the flower and the specific name *aethiopicus* indicates from southern Africa (Van Wyk & Gericke, 2000). The plant is highly valued for its medicinal value and as a result, it has been over harvested from the wild to a point just short of total extinction (Arnold & de Wet, 1993; Hutchings, 1996; Van Wyk, Oudtshoorn & Gericke, 1997; van Wyk & Gericke, 2000).

Rhizomes are chewed fresh to treat asthma, hysteria, colic and flu as well as to treat malaria and also chewed by women during menstruation. The highly aromatic roots have been reported to be used by Zulu people as a protection against lightning and snakes (Van Wyk *et al.*, 1997).

Little is known about the effect of nitrogen, fertigation frequency and growing medium on the enlarged root of wild ginger. Hence, this trial was established to determine the effect of nitrogen nutrition, fertigation frequency and growing medium on the anatomical structure of wild ginger enlarged root.

4.2 Materials and methods

The experiment was conducted in a Laboratory at the Department of Plant Production and Soil Science, University of Pretoria. To determine the effect of N fertilizer on the enlarged root anatomy, wild ginger plants were grown in pine bark under a glasshouse. Treatments used were six levels of nitrogen viz. 0, 50, 100, 150, 200 and 250 kg ha⁻¹. Thus, to determine the response of enlarged root anatomy of wild ginger to fertigation frequency and growing medium, wild

CHAPTER 4

ANATOMY OF WILD GINGER ENLARGED ROOTS IN RESPONSE TO NITROGEN NUTRITION, FERTIGATION FREQUENCY AND GROWING MEDIUM

4.1 Introduction

Wild ginger is an indigenous forest floor plant of southern Africa scientifically known as *Siphonochilus aethiopicus* (Schwerf) B.L. Burt., and belongs to the family Zingiberaceae. The generic name *Siphonochilus* is derived from the Greek name siphono meaning tube and chilus meaning lip in reference to the shape of the flower and the specific name *aethiopicus* means from southern Africa (Van Wyk & Gericke, 2000). The plant is highly prized for its medicinal value and as a result, it has been over harvested from the wild to a point just short of total extinction (Arnold & de Wet, 1993; Hutchings, 1996; Van Wyk, Outdshoorn & Gericke, 1997; van Wyk & Gericke, 2000).

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Little is known about the effect of nitrogen, fertigation frequency and growing medium on the enlarged root of wild ginger. Hence, this trial was established to determine the effect of nitrogen nutrition, fertigation frequency and growing medium on the anatomical structure of wild ginger enlarged root.

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The experiment was conducted in a Laboratory at the Department of Plant Production and Soil Science, University of Pretoria. To determine the effect of N nutrition on the enlarged root anatomy, wild ginger plants were grown in pine bark under a glasshouse. Treatments used were six levels of nitrogen viz. 0, 50, 100, 150, 200 and 250 kg·ha⁻¹. Thus, to determine the response of enlarged root anatomy of wild ginger to fertigation frequency and growing medium, wild

ginger plants were grown in either sand or pine bark with five fertigation frequencies (0.25L/day, 1L/day, 2L/day, 2L/2nd day and 2L/week). Plants were harvested at 224 days after emergence (DAE) for sectioning. Thereafter, enlarged roots were immersed in small test tubes and fixed in a solution of F.A.A [(100 ml acetic acid (CH₃COOH), 100 ml formaldehyde (HCHO) and 1800 ml 50% ethanol (CH₂H₂OH), thereafter were put on a mechanical shaker for 2 to 3 hours. Samples were dehydrated with 30, 50, 70 and 100% ethanol. Samples were dehydrated with 30, 50, 70 and 100% ethanol and each concentration was changed after 2 to 3 hours on a mechanical shaker and the procedure was repeated once for 30, 50 and 70% and twice for 100% alcohol.

Ethanol was extracted from the samples with 30, 50, 70 and 100% xylene and also each and every concentration was changed after 2 to 3 hours, except for the 100% xylene where, after 2 to 3 hours of agitation, 2 small wax granules were added into the small test tubes with samples. Another 2 wax small granules were added only when the previously added granules had dissolved. Wax granules were added up until liquid xylene in the test tube looked milky. Samples were put in an oven at 50 °C and the test tubes were filled with wax granules up to the top. Other wax granules were added only after wax in the samples had dissolved and this procedure was repeated three times.

Wild ginger enlarged root samples were mounted on a Thermolyne Histo – Centre II (Getan, England). Specimens were prepared on silver plates with a protrusion like extended to the outside. Silver plates were spread with glycerin to enable easier removal of the specimens from the plates. A small amount of liquid wax was poured into the protrusion of the plate where the specimen was to be placed. The procedure was done on a hot plate at 63.4 °C and later specimens were put on a cold plate at –9 °C for 2 hours for the wax and the samples to be in contact. Thick wax from the samples was removed with a razor blade so that an adequate part of the sample could be available for cutting.

Samples were cut at 5 – 7 microns in a microtone 2040 Autocut Sterea star Zoom, Reichert Jung – 0.7x to 42x 570 (Leica, Johannesburg, South Africa). Water was poured into an electrothermal E7 9QN (Instec, USA) and was kept at 40 °C with lights on (GEC 30/250V 40W). Cut samples were put in water to stretch in order to enable easier collection with micro slides. Micro slides

were smeared with 5 microlitre of Haupt solution, so that samples could stick on them. Micro slides were labelled with a diamond stick 1 (MafTek, Britain). After putting the samples on the micro slides they were taken to the microscope to check if the specimens came out clear after which micro slides with specimens were put on a hot plate (Kunz instrument aps HP 3, Denmark) at 2.5 °C.

4.2.1 Staining

Nine staining beakers were prepared. The first beaker contained saffranin (water base), the second beaker had toluidien blue solution (alcohol base), the third beaker had 100% distilled water, the fourth beaker had 50% distilled water and 50% ethanol, the fifth beaker had 30% distilled water and 70% ethanol, the sixth beaker contained 100% alcohol (to remove all the available water from the specimens), the seventh beaker had 50% xylene, the eighth and ninth beakers contained 100% xylene (to remove available wax from the samples). Each and every slide was inserted in all the beakers, starting with the beaker with saffranin and ending with the one with 100% xylene. In each solution, samples were inserted for two minutes and about ten seconds in 50, 70 and 100% ethanol as well as 50% xylene. However, samples were kept for five minutes in two beakers with 100% xylene to ensure that all the wax had been removed from the samples.

Slides were removed from the staining bath and wiped to remove water and then taken to the microscope to detect whether the cells were clearly visible and that no air bubbles were present on the slides. Samples were observed with a microscope at 100X magnification. Thereafter, the slides were taken together with specimens and put on a hot plate at 2.5 °C so that the mount could dry off.

4.2.2 Mounting

Samples were mounted with a clear mount and taken to a midi hot plate (CJB glassware, Halfway House, South Africa) at 2.5 °C to enable the mount to dry off so that samples could be preserved for years. Samples were mounted slowly so as to get the air bubbles off the slides.

Slides were mounted with 15 microlitres (μL) of a clear mount and samples on the slides were closed with Menzel glasses and thereafter, put on the midi hot plate.

4.3 Results

4.3.1 Nitrogen nutrition effect on the anatomy of enlarged roots of wild ginger

Differences were detected for the anatomy of roots across nitrogen treatments. Differences were observed based on the number of primary xylems, number of cells between two primary xylems, the size of the pith, number of glandular cells, presence of starch, number of pericycle layers, the size of the endodermis and the surface area of the cortex. Wild ginger enlarged roots had a solid core of xylem, surrounded by a ring of phloem which was surrounded by ground tissue (cortex). The innermost layer of the cortex is the endodermis.

With respect to roots of plants that received no nitrogen, they had 16 primary xylems and ten cells between two primary xylems as well as phloem cells behind the metaxylem. There was a big pith and two glandular cells surrounded by primary xylems. There was a single layer of pericycle protecting the vascular bundles to the inside followed by an endodermis with very thick casparian strips and from the outside followed a big surface area of cortex with starch grains (Table 4.1 and Fig. 4.1).

In roots of plants fertilized with $50 \text{ kg}\cdot\text{ha}^{-1} \text{ N}$, there were 12 primary xylems and 10 cells between two primary xylems and surrounded by a ring of the phloem cells. There was a small pith and two glandular cells as well as a single layer of pericycle followed by an endodermis with very thick casparian strips from the outside followed by a big surface area of cortex with starch grains (Table 4.1 and Fig. 4.2).

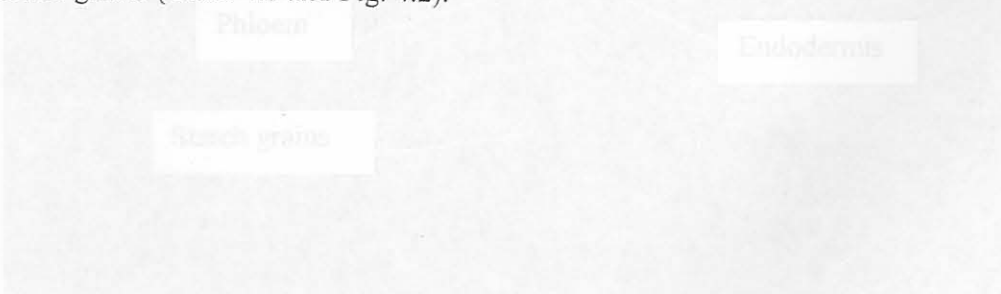


Fig. 4.2 Cross section of wild ginger enlarged root fertilized with $50 \text{ kg}\cdot\text{ha}^{-1} \text{ N}$

