

HERBICIDE EVALUATION FOR WEED CONTROL IN KENAF (HIBISCUS CANNABINUS L.)

by

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DECLARATION

I hereby certify that this dissertation is my own work, except where duly acknowledged. I also certify that no plagiarism was committed in writing this dissertation. This work has never been submitted by me at any other university.

Signed	
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ABSTRACT

Herbicide evaluation for weed control in kenaf (*Hibiscus cannabinus* L.)

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Kenaf (Hibiscus cannabinus L.) was introduced in 2005 as a fibre crop on a commercial scale in the KwaZulu-Natal Province of South Africa. No herbicides have yet been registered for use in this crop. The purpose of this study was to determine the tolerance of kenaf to a total of five pre-emergence and four post-emergence herbicides under semi- and fully controlled conditions. The herbicides were chosen based on their potential safety for use in Hibiscus spp. as well as on the weed spectra they are registered for in other crops. Several additional factors were also taken into consideration, such as: temperature, soil depth and timing of herbicide application. Four pot trials were conducted to determine the separate and combined effects of herbicide, temperature, planting depth and application timing. During the first trial the effects of five pre-emergence herbicides and four post-emergence herbicides were researched. The pre-emergence herbicides were: S-dimethenamid, imazethapyr, fluometuron/prometryn, pendimethalin, S- metolachlor and the post-emergence herbicides were: bentazone, 2,4-DB, monosodium methanearsonate and pyrithiobac sodium. The trials were conducted under either semi-controlled conditions in a glasshouse or in growth cabinets under fully controlled conditions at the Hatfield Experimental Farm of the University of Pretoria. All experiments were conducted with a Hutton soil with 22% clay. Each trial lasted about 40 days to allow for maximum phytotoxicity damage manifestation on the kenaf seedlings.

Measurements that were taken included plant height, herbicide damage, weed control efficiency, fresh plant weight, dried plant weight, and dried root weight. The data were subjected to analysis of variance (ANOVA) to determine the statistical likelihood of damage to plants from the herbicides. In Trial 2, 3 and 4 the interaction effects of herbicide and plant depth, herbicide and temperature, and herbicide and application timing were researched respectively. Neither planting depth nor application timing affected the kenaf seedlings negatively, but low temperature in combination with the



application of herbicides during germination of seed and seedling emergence had serious deleterious effects on the young kenaf seedlings. Based on the findings the majority of the herbicides can be included in further field trials on *Hibiscus cannabinus* L. with the exception of S-dimethenamid and fluometuron/prometryn which caused substantial injury to the kenaf seedlings.



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TABLE OF CONTENTS

1.	INTR	ODUCTION	15
2.	LITEF	RATURE REVIEW	17
	2.1	TAXONOMY AND NOMENCLATURE	17
	2.2	BOTANY	
	2.2.1	Whole plant	18
	2.2.2	Stem (stalk)	18
	2.2.3	Leaves	20
	2.2.4	Inflorescence	20
	2.2.5	Seeds	21
	2.2.6	Growth conditions	22
	2.3	HISTORICAL USE OF KENAF AS FIBROUS CROP	23
	2.3.1	Introduction	23
	2.3.2	United States	23
	2.3.3	China and the Far East	24
	2.3.4	Other parts of the world	25
	2.4	USES OF KENAF	26
	2.4.1	Introduction	26
	2.4.2	Stalks	26
	2.4.3	Usefulness of the whole plant	28
	2.4.4	Leaves	29
	2.4.5	Seed	29
	2.5	THE USE OF HERBICIDES IN KENAF CULTIVATION	30
	2.5.1	Introduction	30
	2.5.2	Literature review	31
3.	GFNI	ERAL MATERIALS AND METHODS	35
	3.1	Introduction	
	3.2	EXPERIMENTAL DESIGN	
	3.3	MATERIALS	
	3.3.1		
	3.3.2	,	
	3.3.3	.,	
	3.3.4	3	
	3.3.5		
	3.3.6		
	3.4	METHODS	51



	3.4.1	Preparation of herbicide solutions	51
	3.4.2	Planting	51
	3.4.3	Herbicide application	52
	3.4.4	Daily chores and monitoring	53
	3.4.5	Weekly chores and monitoring	53
	3.4.6	Other measurements	53
	3.4.7	Harvesting	55
	3.5	Data analysis	56
4.	TRIA	L 1: PRE-EMERGENCE HERBICIDE SCREENING	57
	4.1	Introduction	57
	4.2	MATERIALS AND METHODS	57
	4.3	RESULTS AND DISCUSSION	59
	4.3.1	Emergence	59
	4.3.2	Plant height	60
	4.3.3	Herbicide injury	60
	4.3.4	Weed control: grass species	62
	4.3.5	Weed control: broadleaved species	62
	4.3.6	Kenaf fresh mass: top growth	63
	4.3.7	Kenaf dry mass: top growth	64
	4.3.8	Kenaf dry mass: roots	65
	4.4	Conclusions	66
5.	TRIA	L 1: POST-EMERGENCE HERBICIDES	68
	5.1	Introduction	68
	5.2	MATERIALS AND METHODS	69
	5.3	RESULTS AND DISCUSSION	70
	5.3.1	Emergence	70
	5.3.2	Plant height	70
	5.3.3	Herbicide injury	71
	5.3.4	. Weed control: grass species	73
	5.3.5	. Weed control: broadleaved species	73
	5.3.6	. Kenaf fresh mass: top growth	74
	5.3.7	. Kenaf dry mass: top growth	75
	5.3.8	. Kenaf dry mass: roots	76
	5.4.	Conclusions	77



	6.1.	Introduction	79
	6.2	MATERIALS AND METHODS	79
	6.3	RESULTS AND DISCUSSION.	80
	6.3.1	Emergence	80
	6.3.2	Plant height	81
	6.3.3	B Herbicide injury	81
	6.3.4	Kenaf fresh mass: top growth	82
	6.3.5	Kenaf dry mass: top growth	84
	6.3.6	Kenaf dry mass: roots	85
	6.4	CONCLUSIONS	87
7.	TRIA	L 3: THE ROLE OF TEMPERATURE IN THE SENSITIVITY OF KENAF (HIBISCUS CANNABINUS)	
		S PRE-EMERGENCE HERBICIDES	88
	7.4		00
	7.1	Introduction	
	7.2	MATERIALS AND METHODS	
	7.3	RESULTS AND DISCUSSION Emergence	
	7.3.1		
	7.3.2 7.3.3		
	7.3.3		
	7.3.4	22	
	7.3.6		
	7.3.0	Conclusions	
8.	TRIA	L 4: INVESTIGATING THE ROLE OF APPLICATION TIMING IN HERBICIDE SENSITIVITY OF KENAF	98
	8.1	Introduction	98
	8.2	MATERIALS AND METHODS	99
	8.3	RESULTS AND DISCUSSION.	100
	8.3.1	Emergence	100
	8.3.2	Plant height	101
	8.3.3	B Herbicide injury	101
	8.3.4	Kenaf fresh mass: top growth	102
	8.3.5	Kenaf dry mass: top growth	102
	8.3.6	Kenaf dry mass: roots	102
	8.4	CONCLUSIONS	103
9.	SUM	MARY	105
	0.1	Constant	105
	9.1	CONCLUSION	105
	7.7	CUNCLUSION	(U/



	9.2.1	Herbicide selectivity	. 107
	9.2.2	Influence of soil depth	. 108
	9.2.3	Influence of temperature	. 109
	9.2.4	Influence of application timing of pre-emergence herbicides	. 109
	9.2.5	Recommendations	. 109
REFE	RENCES		.111
APPI	ENDIX A	ANALYSIS OF VARIANCE	.118
APPI	ENDIX B	SUMMARY OF THE MODE OF ACTION OF HERBICIDES	.123
APPI	ENDIX C	SUPPLEMENTARY TABLES	.128



TABLE OF FIGURES

Figure 2-1 Kenaf "Tainung#2" stand at 4 months in the Winterton area. Average height: 2m. (Photo: AS Ma	lan
2008)	18
Figure 2-2 Kenaf stalks ('Everglades 41') at harvest, 150 days after planting, at USDA, ARS South Central	
Agricultural Research Laboratory, Lane, Oklahoma. (Photo: Webber et al. 2002)	18
Figure 2-3 Section of a young kenaf stem (Photo: Courtesy of Prof PJ Robbertse 2009)	19
Figure 2-4 Section of kenaf stem showing first bast fibre bundle ring (Photo: Courtesy of Prof PJ Robbertse	
2009)	19
Figure 2-5 The juvenile leaf of Hibiscus cannabinus (Photo: AS Malan 2008)	20
Figure 2-6 The divided leaf shape (Photo: AS Malan 2008)	20
Figure 2-7 A kenaf flower in full bloom (Webber et al. 2002)	21
Figure 2-8 Kenaf seeds (Webber et al. 2002)	22
Figure 3-1 Experimental layout of one of the kenaf pot experiments (Photo: AS Malan 2008)	36
Figure 3-2 Molecular structure of dimethenamid - formula: $C_{12}H_{18}CINO_2S$	39
Figure 3-3 Molecular structure of imazethapyr - formula: $C_9H_{10}CIN_5O_2$	40
Figure 3-4 Molecular structure of fluometuron - formula: $C_{10}H_{11}F_3N_2O$	41
Figure 3-5 Molecular structure of prometryn - formula: $C_{10}H_{11}F_3N_2O$	41
Figure 3-6 Molecular structure of pendimethalin - formula: $C_{13}H_{19}N_3O_4$	42
Figure 3-7 Molecular structure of metolachlor - formula: $C_{15}H_{22}CINO_2$	43
Figure 3-8 Molecular structure of bentazone - formula: $C_{10}H_{12}N_2O_3S$	45
Figure 3-9 Molecular structure of 2,4 DB - formula: $C_{12}H_{14}Cl_2O_3$	46
Figure 3-10 Molecular structure of monosodium methanearsonate - formula: CH₄AsNaO₃	47
Figure 3-11 Molecular structure of pyrithiobac sodium - formula: $C_{13}H_{10}CIN_2NaO_4S$	48
Figure 3-12 Illustration of how soil depth is determined according to weight of soil	51
Figure 3-13 Herbicide application requires expert technical ability! (Photo: AS Malan 2008)	52
Figure 3-14 No injury to any of the seedlings (Injury factor=0)	54
Figure 3-15 Slight damage visible in chlorosis and deformity of leaves due to herbicide sensitivity (Injury	
factor=1)	54
Figure 3-16 Medium damage (further visible signs of chlorosis/leaf deformity) due to herbicide sensitivity	
(Injury factor=3)	54
Figure 3-17 Severe damage, plants show signs of senescence (Injury factor=4)	54
Figure 3-18 All plants have effectively died (Injury factor = 5) (Photos: AS Malan 2008)	54
Figure 4-1 The daily average day and night temperatures as measured in a semi-controlled glasshouse during	ng a
5 week growth period	58
Figure 4-2 The planting of the seeds (Photo: AS Malan 2008)	58
Figure 4-3 Irrigating the pots to field capacity (Photo: AS Malan 2008)	58



Figure 4-4 Significant kenaf plant height differences, 40 DAP, when treated with different pre-emergence
herbicide at different application rates60
Figure 4-5 Kenaf seedling damage caused by pre-emergence herbicide treatments on a scale from 0 to 5, 20
DAP (0 = no injury, 5 = senescence of seedlings)61
Figure 4-6 Severe injury effect of fluometuron applied at 3x the recommended rate. (Photo: AS Malan 2008) 62
Figure 4-7 Less severe injury caused by imazethapyr at 3x the recommended rate. (Photo: AS Malan 2008) 62
Figure 4-8 The effectiveness of broadleaved weed control in kenaf treated with six pre-emergence herbicides at
three application rates63
Figure 4-9 The fresh top mass of kenaf seedlings as affected by three application rates per herbicide as
compared to the control64
Figure 4-10 The dry top mass of kenaf seedlings as affected by three application rates per herbicide as
compared to the control65
Figure 4-11 The dry root mass of kenaf seedlings as affected by three application rates per herbicide as
compared to the control66
Figure 5-1 Effect of post-emergence herbicides on plant height of kenaf seedlings71
Figure 5-2 Kenaf seedling injury caused by post-emergence herbicides at three application rates72
Figure 5-3 Grass weed species control in kenaf with different post-emergence herbicides at three application
rates
Figure 5-4 Broadleaved weed species control in kenaf with post-emergence herbicides at three application
rates
Figure 5-5 Average fresh mass of kenaf seedling top growth as affected by post-emergence herbicides at three
application rates
Figure 5-6 The average dried mass of kenaf seedling top growth as affected by post-emergence herbicides
applied at three application rates76
Figure 5-7 The average dry root mass of kenaf seedlings affected by post-emergence herbicides applied at three
application rates77
Figure 6-1 Differences in kenaf fresh top growth mass as affected by two planting depths and different
herbicide treatments
Figure 6-2 Average dry top growth mass of kenaf as affected by planting depth. (Data averaged over herbicide
treatment and application rate)85
Figure 6-3 Differences in kenaf root mass as affected by different herbicides and two planting depths86
Figure 7-1 The kenaf containing pots arranged inside the medium temperature range growth chamber (Photo:
AS Malan 2008)89
Figure 7-2 Kenaf seedling mergence rate as affected by temperature (Data averaged over herbicide treatment
and application rate. Count = total amount of seedlings emerged)91
Figure 7-3 Kenaf seedling injury rating as affected by temperature and herbicide93
Figure 7-4 Significant differences in the effect of temperature range on dry root mass (Data averaged over
herbicide treatment and dosage)96



Figure 7-5 Significant effects of herbicide treatment on kenaf dry root mass (Data averaged over dosage and	
temperature)	96
Figure 8-1 The relative safety of pre-emergence herbicides used in Trial 4 on kenaf seedling injury	104



TABLE OF TABLES

Table 2-1 Recommendation of herbicides that were used in earlier research, in the USA, on kenaf (compiled	l
according to company label recommendations in USA)	34
Table 3-1 Properties of the soil used in all the herbicide pot experiments	37
Table 3-2 Pre-emergence herbicides used in kenaf Trials 1 – 4	38
Table 3-3 Post-emergence herbicides used in kenaf Trial 1	39
Table 3-4 The nutrients in Supafeed [®]	50
Table 3-5 List of weed species that emerged during the trials	55
Table 4-1 Emergence of kenaf seed as affected by pre-emergence herbicides	59
Table 4-2 The average grass weed control in kenaf as affected by application rate	62
Table 4-3 Summary of the pre-emergence herbicide efficacy in kenaf under controlled conditions	67
Table 5-1 Kenaf seedling emergence	70
Table 5-2 Summary of the performance of the post-emergence herbicides for use in kenaf	78
Table 6-1 Difference in kenaf emergence between two planting depths	80
Table 6-2 The significant effects of herbicide dosage on average plant height of kenaf seedlings	81
Table 6-3 The average injury rating for kenaf plants as affected by herbicide application rates	82
Table 6-4 Average fresh top growth mass of kenaf seedlings as affected by herbicide. (Data averaged over	
application rate and planting depth)	83
Table 6-5 Average fresh top growth mass of kenaf as affected by application rate. (Data averaged over	
herbicide treatment and planting depth)	83
Table 6-6 Average dry top growth mass of kenaf as affected by herbicide. (Data averaged over application	rate
and planting depth)	84
Table 6-7 Average dry top growth mass of kenaf as affected by application rate. (Data averaged over herbi	cide
treatment and planting depth)	84
Table 6-8 Average dry root mass of kenaf as affected by planting depth. (Data averaged over herbicide	
treatment and application rate)	86
Table 6-9 Average dry root mass of kenaf as affected by application rate. (Data averaged over herbicide	
treatment and planting depth)	87
Table 7-1 Kenaf seedling emergence as affected by temperature (Data averaged over herbicide treatment	and
application rate)	90
Table 7-2 Kenaf plant height as affected by temperature, herbicide treatment and application rate	92
Table 7-3 Kenaf seedling injury rating as affected by temperature and dosage. (Data averaged over herbicides)	de
treatments)	92
Table 7-4 Significant differences in the interaction of herbicide treatment, application and temperature ran	ge
on fresh top mass of kenaf seedlings	94
Table 7-5 Significant differences in the interaction of herbicide treatment, application and temperature ran	ge
on dry ton mass of kenaf seedlings	95



Table 8-1 The final average count of emerged kenaf seedlings per pot as a percentage	. 100
Table 8-2 The average plant height for each treatment and application timing compared to the control	. 101
Table 8-3 Injury rating (0-5)** of kenaf plants as affect by herbicide application rate	. 101
Table 8-4 Significant differences in kenaf seedling fresh top mass due to the interaction between application	า
timing and dosage	. 102
Table 8-5 No significant differences in the effects of any of the parameters that could affect kenaf dry root	
mass	. 103

APPENDIX C TABLE OF TABLES

Appendix C Table 1 Trial 1: Kenaf seedling emergence count expressed as a percentage	128
Appendix C Table 2 Trial 1: Effects of pre-emergence herbicides on kenaf plant height	128
Appendix C Table 3 Trial 1: Effects of pre-emergence herbicides on plant health	129
Appendix C Table 4 Trail 1: Weed control for grass species in kenaf	129
Appendix C Table 5 Trail 1: Weed control for broadleaved species in kenaf	130
Appendix C Table 6 Trial 1: The interaction between herbicide and dosage for kenaf fresh top growth mass	130
Appendix C Table 7 Trial 1: The interaction between herbicide and dosage for kenaf dry top growth mass	130
Appendix C Table 8 Trial 1: The interaction between herbicide and dosage for kenaf dry root mass	131
Appendix C Table 9 Trial 1: The emergence count expressed as a percentage	131
Appendix C Table 10 Trial 1: Significant differences in the interaction of post-emergence herbicide x dosage	e on
kenaf plant health	131
Appendix C Table 11 Trial 1: Significant differences in the post-emergence herbicide x dosage interaction of	n
grass weed control in kenaf	132
Appendix C Table 12 Trial 1: Significant differences in the herbicide x dosage interaction on broadleaved we	eed
control in kenaf	132
Appendix C Table 13 Trial 2: Kenaf seedling emergence count expressed as a percentage	133
Appendix C Table 14 Trial 2: Average kenaf plant height	133
Appendix C Table 15 Trial 3: Kenaf seedling injury rating as affected by temperature and herbicide. (Data	
averaged over application rates)	134
Appendix C Table 16 Trial 3: significant effects of herbicide treatment on kenaf dry root mass	134



1.

INTRODUCTION

The cultivation of kenaf (*Hibiscus cannabinus* L.) on commercial scale in Southern Africa is a fairly recent development. Apart from notes by Dempsey (1975) about some crop farming practices during the 1950's, it has not been grown on a significant scale in South Africa until about 2005, and has in fact been regarded as a noxious weed by most farmers for generations, and is described as such in literature about weeds (Bromilow, 1996). Earlier authors such as Murdock (1959), Wilson and Menzel (1964) and Dempsey (1975) regard kenaf to be a native of Africa.

The introduction of kenaf as fibrous crop in South Africa was spearheaded by a company called SFS (Sustainable Fibre Solutions (Pty) Ltd.). This company was founded due to a scarcity of natural fibres in the region. Research conducted by the South African Agricultural Research Council (ARC) on suitability of growth conditions and on cultivar evaluation directed the company to choose Winterton, KwaZulu Natal, and Tainung II as the respective area and cultivar to cultivate kenaf for the fibre market.

Winterton is situated in an area known as the foothills of the Drakensberg. The veldtype according to Acocks (1988) is the Dohne Sourveld which is very similar to the Highland Sourveld, but it is found at lower altitudes of 600 - 1350 m above sea level. It is warmer and drier than higher up the plateau, receiving 650 - 1000 mm of rain per annum, and no snow in winter except on the top of the mountains (occasional snow precipitation is possible). Soils are more fertile than that of the Highland Sourveld, and slightly less thoroughly leached.

Farmers from the Winterton and Bergville districts were canvassed to introduce kenaf as a crop, especially as a rotational crop with soy bean, or as an addition to existing wheat farms. Unfortunately, within the initial seasons, in November/December of 2006 in particular, the local farmers experienced huge kenaf crop failures. The company had to take a step back and examine the possibilities that could have induced these crop failures. Use of unregistered herbicides were thought to be the main factor responsible, but there also existed the possibility that a sudden drop in temperatures due to a cold front two weeks after planting could have contributed to crop injury¹. The University of Pretoria was subsequently contracted to investigate some of the parameters that would

¹ Personal comments by Mr. Dale van den Aardweg and management team of SFS (Website: http://www.kenaf.co.za; email address: robink@kenaf.co.za)



affect kenaf cultivation. The first project undertaken by the University was to screen a range of herbicides available in South Africa to determine the sensitivity of kenaf towards them. This project was divided into an initial experimental phase with potted plants under controlled conditions, after which the screened herbicides were to be researched further under field conditions in the Winterton area.

The study reported here represents the first phase of a broader project aimed at getting herbicides registered for use in kenaf. The initial phase should be followed up by field trials which may be conducted in conjunction with agro-chemical companies for the purpose of seeking registration of certain herbicides in kenaf. Phase 1, which was the focus of the present investigation, involved pot experiments to assess the selectivity (=safety) of selected pre- and post-emergence herbicides towards kenaf. As part of the herbicide screening process, several other factors were also researched. These were: the role that planting depth of kenaf might play in herbicide injury, the possible aggravation of herbicide injury by low temperatures, and the possible influence of application timing of pre-emergence herbicides on the tolerance of kenaf. Since the present study involves the initial experimental phase, it should provide some of the critical information necessary to take herbicide screening to the next level, the field trials.



2.