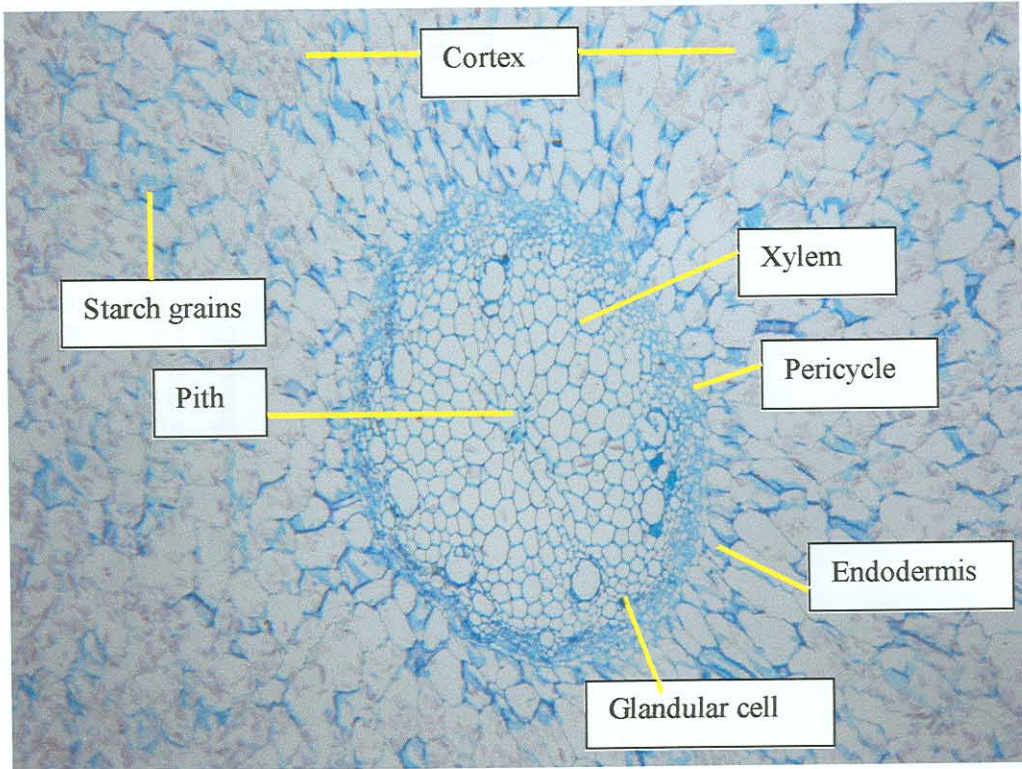


Fig. 4.1 Cross section of wild ginger enlarged root that received no nitrogen

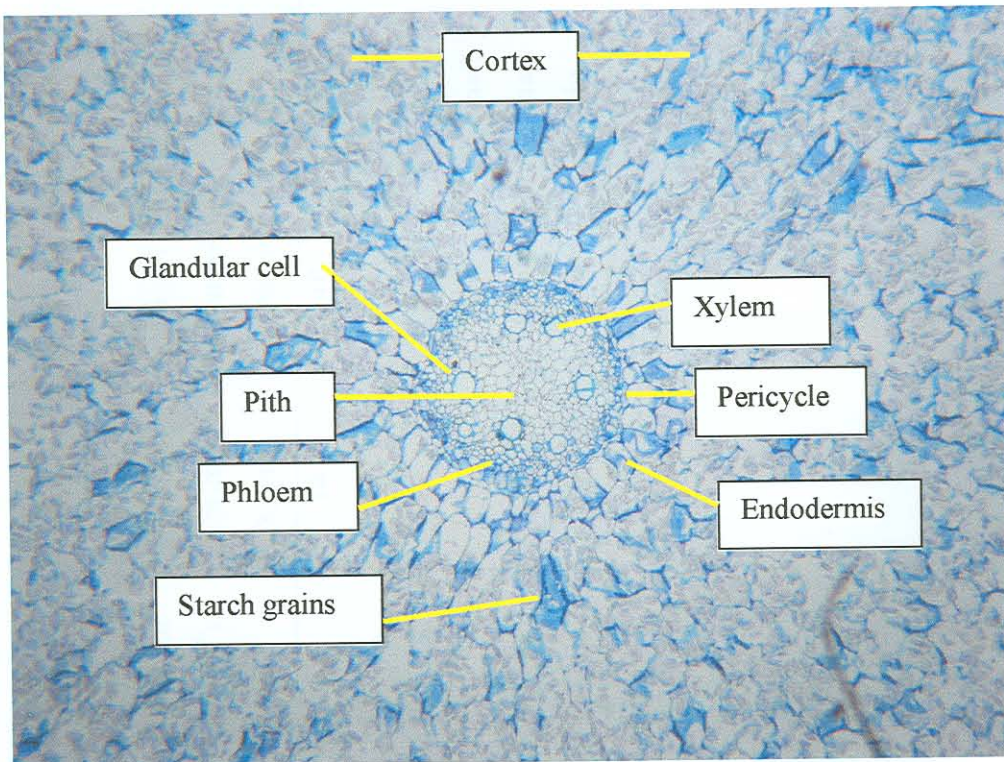


Fig. 4.2 Cross section of wild ginger enlarged root fertilized with 50 kg-ha⁻¹ N

Table 4.1 Anatomical structures of wild ginger enlarged root as affected by N nutrition during 2001/2002 seasons

Nitrogen (kg·ha ⁻¹)	Number of primary xylem	Number of cells between primary xylems	Size of the pith	Number of glandular cells	Number of pericycle layer	Size of endodermis	Size of cortex	Presence of starch grains
0	16	10	big	two	single	thick	big	yes
50	12	9	small	two	single	thick	big	yes
100	19	10	small	four	single	thin	big	yes
150	10	10	small	four	single	thin	big	yes
200	13	undifferentiated	small	six	single	thick	big	yes
250	10	10	big	eight	single	thick	big	yes

For plants that received $100 \text{ kg}\cdot\text{ha}^{-1} \text{ N}$, their enlarged root had 19 primary xylems and 10 cells between two primary xylems and surrounded by a ring of phloem cells. There was a small pith and four glandular cells. Furthermore, there was a single layer of pericycle and endodermis with a thin casparian strip. There was a big surface area of cortex with starch grains (Table 4.1 and Fig. 4.3).

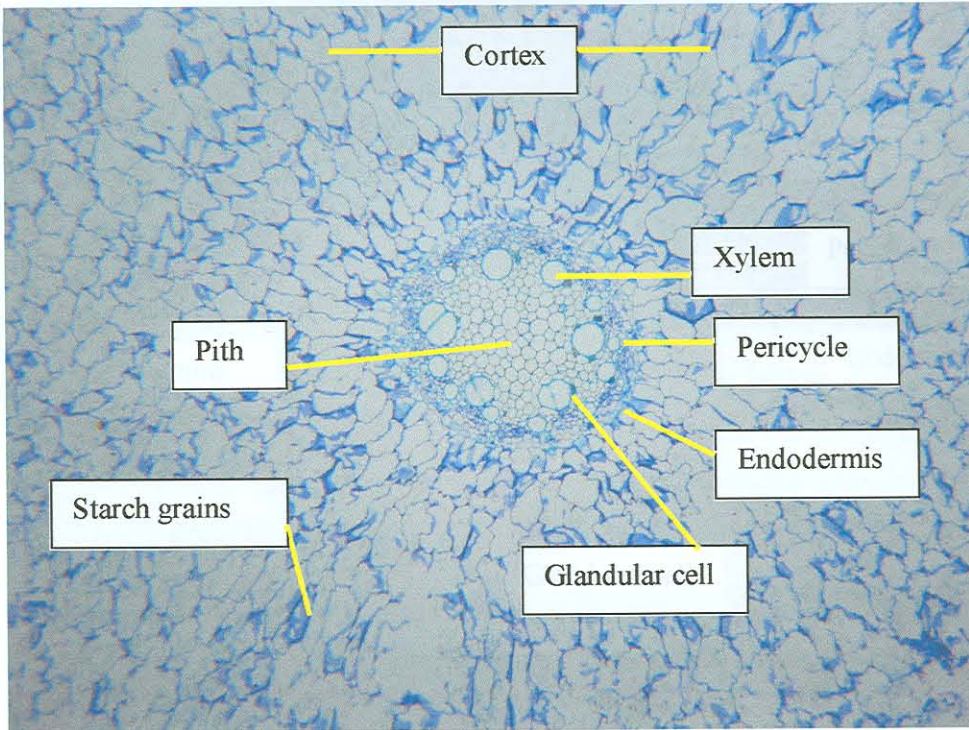


Fig. 4.3 Cross section of wild ginger enlarged root fertilized with $100 \text{ kg}\cdot\text{ha}^{-1} \text{ N}$

For plants that received $150 \text{ kg}\cdot\text{ha}^{-1} \text{ N}$, their root had 10 primary xylems and 10 cells between two primary xylems. There was a small pith and four glandular cells formed on the forefront of the metaxylem. There were many cell layers of pericycle and endodermis with thin casparian strips. There was a big surface area of cortex with starch grains (Table 4.1 and Fig. 4.4).

With respect to plants that received $200 \text{ kg}\cdot\text{ha}^{-1} \text{ N}$, the anatomy of the root had 13 primary xylems and undifferentiated cells between two primary xylems as well as phloem cells behind the metaxylem. Polarization allowed the xylem's thick walls to stand out while the phloem was discreetly tucked within. There was a small pith and six glandular cells. There was a single layer of pericycle protecting the vascular bundles to the inside followed by endodermis with very

thick casparian strips. There was also a big surface area of cortex with starch grains (Table 4.1 and Fig. 4.5).

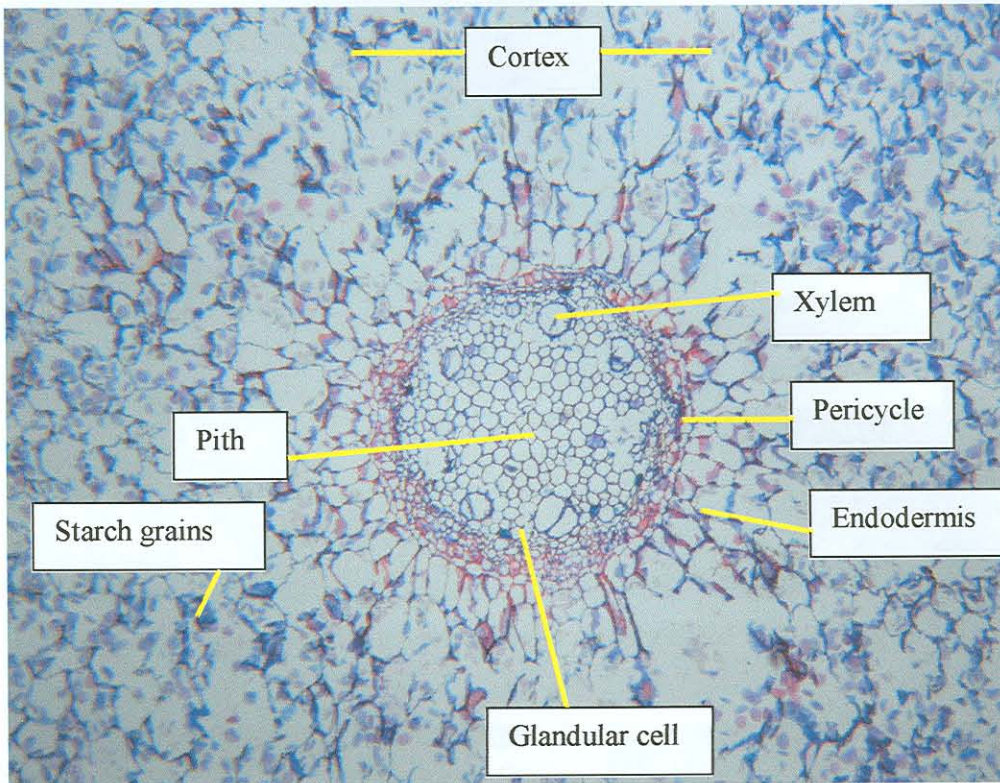


Fig. 4.4 Cross section of wild ginger enlarged root fertilized with $150 \text{ kg}\cdot\text{ha}^{-1} \text{ N}$

For plants fertilized with $250 \text{ kg}\cdot\text{ha}^{-1} \text{ N}$, their root anatomy had 10 primary xylems and 10 cells between two metaxylems. There was a big pith and eight glandular cells as well as the interior was the pericycle of about one cell layer thick followed by an endodermis with very thick casparian strips. There was a big surface area of cortex with starch sheath (Table 4.1 and Fig. 4.6).