LITERATURE REVIEW

2.1 Taxonomy and nomenclature

Kenaf belongs to the Malvaceae or mallow family, which is a moderate-sized family with some 50 genera and 1000 species distributed worldwide. The economic importance of this family lies with those species which are cultivated for their fibre, and of these cotton is undoubtedly the most important fibre-producing plant in the world. Cotton belongs to the genus *Gossypium*, and is closely related to the genus *Hibiscus*, which includes the species *H. cannabinus* (kenaf) and *H. sabdariffa* (roselle), both of which are grown for their fibre content. Also included in the *Hibiscus* family is the species *H. esculentus* (synonym *Abelmoschus esculentus* L. - okra bean), which is grown for its fruit (Berrie 1977). The *Hibiscus* family is divided into 6 sections: *Abelmoschus*, *Alyogen*, *Azanza*, *Calyphyllia*, *Fucaria* and *Ketmia*. Taxonomically, kenaf is classified in the *Fucaria* section of *Hibiscus*; along with 40 to 50 other species that are all closely related morphologically (Dempsey 1975, Taylor 1995). The use of the word kenaf as a common name for *Hibiscus cannabinus* probably originated from the Persians who called the species 'kannaf', which they used for pulp and fibre. In this report, kenaf means or refers to *Hibiscus cannabinus* L.

Common Names²

English: kenaf (Persian origin)

India (Bengal): mesta India (Madras): palungi

India (Bombay): deccan hemp India (Andhra Pradesh): Bimli jute

Taiwan: ambari

Egypt and northern Africa: til, teel, or teal

Indonesia: Java jute

Brazil: papoula de Sao Francisco

South Africa: stokroos

West Africa: dah, gambo, and rama

² According to Miyake and Suzuta (1937), there are more than 129 names for kenaf worldwide (in CropFactsheet: Kenaf - Contributor: Charles S. Taylor, Kenaf International, Ltd., McAllen, TX [based on Dempsey (1975)])



2.2 Botany

2.2.1 Whole plant

Kenaf is an annual or biennial (hardly ever perennial) herbaceous plant with an erect growth form. The plant reaches a height of between 1 and 4 meters (Figures 2.1 and 2.2) during the growth season which lasts about 5-6 months. When grown in dense stands it remains largely unbranched (LeMahieu *et al.* 1991). The plant is photoperiodic, flowering on shortening days of 12.5 hours or less. According to a genetic study conducted by researchers from Fudan and Tongji Universities in Shangai, People's Republic of China, kenaf (*H. cannabinus*) as well as its relative roselle (*Hibiscus sabdariffa* var. *altissima*) originated from Africa. The authors used ALFP (amplified fragment length polymorphism) fingerprinting analysis to determine the origin of kenaf. The study showed that the dissemination of kenaf was from Africa through Asia to Central and North America. (Cheng *et al.* 2004).



Figure 2-1 Kenaf "Tainung#2" stand at 4 months in the Winterton area. Average height: 2m. (Photo: AS Malan 2008)



Figure 2-2 Kenaf stalks ('Everglades 41') at harvest, 150 days after planting, at USDA, ARS South Central Agricultural Research Laboratory, Lane, Oklahoma. (Photo: Webber et al. 2002)

2.2.2 Stem (stalk)

The colour of the stem is mainly green, but there are also red-stemmed and purple-stemmed accessions (LeMahieu *et al.* 1991). The entire stem is covered in fine hairs that can leave an unpleasant burning sensation to the human touch, similar to brambles but not as severe. It is inside the stem where the fibres (bast fibres) are located, which is the



main focus of kenaf cultivation as a crop. The long fibres are found in the bast (bast fibres = phloem cells) (Figures 2.3 and 2.4), which is between the epidermis and the woody core where the shorter core fibres are found (Franck 2005).

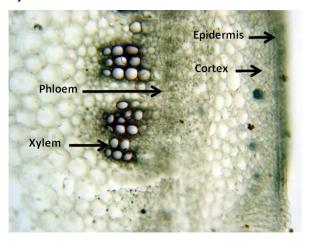


Figure 2-3 Section of a young kenaf stem (Photo: Courtesy of Prof PJ Robbertse 2009)

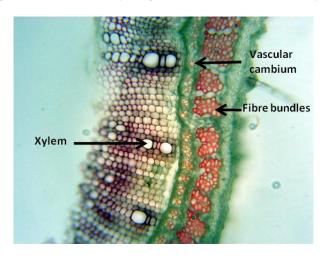


Figure 2-4 Section of kenaf stem showing first bast fibre bundle ring (Photo: Courtesy of Prof PJ Robbertse 2009)

The mean bast fibre diameter of kenaf is 20 μ m with a range from 12 – 36 μ m, and the fibre length varies between 2 and 6 mm (Franck 2005). The plant is usually harvested as a whole unit and then processed by advanced machinery to separate the fibres from the rest of the plant. There are a few key elements that affect kenaf yields. They are: the length of the growing season, average day and night temperatures, and adequate plant available soil moisture (Webber *et al.* 2002).

Stalk yields normally range from 11 to 18 t ha⁻¹ (oven dry weight) and of the different cultivars, Tainung #2 produced the greatest yields in a two-year study with five cultivars (Webber 1993b) and in a three-year study with 16 kenaf cultivars (Webber 1997). The highest stalk yield was 21.8 t ha⁻¹. According to McMillin *et al.* (1998), it was proven in



their study that dry stalk weight and total yield were positively correlated with stalk diameter. Bast : core fibre ratio was inversely correlated with stalk height.

2.2.3 Leaves

The leaves of the kenaf plant have a very similar appearance to that of *Cannabis sativa* or marijuana, hence the species name of *H. cannabinus*. The leaves are simple with serrated edges and grow from the main stem as well as the branches. During the seedling stage the juvenile leaves have a very different appearance from the mature plant, being simple, entire and cordate (Figure 2.5). The different cultivars also vary substantially at times. Plant age also plays a role in the shape of the leaves, but there are mainly two general leaf types: entire ('Everglades 41', 'Guatemala 4', 'Guatemala 45', 'Guatemala 48', 'Cuba 108', 'Cuba 2032' and N7') or divided ('Everglades 71', 'Tainung #1', 'Tainung #2', 'Guatemala 51', ' and 'SF-459') with three, five or seven lobes per leaf (Figure 2.6) (Webber *et al.* 2002). According to a report by Jones *et al.* (1955) the entire leaf shape is due to a recessive gene and the divided leaf characteristic to the dominant gene.



Figure 2-5 The juvenile leaf of Hibiscus cannabinus (Photo: AS Malan 2008)



Figure 2-6 The divided leaf shape (Photo: AS Malan 2008)

A nectar gland is found on the mid-vein of the underside of the leaf (Dempsey 1975), which is visited by a wasp (*Campsomeris trifasciata*) (Jones *et al.* 1955). These wasps visit the plants in large numbers during summer, but they seem to ignore the flowers and to mainly focus on extracting nectar from these glands.

2.2.4 Inflorescence

The flowers of kenaf (figure 2.7) are very similar to those of cotton, okra or the common hollyhock (*Althea rosea*). They are large (8 to 13cm), coloured red, beige or yellow with



crimson centers. They last only one day: they open just before daybreak, begin to close about midday and by mid-afternoon their blossom period is over. Inside the corolla the staminal column surrounds the style with a short stamen. The flowers are borne singly along the stem and branches in the leaf axis (Mabberley 1987).



Figure 2-7 A kenaf flower in full bloom (Webber et al. 2002)

Pollen is released simultaneously with the opening of the flower, and the style emerges shortly afterwards. The corolla closes spirally, which cause the antlers to press against the stigma, and thus enables the plant to self-pollinate, which is a rare occurrence as reported by Ochse *et al.* (1961). According to Jones *et al.* (1955), the nature of the pollen prevents any wind dispersal, and therefore it can be concluded that any cross-pollination must be insect-related.

Nectar is secreted from the base of the corolla, and the flower is mainly visited by common honey-bees (*Apis mellifera*) (Tamargo and Jones 1954). These authors reported that cross-pollination ranged from 2% to 24% for 9 different kenaf strains. In a study by Jones *et al.* (1955), they reported 25 m to be the minimum distance between seed production blocks of different kenaf strains, as 0.16% cross-pollination occurred at this distance. They even found 0.14% crossing at a distance of 302 m. Dubey and Singh (1968), observed that in a hand-pollination experiment, the spiraling action of the corolla can be regarded as a last resort by the species to ensure perpetuation. Studies on the pollination of kenaf have dealt mainly with the effects of cross-pollination, and so far very little have been discovered about the effect of pollination on the total production of seed.

2.2.5 Seeds

After successful pollination, the pointed, ovoid seed capsule is formed that is about 1.9 – 2.5 cm long and 1.3 -1.9 cm in diameter. It is covered with the same fine hairs that also cover the stalk and which cause irritation when in contact with human skin (Webber *et al.*



2002). Within each capsule five segments are found, each containing between four and five seeds (Dempsey 1975). The wedge-shaped seeds are coal black in colour and between 4 and 6 mm long (Figure 2.8). After pollination, the seeds require about four to five weeks to mature (Crane and Acuna 1945).



Figure 2-8 Kenaf seeds (Webber et al. 2002)

2.2.6 Growth conditions

One of the advantages of kenaf as a crop is the fact that it grows under a wide range of climatic and soil conditions. In its natural state, it is mainly found between 45°N and 30°S (Ustinova 1938, in Liu 2005), which indicates a warm, relatively humid tropical and subtropical climate for optimal growth. The plant is definitely not suited to areas where frost occurs, except if planted later during the season when this possibility no longer exists. Kenaf yields have been highest in regions with high temperatures, abundant soil moisture and a long growing season, although yields even in the much cooler Midwest US have been high, but inconsistent (Le Mahieu *et al.* 1991).

Although, like most plant species, kenaf performs better under moist conditions, in a study by Ogbonnaya *et al.* (1997), it was shown that kenaf is able to withstand quite severe deficiencies of moisture in the soil. Although soil moisture stress significantly retarded vegetative growth as analyzed by plant height, stem diameter, leaf development, branching, flowering, and biomass accumulation, water stress had no effects on biomass allocation, in terms of root–shoot and bark–wood ratios. According to Dempsey (1975), a well-distributed rainfall of 100 – 125 mm per month is required for optimum growth, while Crane and Acuna (1945) concur with a required amount of 500 – 625 mm over a period of 5 – 6 months.

As far as soil conditions are concerned, kenaf again seems to be a very adaptable crop with soil types ranging from high organic peat soils to sandy desert soils. It can withstand



late season flooding, low soil fertility as well as a wide range of soil pH values (Dempsey 1975).

The different kenaf cultivars differ in their sensitivity and response to day-length, and most authors agree on the important role day-length plays in the maturing of kenaf plants. Dempsey (1975) divides the cultivars into ultra-early, early to medium, and late-maturing categories. In the US, the distinction is mainly made between photo-sensitive (early to medium maturing) and photo-insensitive cultivars (late maturing cultivars) (Webber *et al.* 2002). Flowering commences when daylight falls below 12.5 hours per day (Liu 2005).

Because of its adaptability, especially to poorer soil conditions, kenaf is often regarded as a weed in South Africa (Bromilow 1996) and probably even more so in the tropical areas, because it is often found in road reserves and on patches of marginal agricultural land. The two main limiting factors in kenaf growth therefore seem to be poor soil drainage and frost.