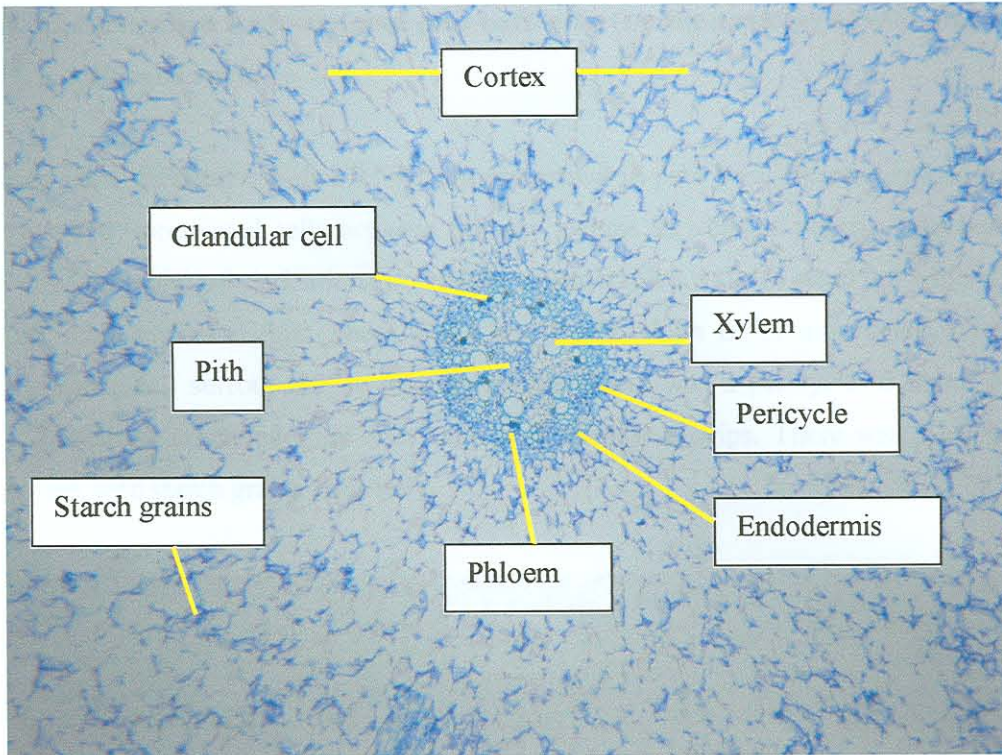


Fig. 4.5 Cross section of wild ginger enlarged root fertilized with $200 \text{ kg}\cdot\text{ha}^{-1} \text{ N}$

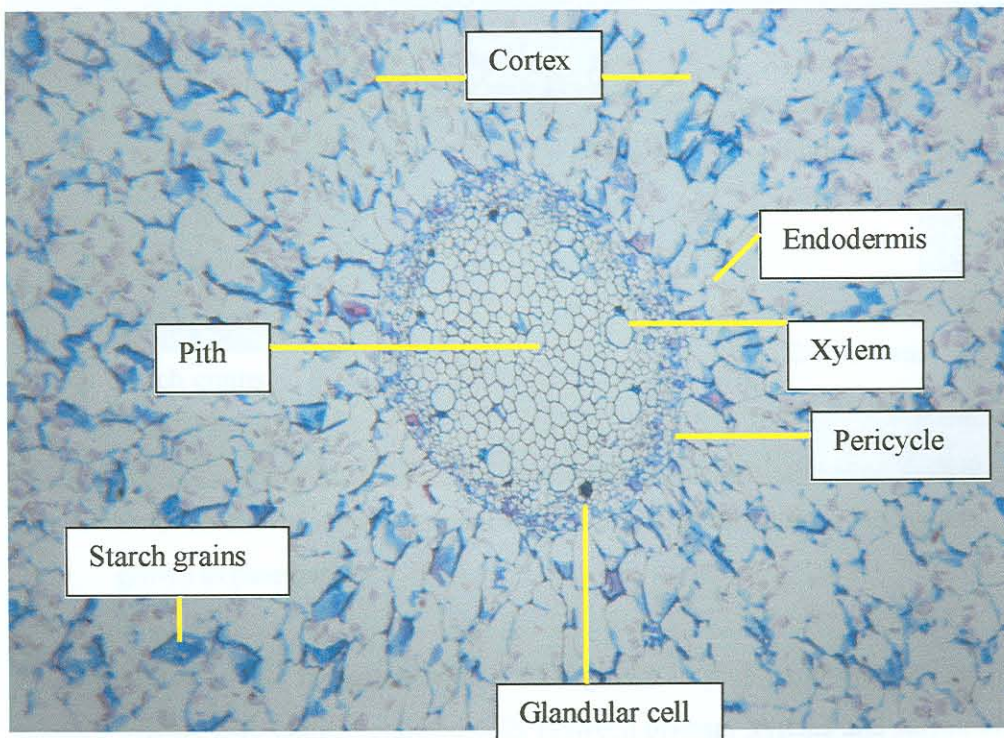


Fig. 4.6 Cross section of wild ginger enlarged root fertilized with $250 \text{ kg}\cdot\text{ha}^{-1} \text{ N}$

4.3.2 Fertigation frequency and pine bark effect on the anatomy of wild ginger enlarged roots

For plants fertigated with a 0.25L/day and grown in pine bark, the root had 13 primary xylems with undifferentiated cells between primary xylems as well as phloem cells behind the xylem cells (Table 4.2). Polarization allowed the xylem's thick walls to stand out while the phloem was discreetly tucked within. There was a small pith which is the most interior feature, and one glandular cell surrounded by primary xylem. There was a pericycle of about one layer thick followed by an endodermis with very thick casparian strips. There was a big surface area of cortex with starch grains (Fig. 4.7).

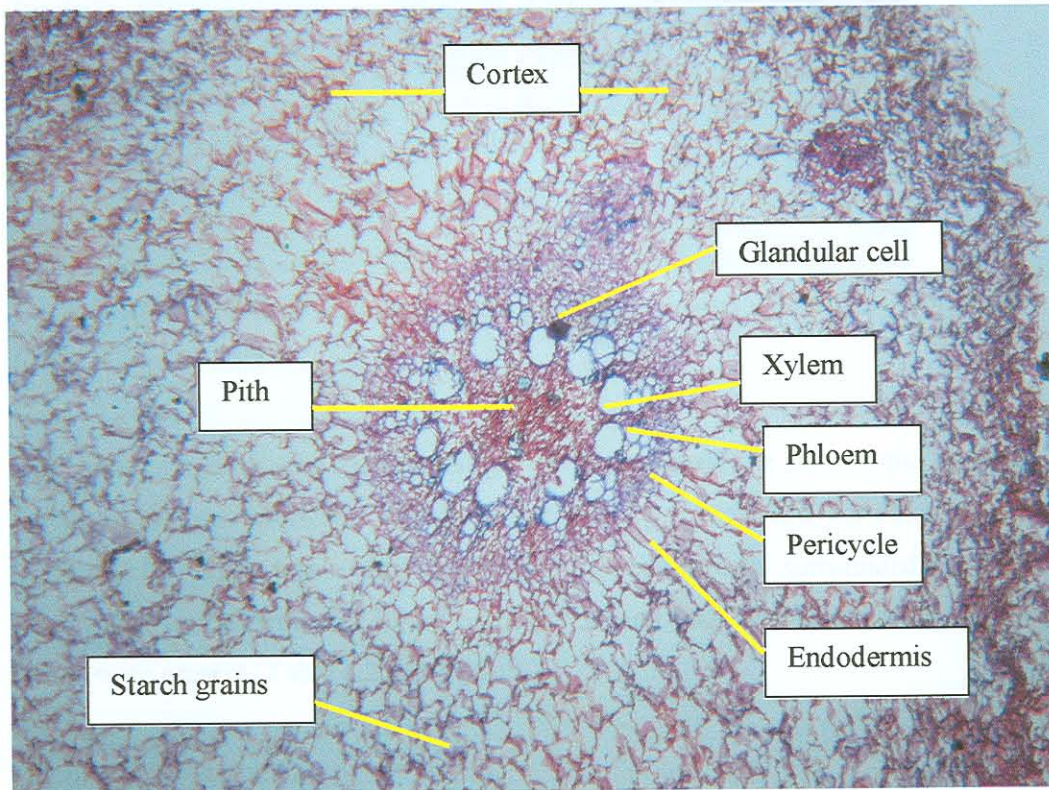


Fig. 4.7 Cross section of wild ginger enlarged root fertigated with 0.25L/day and grown in pine bark

With respect to plants fertigated with 1L/day grown in pine bark, the stele of the root had 14 primary xylems with undifferentiated cells between primary xylems and surrounded by a ring of the phloem cells (Table 4.2). There was a small pith and two glandular cells surrounded by

primary xylem. There was a single layer of pericycle followed by a non-differentiated endodermis and a small cortex. No starch grains were present (Fig. 4.8).

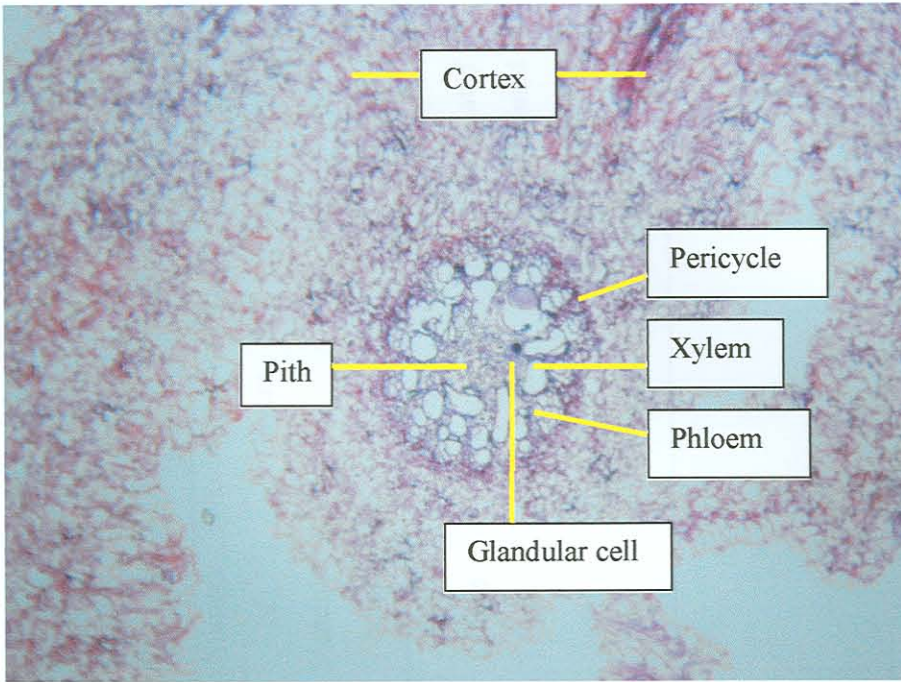


Fig. 4.8 Cross section of wild ginger enlarged root fertigated with 1L/day and grown in pine bark

For plants fertigated with 2L/day and grown in pine bark, the stele of the root had 12 primary xylems with undifferentiated cells between primary xylems and surrounded by a ring of the phloem cells. There was a small pith and no glandular cells surrounded the primary xylems. There were undifferentiated layers of pericycle and endodermis and a big cortex. No starch grains were present (Table 4.2 and Fig. 4.9).

For plants fertigated with 2L/2nd day and grown in pine bark, their root had 18 primary xylems and 10 cells between two primary xylems. There was a big pith and no glandular cells surrounded the primary xylems. There was a small pith which is the most interior feature. There was a pericycle of about one layer thick followed by endodermis with very thick casparian strips. There was a big surface area of cortex with the presence of starch grains (Table 4.2 and Fig. 4.10).

Table 4.2 Anatomical structures of wild ginger enlarged root grown in pine bark as affected by fertigation frequency during 2002/2003 seasons

Fertigation frequency	Number of primary xylem	Number of cells between primary xylem	Size of pith	Number of glandular cells	Number pericycle layers	Size of endodermis	Size of cortex	Presence of starch grains
0.25L/day	13	Undifferentiated	small	one	single	thick	big	no
1L/day	14	Undifferentiated	small	three	single	undifferentiated	small	no
2L/day	12	Undifferentiated	small	no	single	undifferentiated	big	no
2L/2 nd day	18	10	small	no	single	undifferentiated	big	no
2L/week	13	Undifferentiated	small	no	double	undifferentiated	small	no

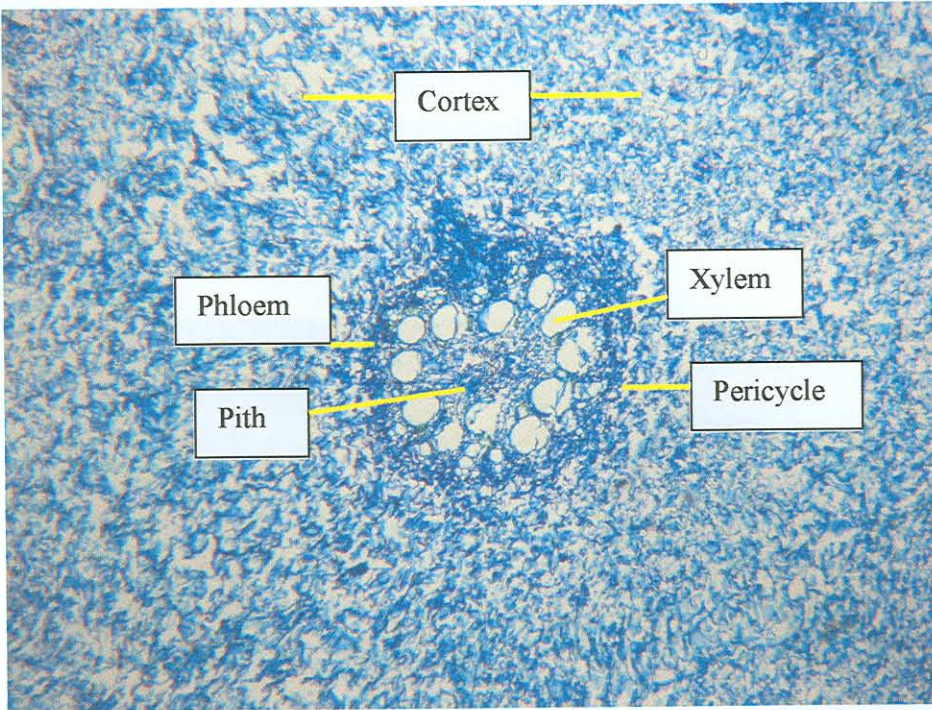


Fig. 4.9 Cross section of wild ginger enlarged root fertigated with 2L/day and grown in pine bark

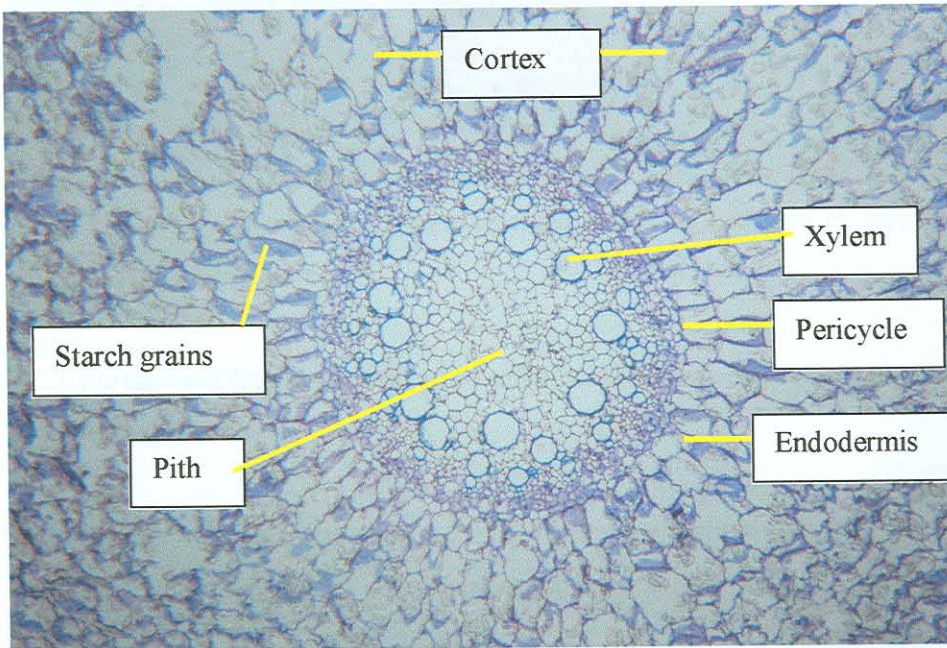


Fig. 4.10 Cross section of wild ginger enlarged root fertigated with 2L/2nd day and grown in pine bark

For plants fertigated with 2L/week and grown in pine bark, the root had 13 primary xylems and undifferentiated cells between primary xylems and surrounded by a ring of phloem cells (Table 4.2). There was a small pith and no glandular cells surrounded the primary xylem. There were two layers of pericycle followed by a thick endodermis and a small cortex. No starch grains were present (Fig. 4.11).

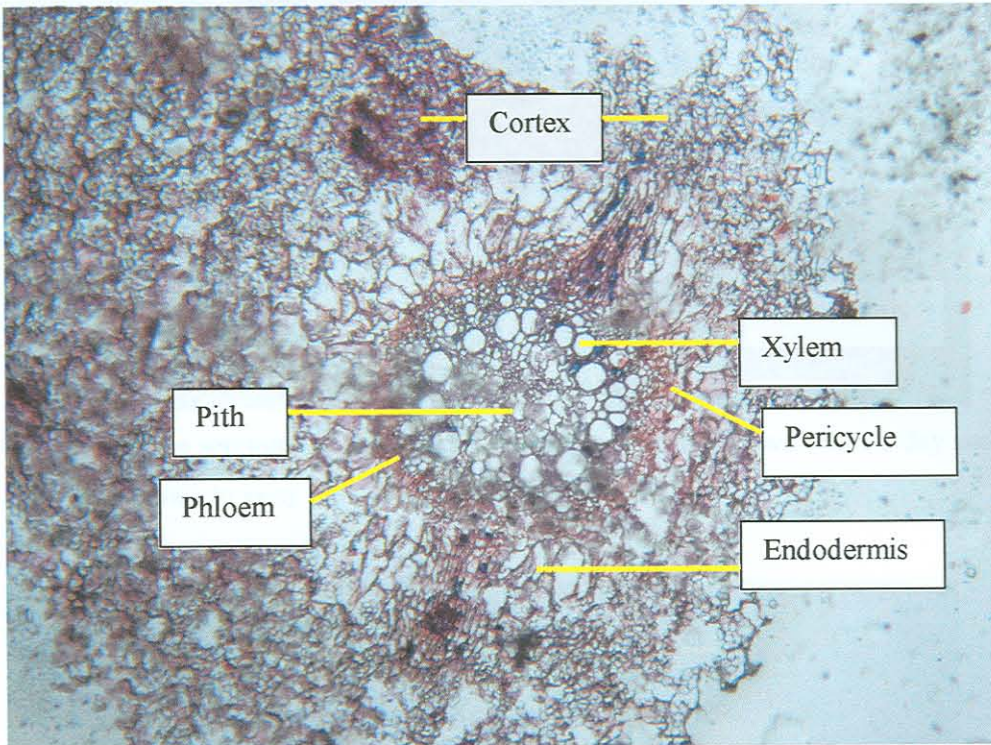


Fig. 4.11 Cross section of wild ginger enlarged root fertigated with 2L/week and grown in pine bark

4.3.3 Fertigation frequency and sand effect on the anatomy of wild ginger enlarged roots

With respect to plants fertigated with 0.25L/day and grown in sand, the root had 16 primary xylems and undifferentiated cells between primary xylems with phloem cells behind the xylem cells (Table 4.3). There was a small pith and no grandular cells surrounded the primary xylems. There was a single layer of pericycle followed by a thick endodermis and a big cortex with scattered starch grains present (Fig. 4.12).