2.3 Historical use of kenaf as fibrous crop

2.3.1 Introduction

According to the Alternative Field Crops Manual published by the Universities of Wisconsin and Minnesota (LeMahieu *et al.* 1991), kenaf has been cultivated as source of food and fibre for thousands of years. McNulty (1995) reports that Sir William Roxburg (1759 – 1819) conducted experiments with kenaf in India where it was used instead of the European hemp he was familiar with. Here its main use was for cordage, rope, twine and sackcloth. She further refers to a big research program conducted in the USSR on fiber crops between 1920 and 1925. Apparently this study proved so successful, that it grew into a 20-year research program. McNulty (1995) further reports that it was Russia who introduced kenaf into China in the mid-thirties.

2.3.2 United States

During the Second World War, there developed a shortage of fibrous products from countries such as the Philippines, and the USA began looking into producing their own fibres for ropes and cordage. This resulted in research into the use of kenaf as possible crop to provide an alternative source of fibre (Wilson *et al.* 1965). After the crop was established, the research was extended to also cover other agronomic aspects of the crop, such as yield, resistance to anthracnose, as well as better farming practices and



improved harvesting machinery (Nieschlag *et al.* 1960, Wilson *et al.* 1965, White *et al.* 1970).

Another wave of kenaf cultivation followed during the 1950's and 1960's when the United States Department for Agriculture was looking for a replacement for wood in paper production and for alternative sources of fibre. It was discovered that kenaf provided an excellent source of cellulose fibre which is used in various types of paper (White *et al.* 1970). Furthermore, the output of energy and chemicals required to process kenaf proved to be less than for standard tree wood (Nelson *et al.* 1962). For the next two decades, the interest in developing this fairly unknown crop grew steadily. Especially Georgia, Texas, Mississipi and New Mexico became the four states where farmers and researchers alike focused their efforts on kenaf (Rymsza 2000).

After a brief period of dormancy during the early eighties, the emergence of the Kenaf Demonstration Project introduced renewed interest in kenaf research. The main focus of this project was to investigate the viability of kenaf as an ingredient in paper pulp. The private/public initiative actually produced rolls of newsprint, which revived the interest in kenaf cultivation (Rymsza 2000). However, by the late 1990's the Agricultural Research Society saw a budget cut of \$8 million in spending on new crops. After this, the Kenaf Society in the US has been struggling to revive the interest of the government in kenaf production.

2.3.3 China and the Far East

According to Dempsey (1975), India has been cultivating kenaf for the past 200 years, whereas Russia started cultivation in 1902 and then introduced the crop to China in 1935. Today, China and Japan are considered the leaders in the research of cultivars and yield of different kenaf cultivars. Fundamental studies in Japan include:

- Comparing absorption of CO₂ between kenaf and trees. These reports were
 published by three researchers in 1999 in Japanese. Every report pointed out that
 the CO₂ accumulation rate of kenaf is several times higher than that of trees (Lam et
 al. 2003).
- Physical property studies of kenaf fibre structures by x-ray diffraction showed that the bark has a clear and well-defined crystal structure and that the core has a completely amorphous structure (Inagaki 2000)

Kenaf fibre and polypropylene were mixed and manufactured into door panels, dashboards, floor mats, package trays, and other automotive parts. The products had



better properties in comparison with those currently in use. Especially bending properties, measurement stability, stamping properties and limitation radius were improved. From the raw materials point of view, species and harvesting season of kenaf were investigated for making door trims. Kenaf varieties in five different countries were tested in tension tests. Vietnamese kenaf showed high fibre strength and therefore these were used in further tests. As to harvesting season, kenaf fibre from the flowering period indicated high fibre strength and thus kenaf fibre harvested during the flowering season were used. Presently, door trims using kenaf are being used in several types of cars in Japan (Hirata *et al.* 2001).

Kenaf was first introduced in Indonesia in 1970 and became familiar to the farmers after being used in the Governmental program "Intensification Scheme for Smallholders", which was launched in the 1979-1980 and continues to this day. It is cultivated in Java, and in South and East Kalimantan (Sujindro 2001). The fibre is required for industries such as gunny sack mills, goo-textiles, fibre drains, and door-trimming for car interiors. The total fibre production fulfills in only about 25 % of the national requirement.

Appropriate sowing time for kenaf on dry lands is November-December while for flooded areas it is September-October, in these parts of the world. Kenaf growing in Indonesia is conducted on dry lands and flooded areas in Java, and on red-yellow podsolic soil and peat soil areas in East and South Kalimantan. The average fibre productivity at farm level is 1.6 -2.0 t ha⁻¹. Based on the results from experiments on agronomic, breeding, environment and industrial aspects, kenaf development in Indonesia has a bright future in supporting the agro-industries and to enhance farmers' income. The research and development of kenaf and allied fibres in Indonesia are handled by the Research Institute for Tobacco and Fibre Crops (RITFC) (Sujindro 2001).

2.3.4 Other parts of the world

Australia is another continent where the kenaf industry is slowly growing in popularity. Currently not much research has been done in this part of the world, with the only significant work being carried out in the 1980's in the Burdekin River Irrigation Area (Sparkes 2007). According to Sparkes, no herbicides have yet been registered on kenaf in Australia. He reports that the lack of large-scale commercial processing facilities is a major obstacle to further development of this industry. However, Japan used 10 000 tons of non-wood fibre (including kenaf) in 1996 alone, and this figure grew to 40 000 by 2002. This could be incentive enough for Australian farmers to export kenaf fibre to Japan (Sparkes 2007).



The South American continent has also seen some research and development and at the Brazilian Weed Conference as well as the Latin American Weed Association, kenaf herbicide research was reported by Salgado and Deuber (1982). As far as South Africa is concerned, very little research has been done prior to the current research connected to the kenaf processing factory in the Winterton area.

2.4 Uses of kenaf

2.4.1 Introduction

Kenaf is certainly one of the most versatile crop species known to man. For six thousand years the long bark fiber strands of kenaf have been a valuable and important resource for use in cordage products (twine, rope, and sackcloth), and although synthetic fibres now often replace the use of the bark fiber strands in cordage material, the newer and more complete usefulness of the entire kenaf plant continues to make kenaf a crop of worldwide interest.

The kenaf plant components that have proved useful include the following: the stalks (bark and core), leaves, and seeds. There exists ample product diversity in the combined attributes of these components (bark fiber strands and bast fibres, the core material and individual core fibres, and leaf and oil chemistry) to encourage continued use and development of the crop. Beyond the diverse new uses for kenaf including its utilization in paper products, building materials, absorbents, textiles, and livestock feed, additional benefits in the areas of soil remediation, toxic waste cleanup, removal of oil spills on water, reduced chemical and energy use for paper production, greater recycled paper quality, reduced soil erosion due to wind and water, replacement or reduced use of fiberglass in industrial products, and the increased use of recycled plastics are all included in the attractive package presented by the species.

2.4.2 Stalks

Research in Oklahoma with five kenaf cultivars to determine the yield components indicated a yield of 26% leaves and 74% stalks (Webber 1993b). According to this study the stalk itself contained 35% bark and 65% woody core. The bark of the stalks contains the fibre strands that were originally used to make cordage in Africa. The remainder of the stalk, called the woody core, consists of core fibres. These are shorter and thicker than the bast fibres. The entire stalk section has been identified as suitable material for making paper pulp (Nieschlag *et al.* 1960, White *et al.* 1970). The different components



of bast and core fibres can either be processed separately or together, depending on the pulping process (Kaldor *et al.* 1990).

In 1960, the United States Department of Agriculture (USDA) conducted a study to determine which of 500 non-woody plant species could become a substitute in paper making (Nieschlag *et al.* 1960). The plant that proved the best alternative was kenaf for the following reasons:

- Rapid growth: kenaf plant height reaches 3.5 5.5m in 150 days, while southern pine
 (a species commonly grown on tree plantations) must grow 14 to 17 years before it
 can be harvested.
- High yield: kenaf yields more fibre per hectare than southern pine, producing 11-22 t dry material ha⁻¹, or approximately 3 to 5 times the amount of southern pine.
- Exceptional papermaking characteristics: kenaf fibres contain less lignin (an average kenaf plant contains only 9% lignin, while southern pine contains 29% lignin) and will therefore require far less chemicals, heat and time to pulp kenaf fibres because they are not as tough as woodpulp. Lignin is a resin that binds the cellulose fibres in plants together. Toxic chemicals such as chlorine are predominantly used to delignify and bleach wood pulp. Kenaf can be quickly and easily pulped and bleached with harmless chemicals, such as hydrogen peroxide.

There now exist many different chemical and mechanical processes to assist in the pulping process of kenaf when processed as a whole stalk. These include kraft, soda, neatral-sulfite, mechanical, chemimechanical, thermomechanical and also a combination of the last two called chemitermomechanical processes (Clark and Wolff 1962, Bagby 1989). These pulps have been developed into high quality bond, coated rawstock and newsprint papers (Clark *et al.* 1971, Bagby *et al.* 1979, Bagby 1989). According to Kugler (1988) the whole stalk pulp can also be used as a corrugated medium.

Other non-pulping products such as particleboard (Webber *et al.* 1999) and fibre injection molded and extruded plastics (Webber and Bledsoe 1993) have also become a reality and make use of the whole stalk. In the case of the pulping process, there is an intentional removal of the non-fibrous lignins and sugars, but in the case of non-pulped products, this is not required and therefore almost 100% of the stalk is utilized.

According to Kaldor *et al.* (1990), the bast pulp has greater tear strength and bulk fibre than softwood. Due to fewer chemicals and less energy required as compared to



conventional pulping of wood fibres, this factor proves kenaf processing to be more environmentally friendly than for softwood.

Some of the recent additions to the uses of kenaf bark fibre strands include the use in automobile dashboards, carpet padding, as a substitute for fibreglass and other synthetic fibres (Scott and Taylor 1988). It can also be used for making of textiles (Ramaswamy and Boyd 1994), fibre lawn mats impregnated with grass seeds, and spray-on soil mulches for use along roads to prevent run-off and wind erosion (Webber and Bledsoe 2002).

According to Karlgren *et al.* (1991), approximately 41% core fibre can be extracted chemically from the original stalk. These fibres vary in length of between 0.49 mm to 0.79 mm (Nieschlag *et al.* 1961, Adamson and Bagby 1975, Kaldor *et al.* 1990), and the pulp has lower tear strength than those of hardwood pulps, but greater tensile and burst strength (Kaldor *et al.* 1990). Research done on uses for the woody core fibres include the use of the core fibres as an absorbent (Goforth 1994), poultry litter and animal bedding (Tilmon *et al.* 1988), as a bulking agent for sewage sludge compost (Webber 1994) and as potting soil replacement (Laiche and Newman 1994, Webber *et al.* 1999). In addition to these, which are already available as products on the market, other core products that are available include products used for toxic waste cleanup, oil spills on water and the remediation of chemically contaminated soils (Webber and Bledsoe 2002).