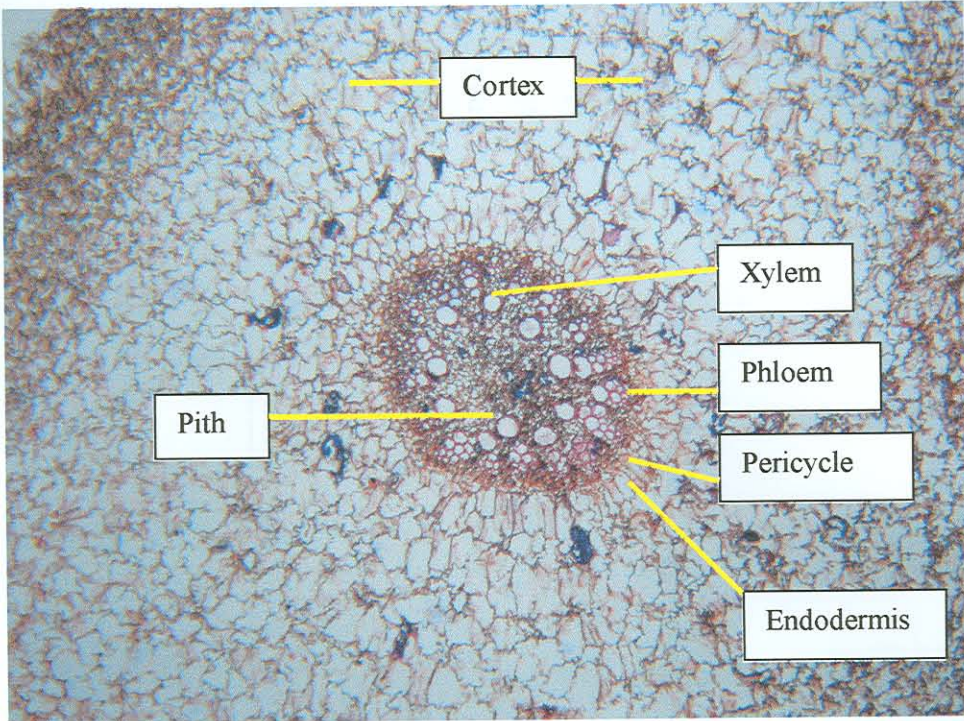


Fig. 4.12 Cross section of wild ginger enlarged root fertigated with 0.25L/day and grown in sand

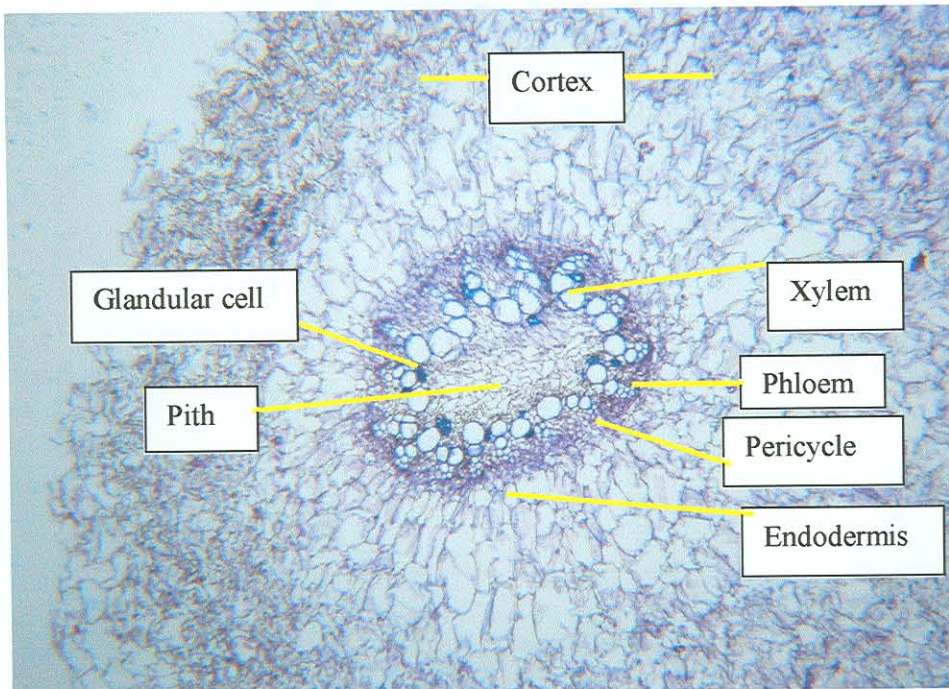


Fig. 4.13 Cross section of wild ginger enlarged root fertigated with 1L/day and grown in sand

Table 4.3 Anatomical structures of wild ginger enlarged root grown in sand as affected by five fertigation frequency in sand during 2002/2003 seasons

Fertigation frequency	Number of primary xylem	Number of cells between primary xylem	Size of pith	Number of glandular cells	Number of Pericycle layers	Size of endodermis	Size of cortex	Presence of starch grains
0.25L/day	16	undifferentiated	small	no	Single	thick	big	yes
1L/day	14	undifferentiated	small	four	single	thick	small	yes
2L/day	15	undifferentiated	small	no	double	thick	big	no
2L/2 nd day	14	undifferentiated	small	eight	undifferentiated	undifferentiated	big	no
2L/week	undifferentiated	undifferentiated	small	sixteen	double	undifferentiated	small	no

For plants that received 1L/day and grown in sand, the stele of the root had 14 primary xylems with undifferentiated cells between primary xylems with phloem cells behind the xylem cells (Table 4.3). There was a small pith and four glandular cells surrounded the primary xylem. There was a pericycle of about one layer thick followed by an endodermis with very thick casparian strips. There was a small surface area of cortex with starch grains (Fig. 4.13).

With plants that received fertigation frequency of 2L/day and grown in sand, the root had 15 primary xylems and undifferentiated cells between primary xylems with phloem cells behind the xylem cells (Table 4.3). There was a small pith which is the most interior feature and no glandular cells surrounded the primary xylems. There was a pericycle of about two layers thick followed by endodermis with very thick casparian strips. There was a big surface area of cortex with the absence of starch grains (Fig. 4.14).

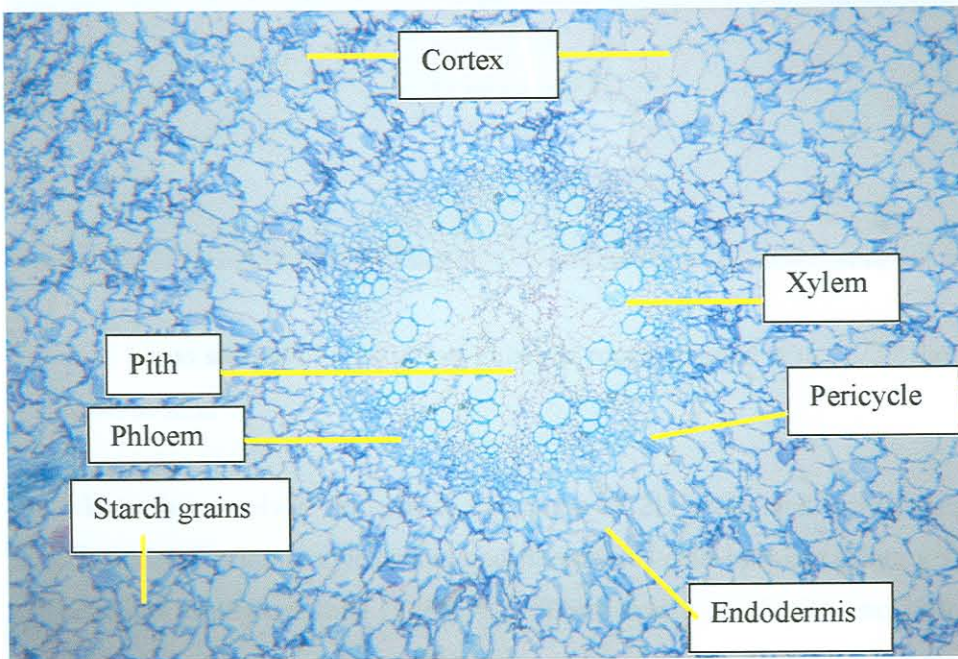


Fig. 4.14 Cross section of wild ginger enlarged root fertigated with 2L/day and grown in sand

For plants that received fertigation frequency of 2L/2nd day and grown in sand, the root had 14 primary xylems and undifferentiated cells between primary xylems and surrounded by a ring of phloem cells (Table 4.3). There was a small pith and eight granular cells surrounded the primary

xylem. There were undifferentiated layers of pericycle and endodermis and a big cortex. No starch grains were present (Fig. 4.15).

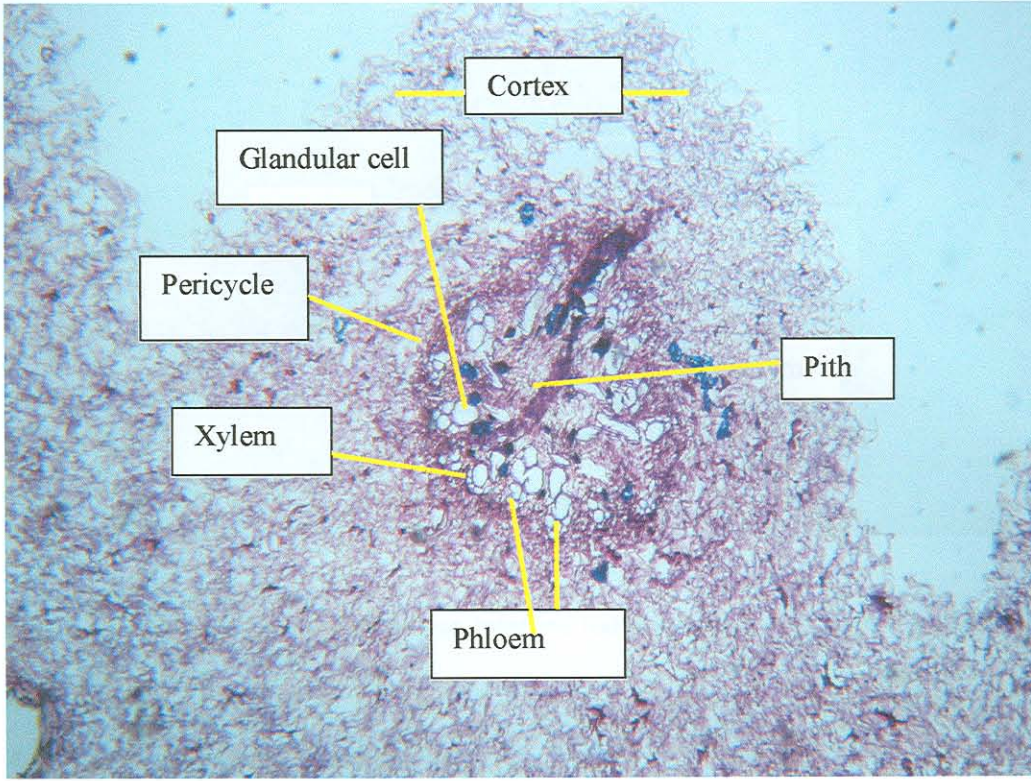


Fig. 4.15 Cross section of wild ginger enlarged root fertigated with 2L/2nd day and grown in sand

For plants that received fertigation frequency of 2L/ week and grown in sand, the root had undifferentiated primary xylems and undifferentiated cells between primary xylems with phloem cells behind the xylem cells (Table 4.3). There was a small pith and sixteen glandular cells surrounded the primary xylems. There was a pericycle of about double layer thick followed by an undifferentiated endodermis. There was a small surface area of cortex with no starch grains present (Fig. 4.16).

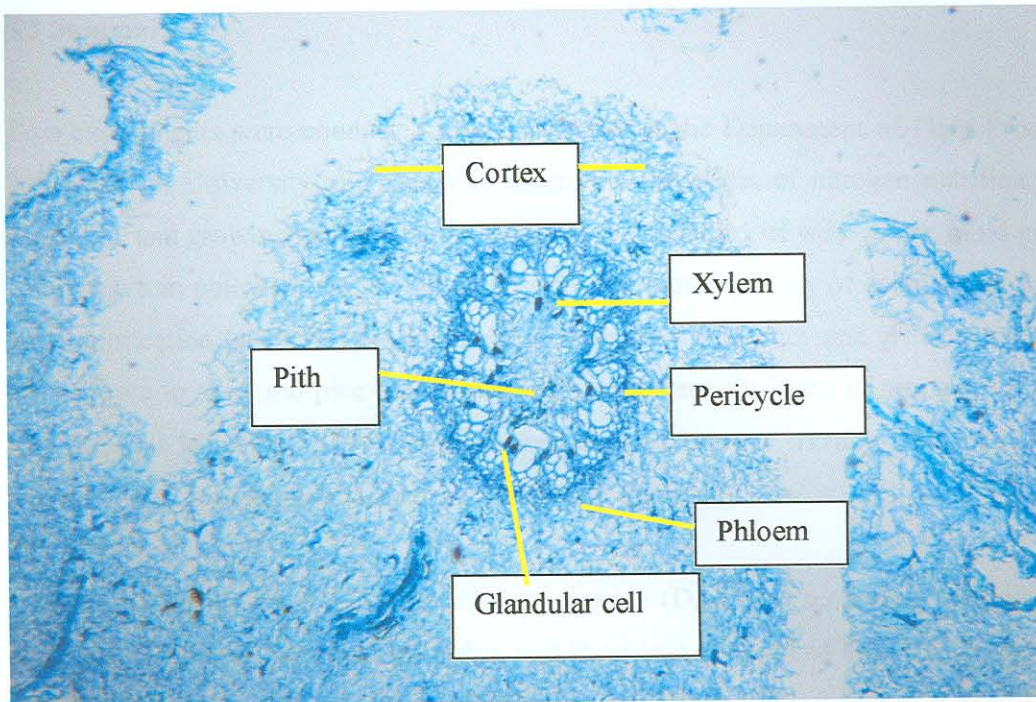


Fig. 4.16 Cross section of wild ginger enlarged root fertigated with 2L/week and grown in sand

4.4 Discussion and Conclusions

Siphonochilus aethiopicus (Schweif.) B.L. Burt, when compared with the typical monocot, such as the normal ginger (*Zingiberaceae officinale*) showed a lot of similar characteristics. The root showed the typical arrangement of the amphivasal vascular bundles and the protective cell layers such as the pericycle and endodermis. More interesting was the presence of glandular cells.

This study demonstrated that N nutrition for wild ginger is important for increasing glandular cells that are important for essential oil production. More glandular cells were, therefore, produced in plants grown in sand with the least fertigation frequency (2L/week).

4.5 Summary

Two experiments were conducted in a Laboratory at the Department of Plant Production and Soil Science, University of Pretoria to determine the effect of nitrogen nutrition, fertigation frequency and growing medium on the enlarged root anatomy of wild ginger. Wild ginger plants were grown in pine bark under a glasshouse for the anatomy study of enlarged roots as affected by N application levels and for the effect of fertigation frequency and growing medium, plants were grown in sand and pine bark. Treatments used were six levels of nitrogen viz. 0, 50, 100, 150, 200 and 250 kg·ha⁻¹, five fertigation frequency (0.25L/day, 1L/day, 2L/day, 2L/2nd day and 2L/week) and growing media used were pine bark and sand.

Plants were harvested at 224 days after emergence (DAE) for sectioning thereafter, enlarged roots were immersed in small test tubes and fixed in a solution of F.A.A [(100 ml acetic acid (CH₃COOH), 100 ml formaldehyde (HCHO) and 1800 ml 50% ethanol (CH₂H₂OH), thereafter were put on a mechanical shaker for 2 to 3 hours. Samples were dehydrated with 30, 50, 70 and 100% ethanol and ethanol was extracted from the samples at 30, 50, 70 and 100% xylene. Samples were mounted so that they could be preserved for years and stained with toluidine solution. Anatomical structures of wild ginger enlarged root were observed with a light microscope at 100X magnification. Anatomical structures observed in wild ginger enlarged roots were primary xylems and the cells between them, glandular cells, pericycle layer, cortex, endodermis and pith.

The number of glandular cells increased from two with zero nitrogen to eight with 250 kg·ha⁻¹ N, indicating improved capacity for the production of essential oil. Enlarged roots from all the nitrogen treatments had a single pericycle layer, a thick endodermis, and a large cortex containing starch grains. In all the treatments, there were 10 cells between the primary xylems. The number of primary xylems varied from 10 to 19, but there was no clear relationship with the level of nitrogen applied. This anatomical study has demonstrated that N nutrition of wild ginger is important for increasing the number of glandular cells that are important for essential oil production.

With plants grown in pine bark, a fertigation frequency of 1L/day increased glandular cells. Glandular cells increased from one with plants that received 0.25L/day to three with plants that

received 1L/day, indicating improved capacity for production of essential oils by wild ginger roots. For plants grown in sand, fertigation frequency of 2L/week increased the number of glandular cells in the root anatomy of wild ginger. Growers should fertigate wild ginger more frequently when grown in pine bark and with a low frequent fertigation when grown in sand in order to increase glandular cells essential for oil production, which will then saves water and nutrients. More glandular cells were produced, therefore, produced in plants grown in sand with the least fertigation frequency (2L/week).

It is important to note that the N treatments were applied at planting in the form of limestone ammonium nitrate (LAN). Measurements were made of plant emergence, plant height, fresh rhizome and enlarged root, fresh rhizome circumference, length of enlarged root and the number of rhizomes and enlarged roots. N level of 200 kg/ha¹ was effective in increasing wild ginger plant height and 250 kg/ha¹ was effective in increasing plant emergence. There was a positive linear relationship in all yield parameters except for the number of rhizomes per plant which had shown a no relationship to applied nitrogen. In this study, in terms of yield, it was not clear which N level was optimum, since the trend have shown an increased up to the highest N treatment applied (250 kg ha⁻¹).

A useful study was undertaken to determine the influence of fertigation frequency and growing medium on the growth, oil quality and yield of wild ginger. Treatments used were five fertigation frequencies (0.25L/day, 1L/day, 2L/day, 2L/2nd day and 2L/week) and two growing media (Pine bark and sand). Measurements were made of plant growth such as plant height, number of leaves and stem at 56, 112, 168 and 224 days after emergence (DAE) and fresh and dry leaf mass and leaf area at 112 and 224 DAE. Yield such as fresh and dry rhizomes mass, fresh and dry enlarged root mass, length of enlarged roots and the number of rhizomes and enlarged roots.

Wild ginger growth was reduced with a fertigation frequency of 0.25L/day during initial growth stages (56 DAE) and at later stages of development (112, 168 and 224 DAE), a fertigation frequency of 2L/week was inadequate to sustain wild ginger growth and development. Plants grown in pine bark have shown improved growth during the initial growth stages (56 and 112 DAE) and at later stages of growth, plants grown in sand have shown an increased in wild ginger growth and development. Fertigation frequency of 1L/day improved fresh and dry leaf mass and plants grown in pine bark increased fresh and dry leaf mass at 112 and 224 DAE. Greater leaf areas were improved when fertigated with 2L/2nd day at 112 and 224 DAE.

GENERAL DISCUSSION AND CONCLUSIONS

An experiment was conducted in the field to determine the response of wild ginger growth and yield to nitrogen nutrition. Planting materials used were sprouted wild ginger rhizomes obtained from the Centre of Scientific and Industrial Research (CSIR). Treatments used were six levels of nitrogen (0, 50, 100, 150, 200 and 250). All N treatments were applied at planting in the form of limestone ammonium nitrate (LAN). Measurements were made of plant emergence, plant height, fresh rhizome and enlarged root, fresh rhizome circumference, length of enlarged root and the number of rhizomes and enlarged roots. N level of 200 kg·ha⁻¹ was effective in increasing wild ginger plant height and 250 kg·ha⁻¹ was effective in increasing plant emergences. There was a positive linear relationship in all yield parameters except for the number of rhizomes per plant which had shown a no relationship to applied nitrogen. In this study, in terms of yield, it was not clear which N level was optimum, since the trend have shown an increased up to the highest N treatment applied (250 kg·ha⁻¹).

A tunnel study was undertaken to determine the influence of fertigation frequency and growing medium on the growth, oil quality and yield of wild ginger. Treatments used were five fertigation frequencies (0.25L/day, 1L/day, 2L/day, 2L/2nd day and 2L/week) and two growing media (Pine bark and sand). Measurements were made of plant growth such as plant height, number of leaves and stem at 56, 112, 168 and 224 days after emergence (DAE) and fresh and dry leaf mass and leaf area at 112 and 224 DAE. Yield such as fresh and dry rhizomes mass, fresh and dry enlarged root mass, length of enlarged roots and the number of rhizomes and enlarged roots.

Wild ginger growth was reduced with a fertigation frequency of 2L/day during initial growth stages (56 DAE) and at later stages of development (112, 168 and 224 DAE), a fertigation frequency of 2L/week was inadequate to sustain wild ginger growth and development. Plants grown in pine bark have shown improved growth during the initial growth stages (56 and 112 DAE) and at later stages of growth, plants grown in sand have shown an increased in wild ginger growth and development. Fertigation frequency of 1L/day improved fresh and dry leaf mass and plants grown in pine bark increased fresh and dry leaf mass at 112 and 224 DAE. Greater leaf areas were improved when fertigated with 2L/2nd day at 112 and 224 DAE.