2.4.3 Usefulness of the whole plant

Apart from its usefulness as fibre crop, kenaf researchers have also discovered a high protein content throughout the entire plant. The crude protein content in kenaf leaves varies between 14% and 34% (Killinger 1969, Suriyajantratong *et al.* 1973, Swingle *et al.* 1978, Webber 1993a). The crude protein levels in the stalks vary between 2% to 12% (Swingle *et al.* 1978, Webber 1993a) and the crude protein levels measured over the entire plant range from 6% to 23% (Killinger 1969, Swingle *et al.* 1978, Webber 1993a). The digestibility of kenaf (high percentage of digestible protein (Wing 1967)) ranges from 53% to 71% (Wing 1967, Suriyajantratong *et al.* 1973, Swingle *et al.* 1978) ensuring that it is also used very successfully as a livestock feed. In a study by Suriyajantratong *et al.* (1973) kenaf compared favourably with alfalfa meal as fodder supplement. This research was confirmed by a study done at Al Reno Agricultural research station in Oklahoma by a team of agricultural scientists from the USDA (Webber and Bledsoe 1993). Research into kenaf as feed source for Spanish goats also determined that chopped kenaf (29%



dry matter, 15.5% crude protein and 25% acid detergent fibre) was proven to be suitable replacement fodder instead of pasture (Wildeus *et al.* 1995).

2.4.4 Leaves

Research indicated that although the apical part of a young kenaf plant is suitable for fodder, the combined stalks and leaves as it would usually be harvested, were very difficult to digest and low in nutritive value (Xiccato et al. 1998). In one of the research projects on the possibility of using whole-plant kenaf as mulch in vegetable production, the suspicion arose that the kenaf plant may be a source of allelopathic chemicals since weed populations were significantly reduced (Russo et al. 1997). The researchers extracted chemicals from the leaf portions of the kenaf plants and analyzed the composition of the essential oils to determine its natural ability to inhibit plant or fungal growth. Fifty-eight components were identified and characterized and it was determined that the essential oil was phytotoxic to lettuce (Lactuca sativa) and bentgrass (Agrostis stolonifera) and it also inhibited fungal growth of Colletotrichum species (Kobaisy et al. 2001). The major components identified were (E)-phytol (28.16%), (Z)-phytol (8.02%), nnonanal (5.70%), benzene acetaldehyde (4.39%), (E)-2-hexenal (3.10%), and 5methylfurfural (3.00%). This confirmed earlier research by Osman et al. (1976) on the leaf volatiles. They isolated 10 components, including ethyl alcohol, isobutyl alcohol, limonene, phellandrene, R-terpenyl acetate, citral and four unidentified components (Kobaisy et al. 2001).

2.4.5 Seed

The leaves are not the only parts of the plant that contain significant levels of oil. The seed is also regarded an excellent source of oil. Research of nine kenaf genotypes ('Cubano', 'Everglades 41', 'Everglades 71', 'GR2563', 'Guatemala 48', 'Indian', 178-18RS-10, 'Tainung #1', and 'Tainung #2') determined the quality and quantity of oil, fatty acids, phospholipids and sterols (Mohamed *et al.* 1995). The oil content varied between 21.4% and 26.4%; the phospholipids between 3.9% and 10.3% of the oil and the total sterol percentage were similar to that of soybean and cottonseed oil. This similarity suggests that the seed oil may be used as an excellent source of edible oil for human consumption (Webber and Bledsoe 2002). This high oil content can affect the seed's viability if not stored at correct temperature levels. Research by Toole *et al.* (1960) reported that kenaf seed stored at 8% RH and either at three different temperature regimes, -10°, 0° or 10°C, remained fully viable for 5.5 years. Too warm or too cold storage conditions had a negative impact on seed germination.



2.5 The use of herbicides in kenaf cultivation

2.5.1 Introduction

The term herbicides, as used in this document, refer to compounds which combat or control undesired plant growth. This class of compounds may be divided into subclasses according to the primary type of mode of action the herbicide has on the plant. For example, according to Taylor and Warren of Purdue University, Indiana, USA, herbicides can be classified as auxin-transport inhibitors, growth regulator herbicides, photosynthesis inhibitors, pigment inhibitors, growth inhibitors, amino acid synthesis inhibitors, lipid biosynthesis inhibitors, cell wall biosynthesis inhibitors, rapid cell membrane disruptors as well as "miscellaneous" herbicides which do not come under one of the preceding categories (1970).

Although kenaf is capable of outgrowing most weeds because of its high growth rate, the crop is vulnerable during the early stages, before and after emergence of the seedlings. According to Burnside and Williams (1968), kenaf is regarded as a good competitor with weeds once the canopy is sufficiently grown to shade the ground.

When it comes to herbicides, a distinction is made between the different application timings. Herbicides that target either the seed or the submerged weed seedling whilst still in the soil are called pre-emergence herbicides. The main modus operandi for pre-emergence herbicides is to target the submerged parts of the plant which are the roots, the seeds and the submerged seedling. These herbicides are generally absorbed by the roots or seeds together with water, where they inhibit cell wall biosynthesis, or they disrupt cell membranes, they retard the growth of the seedlings or they inhibit lipid biosynthesis (Cobb 1992).

Herbicides that are applied after emergence of the weed seedlings are called postemergence, post-directed or over-the-top herbicides. The function of this type of herbicide is to enter the transport systems of the plant, usually through the foliage, to inhibit photosynthesis processes, to inhibit amino acid synthesis or to inhibit pigment formation of the cells, as well as various other harmful operations.

Substantial research has already been done in the United States on the effects of herbicides on kenaf. The choice of herbicides in the current pre-emergence study was mainly guided by the outcomes of these American studies, since either the same herbicides were available in South Africa or herbicides with different names but with the same chemical composition are issued locally. A vast amount of research has also been



conducted in the Far East, especially China, but unfortunately very little of this research has yet been translated into English.

2.5.2 Literature review

In cooler climates and with earlier planting dates, cultural and/or chemical weed control measures are more important. According to Kurtz (1994), moderate to large reductions in yield have been reported due to weed competition. In a study by the United States Department of Agriculture in 1970, yield was diminished by 85% from competition with johnsongrass (*Sorghum halepense*) and common cocklebur (*Xanthium strumarium*) (Kurtz 1994). Williams (1966) reported a 907 kg ha⁻¹ loss of yield from weed competition; whereas Hickman and Scott (1989) observed substantial competition from parthenium ragweed (*Parthenium hysterophorus*) and Fageiry (1978) reported almost 38% yield reduction because of weed competition. Burnside and Williams (1968) reported significantly reduced yields by an average of 69% as well as reduced plant height and stalk diameter in their three year study in Nebraska, USA.

Some of the earlier research initiatives that were conducted reported on many herbicides that are no longer available or that had caused excessive injury (Burnside and Williams 1968, Dean and Parker 1971, Fageiry 1978, Hickman and Scott 1989, Malone *et al.* 1990). There is, however, also substantial research evidence of studies that have been conducted with herbicides that are still available on the market. Already in 1964, Orsenigo conducted research on the reaction of kenaf to diuron during a pre-emergence experiment. He reported a 50% stand reduction during this trial and a 25% and 38% kenaf stand loss from Aatrex[®] (atrazine) at 3 kg ha⁻¹ from granular and sprayable applications, respectively (Orsenigo 1964).

A few years later Burnside and Williams (1968) tested seven herbicides and found that kenaf was most tolerant to trifluralin and this herbicide also provided excellent weed control. However, during the first year the trifluralin, at 2.2 kg ha⁻¹, resulted in significantly reduced kenaf yields by 25%, even though stalk heights and diameters were unaffected by the application. Orsenigo (1964) had also researched trifluralin and reported a 100% tolerance by kenaf to the herbicide at dosages of 2.2, 3.4 and 4.5 kg ha⁻¹.

Not long after this, in 1970, White and other researchers conducted trials and found two herbicides to have registration potential (White *et al.* 1970). They were trifluralin and metolachlor. This was confirmed by Hickman and Scott (1989), who found that trifluralin, at 0.9 and 1.7 kg ha⁻¹, and metolachlor at 3.4 kg ha⁻¹, provided excellent grass control and acceptable broadleaf weed control. Kurtz and Neill (1990) also found that



metolachlor, at 3.0 kg ha⁻¹, gave no visual injury to the kenaf, although stalk yields "may have been reduced". Webber (1993a) confirmed these findings.

At the 14th Brazilian congress on herbicides and herbaceous weeds in Campinas it was reported that 6 herbicides had caused no injury or yield loss to kenaf over two seasons, but that the weed control was not very effective. The six pre-emergence herbicides were: trifluralin (0.72 and 0.96 kg ha⁻¹), pendimethalin (0.75 and 1.25 kg ha⁻¹), napropamide (2 kg ha⁻¹), chlorthal-dimethyl (7.5 and 9.0 kg ha⁻¹), alachlor (1.92 and 2.88 kg ha⁻¹) and metolachlor (2.88 and 4.32 kg ha⁻¹) (Salgado and Deuber 1982).

Between 1990 and 1994, Kurtz and Neill conducted intensive herbicide studies to determine the sensitivity of kenaf towards a range of herbicides found in the United States. At times the results were almost contradictive because application timing appeared to play a decisive role. In 1990, Kurtz and Neill observed 60% injury with a post-emergence application of diuron 28 days after emergence, cyanazine caused little or no injury and fluometuron caused 18% kenaf injury (Kurtz and Neill 1990). Post-emergence applications of diuron (0.56 kg ha⁻¹) and cyanazine (0.9 kg ha⁻¹) caused <10% injury 14 days after treatment with no yield loss in 1994 (Kurtz 1994). In another experiment in 1992, Kurtz and Neill applied over-the-top applications of lactofen on cotyledonary and 36 cm tall kenaf, which caused >85% injury at both growth stages (Kurtz and Neill 1992), but when in 1994 Kurtz applied this herbicide post-emergence at 0.2 kg ha⁻¹, it resulted in <10% injury 14 days after treatment with no report of yield loss (1994).

Kurtz (1994) prepared solutions of MSMA (2.25 kg ha⁻¹) mixed with one rate of cyanazine (0.9 kg ha⁻¹), diuron (0.56 kg ha⁻¹) or lactofen (0.2 kg ha⁻¹). Initially different rates of injury occurred to the lower third of the kenaf stems, but all of these injury symptoms were <10% by 14 days after emergence and there was no loss of yield. An experiment conducted with acifluorfen (0.43 kg ha⁻¹) with over-the-top applications on cotyledonary and 35.5 cm tall kenaf plants caused 95% injury. During the same experiment lactofen (0.17 kg ha⁻¹) and formasafen (0.28 kg ha⁻¹) caused 88% injury to 36 cm tall kenaf plants.

In further tests Kurtz (1994) found that the following post-emergence herbicides posed no threat to the health and yield of kenaf: Bladex® (cyanazine), Direx® (diuron), Meturon® (fluometuron), Cobra® (lactofen), and Cotton Pro® (prometryn). These herbicides also control a broad spectrum of weeds.



Baldwin (2000) reported the effects of pyrithiobac sodium on kenaf. The rate of application was the same as that for cotton (0.084 kg ha⁻¹) and half that, and although kenaf showed some injury after 2 weeks (stunting and yellowing), the plants outgrew this and no loss of yield was reported. The recommendations of the study were to approve pyrithiobac as an over-the-top post-emergence herbicide for kenaf, especially since the weed control was excellent.