However, plants grown in pine bark improved leaf area at 112 DAE and at 224 DAE, wild ginger leaf area were improved with plants grown in sand.

Although there were differences in wild ginger growth and development, fertigation frequency as well as growing medium did not affect yield at 112 DAE. Number of enlarged roots were increased when wild ginger plants were fertigated with 1L/day at 112 DAE and 2L/day at 224 DAE. Plants grown in pine bark increased number of enlarged roots at 112 DAE and those grown in pine bark improved the number of enlarged roots at 224 DAE. Fertigation frequency as well as growing medium did not affect the number of rhizomes and enlarged roots at both 112 and 224 DAE. Wild ginger fresh rhizomes was increased when fertigated with 2L/2nd day at 224 DAE and fresh enlarged root yield was increased when fertigated with 2L/day at 224 DAE. Fertigation frequency as well as growing medium did not affect fresh rhizome and enlarged root oil yield at 224 DAE. Response of wild ginger growth and yield to fertigation frequency and growing medium is highly depended on different sampling dates.

Two experiments were conducted in a Laboratory at the Department of Plant Production and Soil Science, University of Pretoria to determine the effect of nitrogen nutrition and the effect of fertigation frequency and growing medium on the enlarged root anatomy of wild ginger. Wild ginger plants were grown in pine bark under a glasshouse for the anatomy study of enlarged roots as affected by N application levels and for the effect of fertigation frequency and growing medium, plants were grown in sand and pine bark. Treatments used were six levels of nitrogen viz. 0, 50, 100, 150, 200 and 250 kg·ha⁻¹, five fertigation frequency (0.25L/day, 1L/day, 2L/day, 2L/2nd day and 2L/week) and growing media used were pine bark and sand.

Plants were harvested at 224 days after emergence (DAE) for sectioning thereafter, enlarged roots were immersed in small test tubes and fixed in a solution of F.A.A [(100 ml acetic acid (CH₃COOH), 100 ml formaldehyde (HCHO) and 1800 ml 50% ethanol (CH₂H₂OH), thereafter were put on a mechanical shaker for 2 to 3 hours. Samples were dehydrated with 30, 50, 70 and 100% ethanol and ethanol was extracted from the samples at 30, 50, 70 and 100% xylene. Samples were mounted so that they could be preserved for years and stained with toluidine solution. Anatomical structures of wild ginger enlarged root were observed with a light microscope at 100X magnification. Anatomical structures observed in wild ginger enlarged

GENERAL SUMMARY

roots were primary xylems and the cells between them, glandular cells, pericycle layer, cortex, endodermis and pith.

N application levels increased the number of glandular cells, important for the production of essential oil. Glandular cells increased from one where no nitrogen was applied to eight where the highest level ($250 \text{ kg}\cdot\text{ha}^{-1}$) was used. N application rates also increased the number of primary xylems, the size of the pith and endodermis. Enlarged roots from all the nitrogen treatments had a single pericycle layer, a thick endodermis, and a large cortex containing starch grains. In all the treatments, there were 10 cells between the primary xylems.

For plants grown in pine bark, a fertigation frequency of 1L/day increased the number of glandular cells in wild ginger enlarged roots. Fertigation frequency also had improved number of primary xylems and cells between them and the size of the endodermis and cortex. There were no starch grains present in all fertigation frequencies applied. With plants grown in sand, a the lowest frequent fertigation (2L/week) improved the number of glandular cells, indicating improved capacity for production of essential oils by wild ginger roots. There were no starch grains present in all fertigation frequencies applied.

Also fertigation helps in improving the quality of products, which could fetch better prices than in domestic and overseas markets. However, the response of fertigation frequency and growing medium to wild ginger growth and development, oil quality and yield was not known. Hence, a master study was undertaken to determine the influence of fertigation frequency and growing medium on the growth, oil quality and yield of wild ginger. Treatments used were five fertigation frequencies (0.25L/day, 1L/day, 2L/day, 2L²nd day and 2L/week) and two growing media (pine bark and sand). Measurements were made of plant growth such as plant height, number of leaves and stem at 36, 112, 168 and 224 days after emergence (DAE) and fresh and dry leaf mass and leaf area at 112 and 224 DAE.

All fertigation frequencies increased wild ginger plant growth and development except with a fertigation frequency of 2L/day during the initial growth stages (36 DAE) and a fertigation frequency of 2L/week reduced plant growth during later stages of development (112, 168 and 224 DAE). Growth of plants grown in pine bark were improved during initial sampling dates (36 and 112 DAE) and at later sampling dates (168 and 224 DAE) growth of plants grown in

GENERAL SUMMARY

The valuable role of wild ginger as an important medicinal and aromatic plant has been known for decades by traditional practitioners. The effect of nitrogen on growth and medicinal value of wild ginger is, however, not known. A field trial on a sandy loam soil was established to determine the response of wild ginger growth and yield to nitrogen nutrition. Treatments used were six levels of nitrogen (0, 50, 100, 150, 200 and 250). All N treatments were applied at planting in the form of limestone ammonium nitrate (LAN). Measurements were made of plant emergence, plant height, fresh rhizome and enlarged root, fresh rhizome circumference, length of enlarged root and the number of rhizomes and enlarged roots. Nitrogen application level of 200 kg·ha⁻¹ was effective in increasing wild ginger plant height and 250 kg·ha⁻¹ was effective in increasing plant emergences. There was a positive linear relationship in all yield parameters except for the number of rhizomes per plant which had shown a no relationship to applied nitrogen. It is recommended that wild plants should be fertilized with highest N (250 kg·ha⁻¹) level to increase its growth and yield.

Fertigation may usher solution with water use efficiency in the range of 70-95% and improvement in crop yields by approximately 25% at a lesser cost. Also fertigation helps in improving the quality of produce, which could fetch better price both in domestic and overseas markets. However, the response of fertigation frequency and growing medium to wild ginger growth and development, oil quality and yield was not known. Hence, a tunnel study was undertaken to determine the influence of fertigation frequency and growing medium on the growth, oil quality and yield of wild ginger. Treatments used were five fertigation frequencies (0.25L/day, 1L/day, 2L/day, 2L/2nd day and 2L/week) and two growing media (pine bark and sand). Measurements were made of plant growth such as plant height, number of leaves and stem at 56, 112, 168 and 224 days after emergence (DAE) and fresh and dry leaf mass and leaf area at 112 and 224 DAE.

All fertigation frequencies increased wild ginger plant growth and development except with a fertigation frequency of 2L/day during the initial growth stages (56 DAE) and a fertigation frequency of 2L/week reduced plant growth during later stages of development (112, 168 and 224 DAE). Growths of plants grown in pine bark were improved during initial sampling dates (56 and 112 DAE) and at later sampling dates (168 and 224 DAE) growths of plants grown in

sand were improved. At both initial (112 DAE) and later sampling dates (224 DAE) fresh and dry leaf were increased when fertigated with 1L/day, whereas leaf area was improved with a fertigation frequency of 2L/2nd day.

Yield was determined at 112 and 224 DAE and parameters measured were fresh and dry rhizomes mass, fresh and dry enlarged root mass, length of enlarged roots and the number of rhizomes and enlarged roots. Although, wild ginger plant growth showed differences in growth, fertigation frequency as well as growing medium did not affect the yield of fresh rhizome and enlarged root at 112 DAE. At 224 DAE, a fertigation frequency of 0.25L/day increased fresh rhizome mass and a fertigation frequency of 2L/day increased the yield of fresh enlarged root. At both 112 and 224 DAE, fresh rhizome and enlarged root were not affected by either pine bark or sand.

Fertigation frequency and growing medium did not affect the length of enlarged roots and the number of rhizomes. To increase the number of enlarged roots it was wised to fertigate plants with 2L/2nd day at 112 DAE as well as at 224 DAE. Fresh rhizome and enlarged root oil yield were not affected by fertigation frequency and growing medium.

During the initial sampling date (56 DAE), exceptionally a fertigation frequency of 2L/day is not recommended to improve wild ginger growth in that plants are still developing roots and unable to utilized too much fertigation supplied to them and at later sampling dates (112, 168 and 224 DAE) as well, a fertigation frequency of 2L/week is not recommended to improve wild ginger growth in that plants were big, therefore required more rather adequate fertigation to improve their growth. Wild ginger plants should be grown in pine bark during initial growth stages (56 and 112 DAE) and at later stages of growth (168 and 224 DAE) should be produced in sand. For the production of fresh rhizome yield, wild ginger plants should be fertigated with 2L/2nd day and for the fresh enlarged root yield, plants should be fertigated with 2L/day at later stages of growth. Either pine bark or sand is recommended to produce yield of wild ginger at initial as well as later stages of development.

An experiment was conducted in a Laboratory at the Department of Plant Production and Soil Science, University of Pretoria to determine the effect of nitrogen nutrition on the enlarged root anatomy of wild ginger. Plants were grown in pine bark under a glasshouse Treatments used

were six levels of nitrogen viz. 0, 50, 100, 150, 200 and 250 kg·ha⁻¹. Enlarged roots for the N nutrition study as well as the fertigation frequency and growing medium study were harvested at 224 days after emergence (DAE) for sectioning thereafter, enlarged roots were immersed in small test tubes and fixed in a solution of F.A.A [(100 ml acetic acid (CH₃COOH), 100 ml formaldehyde (HCHO) and 1800 ml 50% ethanol (CH₂H₂OH), thereafter were put on a mechanical shaker for 2 to 3 hours. Samples were dehydrated with 30, 50, 70 and 100% ethanol and ethanol was extracted from the samples at 30, 50, 70 and 100% xylene. Samples were mounted so that they could be preserved for years and stained with toluidine solution. Anatomical structures of wild ginger enlarged root were observed with a light microscope at 100X magnification. Anatomical structures observed in wild ginger enlarged roots were primary xylems and the cells between them, glandular cells, pericycle layer, cortex, endodermis and pith.

N application level of 250 kg·ha⁻¹ increased the number of glandular cells in the anatomy of wild ginger enlarged root, indicating improved capacity for production of essential oils by wild ginger roots. Glandular cells increased from two for plants that received no nitrogen to eight for plants that was fertilized with 250 kg·ha⁻¹. Glandular cells increased from two for plants that received no nitrogen to eight at the highest (250 kg·ha⁻¹) level applied. This study demonstrated that N nutrition is important for increasing glandular cells important for essential oil production.