A summary of other research projects in the USA include the following:

- Lasso[®] (alachlor, 1 kg ha⁻¹) was reported to have no negative effects on kenaf (Dean and Parker 1971) even at higher dosages (2.24 or 4.48 kg ha⁻¹) (Hickman and Scott 1989).
- Hickman and Scott (1989) reported no yield loss or injury with Sonalan® (ethalfluralin) applied as a pre-emergence herbicide at rates of 1.1 and 1.7 kg ha⁻¹.
- Kurtz and Neill (1992) reported substantial injury to kenaf after post-emergence treatments of Scepter[®] (imazaquin, 0.07 kg ha⁻¹) and Pursuit[®] (imazethapyr, 0.03 kg ha⁻¹).
- Webber (1992) and Hickman and Scott (1989) both found no reduction in kenaf yield when treated with Dual[®] (metolachlor), although Webber did observe significant stand reductions when compared to the weed-free control.
- Fageiry (1978) found Prowl® (pendimethalin, 1.12 kg ha⁻¹) to be safe and an effective herbicide to use with kenaf.
- Kurtz and Neill (1990) reported in a preliminary project that the following herbicides caused too much injury to be used safely on kenaf: Canopy[®] (0.67 kg ha⁻¹), Direx[®] (1.34 kg ha⁻¹), Scepter[®] (1.29 kg ha⁻¹), Sencor[®] (0.5 kg ha⁻¹) and Zorial[®] (1.8 kg ha⁻¹).

The following table summarizes the outcomes of all the herbicide research conducted over 40 years in the USA. When research indicated an injury percentage below 20, the herbicide is regarded as safe in this summary.



Table 2-1 Recommendation of herbicides that were used in earlier research, in the USA, on kenaf (compiled according to company label recommendations in USA)

Registered Trademark	Active ingredient	Proposed Dosage (kg ha ⁻¹)	Recommendation
Aatrex [®]	Atrazine	2.68	Not safe
Bladex®	Cyanazine	0.89	Safe
Blazer [®]	Acifluorfen	0.42	Not safe
Bueno-6®	monosodium methanearsonate	1.12	Safe
Canopy®	Metribuzin	0.67	Not safe
Cobra [®]	Lactofen	0.22	Not safe
Command®	Clomazone	1.68	Safe but risky
Cotton Pro®	Prometryn	0.56	Safe
Direx [®]	Diuron	0.66	Contradictive findings
Dual [®]	metolachlor	3.13	Safe
Lasso®	Alachlor	2.24	Safe
Meturon [®]	fluometuron	1.12	Safe
Prowl®	pendimethalin	1.12	Safe
Pursuit [®]	imazethapyr	0.03	Not safe
Reflex [®]	Fomesafen	0.42	Not safe
Scepter®	Imazaquin	0.06	Not safe
Sencor®	Metribuzin	0.50	Not safe
Sonalan®	Ethalfluralin	1.45	Safe
Staple [®]	pyrithiobac-sodium	2.01	Safe, very effective
Zorial [®]	Norflurazon	1.79	Not safe



3.

GENERAL MATERIALS AND METHODS

3.1 Introduction

The study described in this dissertation formed the first phase of a broader project to determine the sensitivity of kenaf (*Hibiscus cannabinus*) towards herbicides currently registered in South Africa. These herbicides were selected according to the guidelines described in Chapter 2. During this phase of the project, the effect that the herbicides would potentially have was researched under controlled conditions at the Hatfield Experimental Farm of the University of Pretoria. A second research phase, which focuses on the response of the crop toward those herbicides under field conditions was subsequently carried out but is not reported here.

Since the experiences of the farmers in the Winterton area were also considered when the project was conceptualized, it was decided to take the factors that they identified *in situ* into account and to incorporate some of these into the project. This resulted in testing not only the sensitivity of kenaf to the herbicides examined, but also the way the herbicides interacted with some of the environmental factors which might play a role in this particular production area. These factors could, however, also occur in other areas of South Africa and could therefore be useful not only to farmers of the Winterton area, but also to other production areas in the country or region.

Not only pre- and post-emergence herbicides were researched, but also the combined effect of herbicide application and temperature, light, planting depth and application timing of pre-emergence herbicides. In addition, data regarding the effectiveness of the herbicides in controlling weeds were also recorded and analyzed as a peripheral aspect of the study.

3.2 Experimental design

The project was divided into four separate experiments. The same pre-emergence herbicides were used throughout the four experiments, apart from one that was eliminated after the first herbicide trial. Only in the first experiment, which determined the sensitivity of kenaf to the chosen herbicides, was the reaction to post-emergence herbicides also researched. The four experiments will each be explained in the following chapters, but they can be summarized as follows:



- Experiment/trial 1: Selectivity of five pre-emergence herbicides, one combination of pre-emergence herbicides and four post-emergence herbicides towards kenaf.
- Experiment/trial 2: The role of planting depth on the effect of four pre-emergence herbicides in kenaf.
- Experiment/trial 3: The role of temperature on the effect of four pre-emergence herbicides in kenaf.
- Experiment/trial 4: The effect of different herbicide application timings on the sensitivity of kenaf. Only pre-emergence herbicides were researched.

The broad experimental approaches for the different experiments were similar, but the different factors in each experiment will be dealt with in the particular chapters. Experimental design for each was based on a completely randomized block arrangement of treatments. In the majority of the experiments, four herbicides at three levels of application were replicated four times each. This will also be developed further in the specific chapters. Figure 3.1 shows the random block design of one of the experiments in a glasshouse on the Hatfield Experimental Farm.



Figure 3-1 Experimental layout of one of the kenaf pot experiments (Photo: AS Malan 2008)

The different coloured tags (Figure 3.1) represented the different herbicides/combinations, and pots were also replaced randomly in the respective blocks after the weekly weighing session for irrigation scheduling.

3.3 Materials

3.3.1 Soil

A Hutton sandy loam soil was used to conduct all the experiments. The soil was taken from the Hatfield Experimental farm of the University of Pretoria. The diagnostic horizons which characterize a Hutton soil are an orthic A-horizon, which is underlied by a red



apedal B-horizon according to the Soil Classification System developed by The Soil Classification Workgroup under the leadership of MacVicar (1991). Soil samples were taken for analysis prior to the execution of the experiment. The soil was analyzed for Ca, Mg, K, Na [Extractable Cations: Ammonium acetate (1 mol dm⁻³, pH 7)], extractable phosphorus (Bray-1), and pH of the soil when dissolved in water. The clay percentage was 22%. Kenaf is adaptable to a variety of soils, best being a deep, friable, well-drained, sandy loam with humus; light sandy soils are not recommended and the clay percentage should be in the region of 20%. A pH of neutral to slightly acid is suggested (Duke and Ducellier 1993). Table 3.1 shows the results of the soil analysis conducted by the Soil Analysis Laboratory of the University of Pretoria. The phosphorus, but especially the potassium is on the low side for kenaf production. This as well as the N requirements was, however, addressed with the application of Supafeed® (Table 3.2). The sodium content of the soil was low enough and no problems with salinity were to be expected.

Table 3-1 Properties of the soil used in all the herbicide pot experiments

P-Bray I	Ammonium Acetate Extractable			Water pH	Clay %	
P mg kg ⁻¹	K mg kg ⁻¹	Ca mg kg ⁻¹	Mg mg kg ⁻¹	Na mg kg ⁻¹		
18	41	341	130	15	5.7	22

Each pot in all the experiments contained 3 kg soil. Since one experiment had as treatment factor the effect of planting depth on the sensitivity of kenaf to herbicides, seeds were planted at two different soil depths (2.5 cm and 5 cm) in that experiment. However, the total amount of soil per pot remained 3 kg. Since the trials were focusing on the effectivity of herbicides on weeds, the soil contained random amounts of both grass and broadleaved weed seeds, and these germinated simultaneously with the kenaf seedlings.

3.3.2 Herbicides used in this study

Several factors had to be taken into consideration before it was decided which herbicides would be used during the trials. They were:

Availability of the herbicide in South Africa



- Herbicides that had been used by the farmers within the Winterton area previously, either with or without success. Recommendations were made by Mr. Dale van den Aardweg³.
- Herbicides that had already been approved for use on cotton (Gossypium hirsutum)
 might be suitable since cotton is the closest crop to kenaf as far as botanical
 composition is concerned.
- Herbicides that had already proved safe on kenaf in other parts of the world, or had
 already been registered for use on kenaf would be considered. These herbicides also
 had to be available locally. An exception was made in the case of the postemergence pyrithiobac sodium, which had to be imported from the USA, since this
 herbicide proved very successful with elimination of morning glory (*Ipomoea hederacea*), a particularly noxious problem in the Winterton area.
- Herbicides that were also declared safe on the crops that might possibly be farmed in rotation with kenaf, such as soy beans or wheat. The advice of experts from the agrochemical industry was also taken into consideration.

A distinction was made between herbicides that were appropriate to use before the kenaf seedlings emerge (pre-emergence herbicides), and herbicides that were appropriate for use after the seedlings had already emerged from the soil, but while they were too small to form a proper canopy (post-emergence herbicides). The following tables (Table 3.2 and 3.3) give a summary of the active ingredient as well as the prescribed dosage (at x1 strength) for use in South African conditions. A "cocktail" included in the pre-emergence trials was a combination of imazethapyr and S-metolachlor.

Table 3-2 Pre-emergence herbicides used in kenaf Trials 1 – 4

Active ingredient	Proposed Dosage (rate per hectare)	Availability in South Africa
S-dimethenamid	750 ml	Freely available
Imazethapyr	200 ml	Freely available
fluometuron/prometryn	2 /	Freely available
Pendimethalin	21	Freely available
S-metolachlor	500 ml	Freely available
Imazethapyr + S- metolachlor	200 ml + 500 ml	Freely available

³ Personal comments by Mr. Dale van den Aardweg and management team of SFS (Website: http://www.kenaf.co.za; email address: robink@kenaf.co.za)

38



Table 3-3 Post-emergence herbicides used in kenaf Trial 1

Active ingredient	Proposed Dosage (rate per hectare)	Availability in South Africa
Bentazone	21	Freely available
2,4D-B (Butyrac), dimethylamine salt	80 ml	Freely available
monosodium methanearsonate	3 /	Freely available
pyrithiobac-sodium	60 g	Not available

3.3.3 The pre-emergence herbicides

S-Dimethenamid

Chemical family: Chloroacetamide

Chemical information: Active ingredient: 63,9% S-dimethenamid.

<u>Chemical designation</u>: 2-chloro-N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl)-acetamide. Dimethenamid consist of 4 sterioisomers due to two chiral elements and can then also exist in the form of the individual isomers as diasteromeric mixtures (1S, aRS (known as S-dimethenamid) and 1R, aRS (known as R-dimethenamid) and as a racemic mixture (1RS, aRS) (Figure 3.2). References herein to dimethenamid refer to its various forms unless otherwise stated. Of the diasteromeric mixtures S-dimethenamid is preferred (US Patents n.d.)

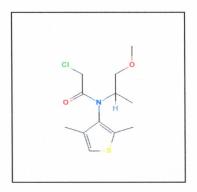


Figure 3-2 Molecular structure of dimethenamid - formula: C₁₂H₁₈CINO₂S

Description: dark, brown viscous liquid, weak 'tar-like' odour.

⁴ Chemical data from http://www.pubchem.ncbi.nlm.nih.gov



<u>Biochemistry:</u> it is not yet that clearly understood, but the main belief is that the herbicide induces an inhibition of very long chain fatty acid synthesis.

<u>Mode of action:</u> S-dimethenamid is absorbed via the coleoptile the moment the young seedling breaks through the soil. It is also taken up through the roots of annual one-seed or two seed lobed weeds.

<u>Uses</u>: this is a soil herbicide mainly targeting grass species. It should be applied as a pre-emergence herbicide.

Imazethapyr

Chemical family: Imidazolinone

<u>Chemical information:</u> Active ingredient: imazethapyr. This herbicide contains 9,35% imazethapyr and 9,35% of the salt ethyleneglycol.

<u>Chemical designation:</u> IUPAC name: (*RS*)-5-ethyl-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)nicotinic acid. Chemical abstracts name: (?-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid. (Figure 3.3)

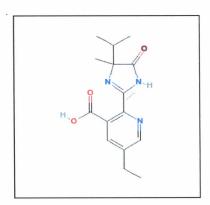


Figure 3-3 Molecular structure of imazethapyr - formula: C₉H₁₀CIN₅O₂ ⁵

Description: green to dark brown liquid, slightly pungent odour.

<u>Biochemistry</u>: Imazethapyr is a branched chain amino acid inhibitor. It therefore reduces the plant's levels of valine, leucine and isoleucine, which leads to a disruption of protein and DNA synthesis. Selectivity in soy beans and peanuts is attributed to rapid detoxification via hydroxylation and glycosylation (Tecle *et al.* 1993).

⁵ Chemical data from http://www.pubchem.ncbi.nlm.nih.gov



<u>Mode of action</u>: It is a systemic herbicide, absorbed by the roots and foliage, with translocation in the xylem and phloem, and accumulation in the meristematic regions.

<u>Uses</u>: Control of many annual and perennial grass and broadleaved weeds found amongst soy beans and other leguminous crops. It can be applied pre-plant incorporated, pre-emergence or post-emergence. It is non-phytotoxic to soy beans and other leguminous crops (taken from www.chinese-esticide.com/herbicides/imazethapyr).

Fluometuron / prometryn

Chemical family: Phenylurea, substituted urea, or urea.

<u>Chemical information:</u> Active ingredient: fluometuron (urea-derivative) / prometryn (triazine).

Chemical designation:

Fluometuron: 1,1-dimethyl-3-[3-(trifluoromethyl)phenyl]urea (Figure 3.4) **Prometryn**: 6-methylsulfanyl-N,N'-dipropan-2-yl-1,3,5-triazine-2,4-diamine (Figure 3.5).

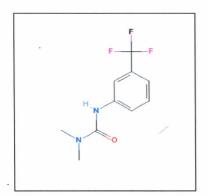


Figure 3-4 Molecular structure of fluometuron - formula: C₁₀H₁₁F₃N₂O

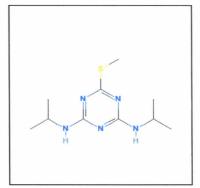


Figure 3-5 Molecular structure of prometryn - formula: C₁₀H₁₁F₃N₂O

<u>Description:</u> white to beige coloured viscous liquid, odourless.

<u>Biochemistry</u>: fluometuron undergoes successive *N*-demethylation as the primary detoxification process in plants. Competitive or subsequent hydroxylation may occur allowing the formation of sugar conjugates. Rapid metabolism is an important means of selectivity in tolerant plant species (Vencill *et al.* 2002).

<u>Mode of action</u>: This herbicide is part of the Group C herbicides and is an inhibitor of photosynthesis at the photosystem II part of mostly cotolydenous plant species. Death

⁶ Chemical data from http://www.pubchem.ncbi.nlm.nih.gov



occurs from oxydation of proteins and lipids. (Langham *et al.* 2007). It is readily absorbed by roots after soil application and translocated via the apoplast (including xylem) to the shoots. It is not as well absorbed when applied to the foliage and is not properly translocated from the treated leaf via the phloem.

<u>Uses</u>: This is mainly a selective pre-emergence herbicide to control broadleaved weeds. It may also be used as a post-emergence directed application. According to some safety data sheets it even controls annual grasses and Langham *et al.* (2007) states that it controls the following broadleaved and grass species: barnyard grass, crabgrass, fall panicum, foxtail, goose grass, broadleaved signal grass, cocklebur, Florida pusley, morning-glory, lambsquarters, prickly sida, common ragweed, sesbania, sicklepod, smartweed, and spurge.

Pendimethalin

Chemical family: Dinitroaniline

Chemical information: Active ingredient: pendimethalin.

Chemical designation: 3,4-dimethyl-2,6-dinitro-N-pentan-3-yl-aniline (Figure 3.6)

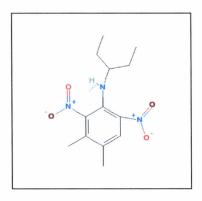


Figure 3-6 Molecular structure of pendimethalin - formula: C₁₃H₁₉N₃O₄

Description: crystalline orange-yellow solid with faint nutty odour.

<u>Symptoms</u>: Susceptible seedlings (grasses as well as broadleaves) fail to emerge. The coleoptile growth in grasses is inhibited. Emerged grass shoots are deformed. Stems of broadleaves can become brittle at the soil line and hypocotyls may swell. The most easily recognized system is root growth inhibition, especially in lateral (secondary) roots. Root tips become thickened and stubby.



<u>Mode of Action:</u> when applied as a pre-emergence solution it is absorbed by the roots and coleoptiles where most of the damage is done. Mitosis of the cells at the ends of the roots is affected.

<u>Uses</u>: pendimethalin has been used both as a pre-emergence as well as a post-emergence herbicide. It controls primarily the grass species like Panicum, foxtail, johnsongrass, signal grass, goose grass and crabgrass. Some broadleaved species such as velvetleaf, lambsquarters and redoot pigweed can also be controlled by this herbicide. It can be applied through liquid fertilizer; it can be impregnated on dry bulk fertilizer and through chemigation systems (Anonymous Purdue University).

S-metolachlor

Chemical family: Chloroacetamide, chloroacetanilide or acetanilide

Chemical information: Active ingredient: S-metolachlor

<u>Chemical designation:</u> 2-chloro-N-(2-ethyl-6-methyl-phenyl)-N-(1-methoxypropan-2-yl) acetamide (Figure 3.7).

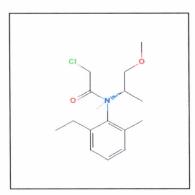


Figure 3-7 Molecular structure of metolachlor - formula: C₁₅H₂₂CINO₂

<u>General information</u>: There are two isomers of metolachlor: S-metolachlor and R-metolachlor. Both are effective against weeds, but S-metolachlor is more effective at a lower rate (35% less active ingredient). (Langham *et al.* 2007)

<u>Description</u>: white to tan liquid, odourless.

<u>Symptoms</u>: Most susceptible weeds fail to emerge from the soil. Injury to grasses appears as malformed and twisted seedlings. Leaves are tightly rolled in the whorl and may not unroll properly. Injured broadleaved weeds have cupped or crinkled leaves with a drawstring or heart shaped appearance.



Mode of action: S-metolachlor inhibits the biosynthesis of several plant components such as fatty acids, lipids, proteins, isoprenoids, and flavonoids. It is absorbed by emerging shoots (grass coleoptile, broadleaved hypocotyl or epicotyl). Some root absorption occurs also. Plants beyond the seedling stage can absorb it through the roots and translocate it to the shoots through the xylem and phloem and it can accumulate in the vegetative parts, but to a lesser extent in the reproductive parts. However, it is phytotoxic only to emerging weed seedlings.

<u>Biochemistry:</u> it is a chloroacetanilide herbicide that inhibits the biosynthesis of several plant components like fatty acids, lipids, proteins, isoprenoids and flavonoids. The conjugation of acetyl coenzyme A seems to be involved (Vencill *et al.* 2002)

<u>Uses</u>: Controls many annual grasses like foxtail, barnyard grass, crabgrass, fall panicum, signal grass, witchgrass and red rice, but not Texas panicum, shattercane or johnsongrass. It also controls a few broadleaved species such as redroot pigweed, Florida pusley and carpetweed. The herbicide can be applied through pivot irrigation systems or in liquid or dry bulk fertilizer.

Composite herbicide

A combination of two herbicides was also included in all pre-emergence herbicide trials: imazethapyr and S-metolachlor. The combination of these herbicides was at the request of the kenaf production plant. The farmers of the Winterton area and the managers of the production plant were curious about the weed control and the effect on kenaf should these two herbicides be combined. The characteristics of both have already been described under the respective headings.

For details about the dilution rates for these herbicides, turn to the Appendix B Table 3.

3.3.4 Post-emergence herbicides

Bentazone

Chemical family: Benzothiadiazole

Chemical information: Active ingredient: bentazone.

<u>Chemical designation:</u> 2,2-dioxo-3-propan-2-yl-1H-benzo[d][1,2,6]thiadiazin-4-one or another option: sodium salt of 3-(1-isopropyl)-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide (Figure 3.8).



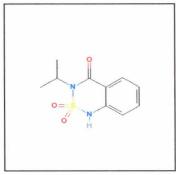


Figure 3-8 Molecular structure of bentazone - formula: C₁₀H₁₂N₂O₃S ⁷

Description: white, crystalline solid, odourless.

<u>Symptoms:</u> chlorosis begins 3-5 days after application. This is followed by desiccation and necrosis of the leaves. Sometimes bronzing may occur in tolerant crops.

Biochemistry: bentazone is a salt of organic acids.

Mode of Action: bentazone is classed as a PSII inhibitor because it inhibits the photosynthetic photosystem II. PSII inhibitors act by preventing the transfer of electrons during photosynthesis. The inhibition blocks photosynthesis, the fixation of CO₂ and the production of ATP or NADPH. Plant death is caused by the production of free radical species which are able to initiate lipid peroxidation, and eventually cell death (Silverman et al. 2004). Basically the metabolism of the plants is severely affected (LeBaron and Gressel 1982, Corbett 1994).

<u>Uses</u>: bentazone is a contact herbicide that only affects the portion of green tissue that is exposed to the herbicide spray (Unruh *et al.* 2004). It is therefore used as a broadleaved chemical herbicide.

2,4-DB

Chemical family: Phenoxy or phenoxyacetic acid

Chemical information: Active ingredient: 2,4-DB. Chemical name: dimethylamine salt.

<u>Chemical designation</u>: 4-(2,4-dichlorophenoxy)butanoic acid (Figure 3.9)

⁷ Chemical data from http://www.pubchem.ncbi.nlm.nih.gov



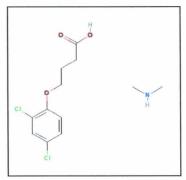


Figure 3-9 Molecular structure of 2,4 DB - formula: C₁₂H₁₄Cl₂O₃ ⁸

<u>Description:</u> white, crystalline solid, odourless when pure.