For plants grown in pine bark, glandular cells increased from one for plants that received 0.25L/day to three for plants that received 1L/day, indicating improved capacity for production of essential oils by wild ginger roots. For plants grown in sand, there were no glandular cells for plants that received 2L/day and increased to sixteen when 2L/week was applied.

Further studies need to be implemented on the effect of N nutrition to the enlarged root anatomy of wild ginger, to determine clear relationships between the number of primary xylems, cells between primary xylems and the optimum N level which could results in the superior number of glandular cells. In this study, the highest N level (250) produced more glandular cells, therefore more N level need to be added in order to determine the optimum N level which will significantly increased the number of glandular cells. It is also recommended that, if one needs to grow wild ginger in pine bark, plants should be fertigated with 1L/day to increase the number of glandular cells essential for oil production, whereas in sand, a fertigation frequency of 2L/week should be used to increased the number of glandular cells.

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APPENDICES

APPENDIX A

SUMMARISED ANALYSIS OF VARIANCES

FIELD NITROGEN EXPERIMENT I

Table A1 Analysis of variance for fresh rhizome and root characteristics of wild ginger as affected by N application in 2001/02 seasons

	Mean squares ^a	
	Fresh rhizome mass (10 ³)	Fresh root mass (10 ³)
Nitrogen	106679*	2727*
Error	990	471

P values significant at 5% level of probability () or highly significant at 1% level of probability (**)

TUNNEL FERTILISER EXPERIMENT 2
APPENDICES
APPENDIX A
 Table A2 Analysis of variance for plant height, number of leaves and number of roots of wild ginger at 56, 112, 168 and 224 DAE as affected by fertilisation frequency and growing medium for Experiment 2

SUMMARISED ANALYSIS OF VARIANCES

FIELD NITROGEN EXPERIMENT 1										
	Mean squares ^z									
	Fresh rhizome		Rhizome		Number of		Fresh enlarged		Number of	
	mass (10 ⁻³)		circumference		rhizomes		root mass		enlarged roots	
	(mg)		(10 ⁻¹) mm		(10 ⁻⁵)		(10 ⁻² mg)		(10 ⁻²)	
Nitrogen	10667**	890	8625**	480	90	2727*	1193*	2539*	199	469
Error										

^zF values significant at 5% level of probability (*) or highly significant at 1% level of probability (**).

TUNNEL FERTIGATION EXPERIMENT 2

Table A2 Analysis of variance for plant height, number of leaves and stems of wild ginger at 56, 112, 168 and 224 DAE as affected by fertigation frequency and growing medium for Experiment 2

	Mean squares ^z											
	Plant height				Number of leaves				Number of stems			
	56	112	168	224	56	112	168	224	56	112	168	224
	(10 ⁴ mm)				(10 ⁻²)				(10 ⁻³)			
Fertigation												
frequency (F)	913*	120*	201*	339*	1791*	767*	351*	1543*	1171*	741*	407	1568
Growing												
medium (M)	5371*	5370*	5768*	3088*	24642*	24640*	11039*	1161*	7320*	7321*	1429	2702
F X M	206	165	313*	183	272	180	103	330	251	159	707	195
Replication	1094	723	849	564	3665	3159	1357	940	1712	1213	651	1009
Error	112	129	89	141	372	396	350	358	347	370	387	469

^z F values significant at 5% level of probability (*) or highly significant at 1% level of probability (**).

Table A3 Analysis of variance for fresh and dry rhizome and root characteristics, fresh and dry leaves and leaf area at 112 DAE as affected by fertigation frequency and growing medium for Experiment 2

		Mean squares ^z									
		Fresh rhizome mass (g)	Dry rhizome mass (10 ⁵ mg)	Number of rhizomes (10 ²)	Fresh root mass (10 ² mg)	Dry root mass (10 ⁵ mg)	Number of enlarged roots (10 ²)	Enlarged root length (10 ³ mm)	Fresh leaf mass (10 ² mg)	Dry leaf mass (10 ² mg)	Leaf area (cm ²)
104	Fertigation frequency (F)	225	26422*	123	6258	2213	2555*	1091	1953*	318*	26418*
	Growing medium (M)	1915	24080*	1215	3023	9475	3081*	5704	666606*	3943*	93576*
	F X M	966	40468*	1298	14343	2369	5494*	4950	17490*	782	24621*
	Replication	132	248*	375	980	836	1401	12240	5447	301	80499
	Error	511	41061	353	8064	349	4216	2712	7301	472	133677

^zF values significant at 5% level of probability (*) or highly significant at 1% level of probability (**)

Table A4 Analysis of variance for fresh rhizome and root characteristics, fresh and dry leaf mass and leaf area of wild ginger at 224 DAE as affected by fertigation frequency and growing medium for Experiment 2

		Mean squares ^z							
		Fresh rhizome mass (g)	Number of rhizomes	Fresh root mass (g)	Number of enlarged roots	Enlarged root length (10 ⁻³ mm)	Fresh leaf mass (10 ⁻³ g)	Dry leaf mass (10 ⁻² mg)	Leaf area (cm ²)
105	Fertigation frequency (F)	72129*	9842	27413*	2566*	1920	3142*	3018	100796*
	Growing medium (M)	8119	12142	12351	2635	5956	2731*	111	20605*
	F X M	27268	10468	19795	1191	5834	1768	851	237755
	Replication	42495	8333	23523	2089	4184	2614	1677	553483
	Error	19067	433	12049	896	2835	1561	605	140372

^zF values significant at 5% level of probability (*) or highly significant at 1% level of probability (**)

Table A5 Analysis of variance for the fresh rhizome and enlarged root oil yield at 224 DAE as affected by fertigation frequency and growing medium for Experiment 2

	Mean squares ^z	
	Fresh rhizome oil yield	Fresh enlarged root oil yield
	(10 ⁻¹⁰ %)	(10 ⁻⁸ %)
Fertigation frequency (F)	91917	1126
Growing medium (M)	720	1008
F X M	46732	5105
Replication	61702	8396
Error	85550	1015

^zF values significant at 5% level of probability (*) or highly significant at 1% level of probability (**)

APPENDIX B

FIELD NITROGEN EXPERIMENT 1

Table B1 Details of nitrogen application used in Experiment 1

Time of application	Nitrogen (kg·ha ⁻¹)	Lime stone ammonium nitrate (LAN)	Number of rows planted	Plot size (m ²)	LAN/plot
Planting	0	0	5	23.75	0
Planting	50	178.6	4	19	339.3
Planting	100	357.1	4	19	678.5
Planting	150	535.7	4	19	1017.8
Planting	200	714.3	4	19	1357.2
Planting	250	892.9	5	23.75	2120.6

FIELD NITROGEN EXPERIMENT 1

Table B2 Fertility status of the soil in Experiment 1 in the field before application of N levels during 2001/02 seasons

Soil level	pH	Resistance(ohm)	P Bray 1 (mg·kg ⁻¹)	Ca (mg·kg ⁻¹)	K (mg·kg ⁻¹)	Mg (mg·kg ⁻¹)	Na (mg·kg ⁻¹)
Topsoil	6.5	3400	24.4	369	37	103	2
Subsoil	6.6	3600	33.4	452	55	132	4

Table B3 Fertility status of the soil in Experiment 1 in the field after N application during 2001/02 seasons

Soil level	Nitrogen (kg·ha ⁻¹)	pH	Resistance (ohm)	P Bray 1 (mg·kg ⁻¹)	Ca (mg·kg ⁻¹)	K (mg·kg ⁻¹)	Mg (mg·kg ⁻¹)	Na (mg·kg ⁻¹)
Topsoil	0	7.2	3000	39.1	534	39	170	2
	50	7.0	3600	38.0	474	40	130	4
	100	7.2	3800	35.5	486	33	170	4
	150	7.0	3200	34.0	449	43	143	0.9
	200	6.7	3300	38.4	382	51	95	4
	250	6.5	2400	34.8	518	54	158	4
Subsoil	0	6.8	3400	37.7	594	47	131	6
	50	6.7	3500	41.6	418	53	110	1
	100	6.7	3600	38.9	498	44	117	2
	150	6.7	3800	33.1	491	46	133	7
	200	6.4	3500	38.9	418	51	105	9
	250	6.4	2000	36.8	489	52	128	11

Table B4 Fertility status of pine bark in Experiment 2 before planting during 2002/03 seasons

	pH	EC	Ca	Mg	K	Na	Cu	Fe	Mn	Zn	NH ₄	NO ₃
		mS/m	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
	5.7	161.0	50.1	48.0	252.5	27.8	0.00	0.07	0.30	0.00	45	198

Table B5 Fertility status of sand in Experiment 2 before planting during 2002/2003 seasons

	pH	Resistance (ohm)	P Bray 1 (mg·kg ⁻¹)	Ca (mg·kg ⁻¹)	K (mg·kg ⁻¹)	Mg (mg·kg ⁻¹)	Mg (mg·kg ⁻¹)
	6.9	18000	1.4	93	364	4	15

Table B6 Wild ginger fresh rhizome and enlarged root oil yield as affected by fertigation frequency and growing medium

Sample number	Fresh rhizome mass (g)	Fresh rhizome oil wet mass (g)	Fresh rhizome oil yield (%)	Fresh root mass (g)	Fresh root oil wet mass (g)	Fresh root yield (%)
1112	471.6	0.63	0.13	706.8	0.35	0.05
2111	781.6	0.49	0.10	496.8	0.17	0.04
1111	436.2	0.34	0.11	873.1	0.38	0.05
2112	408.7	0.31	0.10	632.6	0.31	0.05
1211	398.8	0.51	0.13	492.6	0.19	0.04
2211	426.6	0.16	0.04	630.7	0.26	0.04
1212	371.8	0.36	0.11	601.2	0.57	0.09
2212	606.6	0.38	0.06	698.7	0.61	0.08
2312	545.2	0.28	0.05	575.3	0.25	0.03
1312	694.4	0.29	0.04	775.9	0.41	0.05
1311	772.6	0.36	0.05	610.4	0.38	0.06
2311	476.1	0.41	0.09	322.1	0.27	0.08
2412	663.7	0.27	0.04	560.1	0.17	0.03
1412	818.7	0.39	0.05	482.3	0.27	0.06
1411	277.8	0.16	0.06	276.7	0.23	0.08
2411	856.6	0.52	0.06	767.3	0.34	0.04
2512	22.4	0.02	0.07	587.4	0.22	0.05
2511	431.1	0.23	0.05	298.7	0.20	0.07
1511	321.5	0.32	0.12	51.11	0.10	0.16
1512	474.1	0.32	0.07	435.3	0.41	0.09

First letter {eg 2311} = replication; second = fertigation frequency;

third = plant part [1= rhizome and 2 = root]; fourth letter = growing media

[1= pine bark and 2= sand].