<u>Symptoms:</u> epinastic bending and twisting of stems and petioles stem swelling (particularly at the nodes) and elongation of stems, as well as leaf cupping and curling. This is followed by chlorosis at the growing points, wilting and necrosis (Vencill *et al.* 2002)

<u>Biochemistry:</u> it is not yet fully understood but it appears that these compounds affect cell wall plasticity nucleic acid metabolism. 2,4-DB must first be converted to 2,4-D which acidifies the cell wall by stimulating a membrane-bound ATPase proton pump. The reduction in apoplasmic pH induces cell elongation by increasing the activity of enzymes responsible for cell wall loosening (Vencill *et al.* 2002).

Mode of Action: 2,4-DB is a systemic herbicide that is translocated in the plant's vascular system. This is the vehicle of transport also for the plant's nutrients and water. Systemic herbicides act slower, generally over a period of days (Unruh *et al.* 2004).

<u>Uses</u>: 2,4-DB belongs to the phenoxy family and is mainly used to control broadleaved weeds. It is a selective, systemic foliar-applied herbicide (Unruh *et al.* 2004).

Monosodium methanearsonate

Chemical family: organic arsenical

<u>Chemical information</u>: Active ingredient: monosodium methanearsonate. Common name: sodium methyl-oxido-arsinic acid.

<u>Chemical designation</u>: sodium hydroxy-methylarsinate (Figure 3.10)

⁸ Chemical data from http://www.pubchem.ncbi.nlm.nih.gov





Figure 3-10 Molecular structure of monosodium methanearsonate - formula: CH₄AsNaO₃ ⁹

Description: clear light green to slightly brown liquid.

Symptoms: chlorosis and necrosis of the leaves.

<u>Biochemistry:</u> the herbicide is readily absorbed by the foliage and translocated in the symplast, as well as the apoplast. There is little translocation to the shoots following root absorption from nutrient solutions (Vencill *et al.* 2002)

<u>Mode of Action:</u> not well understood. The rapid desiccation indicates cell membrane destruction.

<u>Uses</u>: the herbicide is mainly applied post-emergence in cotton and non-crop areas. Weeds controlled are generally only grasses.

Pyrithiobac sodium

Chemical information: Active ingredient: pyrithiobac sodium.

Common name: sodium 2-chloro-6-(4,6-dimethoxypyrimidin-2-yl)sulfanyl-benzoate.

<u>Chemical designation</u>: Sodium 2-chloro-6-[(dimethoxypyrimidin-2-yl)thio]benzoate (Figure 3.11)

⁹ Chemical data from http://www.pubchem.ncbi.nlm.nih.gov



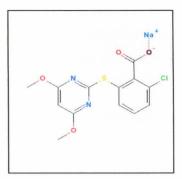


Figure 3-11 Molecular structure of pyrithiobac sodium - formula: C₁₃H₁₀ClN₂NaO₄S ¹⁰

<u>Description:</u> Pyrithiobac sodium is a water soluble powder formulation to be mixed with water.

<u>Symptoms:</u> Target species will develop leaf yellowing, bronzing, leaf crinkling and/or stunting when applied as a post-emergence application. These symptoms may persist from several weeks to mid-season (DuPont label as used on kenaf in USA).

<u>Mode of Action:</u> Pyrithiobac sodium is absorbed by weed roots following a preemergence application. Growth is rapidly inhibited and death of leaf tissue and growing plants will follow in some plants, while others will be stunted and non-competitive.

<u>Uses</u>: This herbicide has been approved for use on kenaf in the state of Tennessee and North Carolina in the USA, for control of a variety of broadleaved weeds such as pigweeds, common cocklebur, pitted morning glory, entire-leaf morning glory, prickly sida, velvetleaf and spurred anoda.

For details about the dilution rates for these herbicides, turn to Appendix B Table 4.

Adjuvants

An adjuvant is usually a liquid that, when added to the dissolved herbicide, it will enhance the performance or handling characteristics of the specific herbicide.

Surfactants, crop oils and crop oil concentrates, anti-foaming agents, drift control agents and compatibility agents are all regarded as adjuvants.

Great care should be exercised in the addition of surfactants, crop oils and crop oil concentrates to herbicides, as indiscriminate use will certainly injure the plants severely, or it might affect the herbicide performance negatively.

¹⁰ Chemical data from http://www.pubchem.ncbi.nlm.nih.gov



Adjuvants are only added to post-emergence herbicides, (and this not always) since they do not improve the performance of pre-emergence herbicides. Sometimes herbicide formulations have pre-mixed surfactants, eliminating the need of additional surfactants to be added (Unruh *et al.* 2004).

For this study, the adjuvant Allgral 94[®] was added to all the post-emergence herbicides. It has the following characteristics:

Chemical information: Active ingredient: an alkylated phenol ethylene oxide condensate.

<u>Mode of action:</u> Allgral is a concentrated wetting and sticking adjuvant for use with most spray materials. It improves the wetting and spreading properties of the herbicide in question on the plant's foliage and it increases fungicidal or insecticidal efficiency.

The prescribed rate of application for control of weeds in Allgral 94[®] is 50 ml/100 / of water, and this dosage was followed throughout the post-emergence experiments.

3.3.5 Fertilizer

A water soluble fertilizer called Supafeed® was applied to every pot on the day of planting and thereafter once a week, starting one week after planting. Supafeed® - Water Soluble Fertilizer is a highly concentrated, completely water soluble fertilizer/foliar feed which is readily taken up by the plant and which is ideally suited for nursery crops to apply through drip or sprinkler irrigation systems. It is also recommended for cotton, which is a close relative of kenaf. The nutrients present in Supafeed® are given in Table 3.4.



Table 3-4 The nutrients in Supafeed®

ACTIVE INGREDIENTS: SUPAFEED®	g kg ⁻¹
Nitrogen (N)	150
Phosphorus (P)	45
Potassium (K)	263
Sulphur (S)	1
Magnesium (Mg)	0.778
Zinc* (Zn)	0.350
Boron (B)	1
Molybdenum (Mo)	0.07
Iron* (Fe)	0.75
Manganese* (Mn)	0.3
Copper* (Cu)	0.075

^{*}EDTA Chelated

Supafeed® - Water Soluble Fertilizer has a mildly acidifying effect on spray water thus making it ideal for use with most insecticides and fungicides. In all the experiments, the method of watering through the fingers was applied when applying the dissolved fertilizer. This method distributes water more gently and evenly over the surface of the soil. The general recommended rate of 2 g fertilizer / liter water was followed throughout the entire herbicide screening process. The same volume (100 ml) of this nutrient solution was applied to all pots per application.

3.3.6 Other materials

- Container: brown plastic pots (volume: 4 ℓ), lined with plastic bags (300 mm x 450 mm) to prevent leaching and contamination of the pots with herbicides.
- Rettweiler balance to weigh pots weekly as well as finer balances to weigh fresh and dried plant material after harvesting.
- Measuring jugs to measure exact amounts of water daily or weekly.
- Drying ovens used for drying plant material at a constant temperature of 65°C over 72 hours.
- A high-pressure Oxford small-plot spray was used to apply each herbicide treatment at a constant pressure of 2 bar. A total spray volume of 50 ml was applied per m² surface area.
- Glasshouse without temperature control (Trial 1 and 2)



- Growth cabinet with controlled light conditions (12 hours light/12 hours dark) and controlled temperatures (Trial 3)
- Glasshouse with controlled temperatures (Trial 4)

3.4 Methods

3.4.1 Preparation of herbicide solutions

The same process was followed for both pre-emergence and post-emergence herbicides. Based on the prescribed rate per hectare the solutions were diluted according to the information in Table 3 and 4 in Appendix B. As indicated in the tables, the final diluted solution was combined with 40 ml of water to give 50 ml of total spray solution in the Oxford small-plot sprayer which was attached to a compressor.

3.4.2 Planting

It was determined that either 0.85 kg of soil had to be removed for a planting depth of 2.5 cm or 1.25 kg soil for a planting depth of 5 cm would be required from a total of 3 kg of soil per pot (Figure 3.12). The correct amount of soil was weighed off before planting, followed by seed placement on the surface of the remaining soil in a pot, depending on the particular soil depth required.

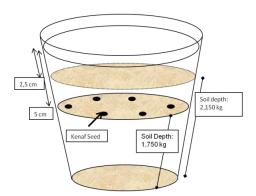


Figure 3-12 Illustration of how soil depth is determined according to weight of soil

Six seeds were placed more or less evenly spaced on the soil (Figure 3.12). For all trials except Trial 2 the planting depth of seeds was 5 cm. Seeds were gently covered with either 1.25 kg (5 cm depth) or 0.85 kg (2.5 cm depth) of soil to bring the weight of the soil to 3 kg per pot. After covering the seeds, 350 ml of water was added to each pot to represent field capacity (11.67 %) for the soil with a clay content of 22%.



3.4.3 Herbicide application

Pre-emergence herbicide solutions were prepared according to prescribed rates (see preparation of herbicides). A frame measuring 1 m x 1 m was placed on the floor of the application shed. The pots to be treated were placed randomly inside the frame. The Oxford sprayer was used to apply each herbicide treatment at a constant pressure of 2 bar (Figure 3.13). A total spray volume of 50 m² was used for each 1 m² application to ensure that the entire surface was moistened, which would allow for even penetration of the herbicide solution. Four herbicide application rates were used: 0, 1x recommended rate, 2x the recommended rate and 3x the recommended rate. The 1x recommended rate represented the prescribed or recommended herbicide rate according to the product label information.



Figure 3-13 Herbicide application requires expert technical ability! (Photo: AS Malan 2008)

After spraying, the outsides of the pots were rinsed with running water to prevent any contamination from the overhanging plastic lining.

A further 100 ml of nutrient solution was added to each pot to represent a possible additional rainfall on the day of planting and herbicide application. Each pot was weighed and the data recorded as initial weight at planting. Each pot was then covered with a plastic bag to prevent moisture loss until the first seedlings emerged.

After planting, the pots were put in their respective experimental blocks inside the glass houses/ growth chambers. Each day after planting the containers were monitored and emerged seedlings recorded.

Watering of pots after seedling emergence was done on the basis of replenishing water lost through evapotranspiration – this was done by weighing pots to determine the amount of water lost and replacing it on the basis of 1 g = 1 ml in order to attempt to



maintain soil moisture content as close as possible to the field capacity level that was established at the start of the experiment. It has already been mentioned that in addition all pots received 100 ml of the nutrient solution once a week.

The application technique for the post-emergence herbicides was exactly the same, the only difference being that the amount of pots was reduced to 64 [4 herbicides x 4 dosages (0x recommended rate (control) 1x the recommended rate, 2x the recommended rate and 3x the recommended rate) x4 replications], and instead of applying herbicides at planting, these were applied 16 days after planting, when the kenaf and weed seedlings had emerged fully and were growing vigorously.

3.4.4 Daily chores and monitoring

A representative, random sample of pots was weighed daily to determine average moisture loss. According to these weights, an average amount of water loss was determined and added to each pot when necessary.

A data logger with 16 sensory probes monitored soil temperature on a half-hourly basis in the glasshouse for the duration of Trial 1. Temperatures inside the glasshouse were monitored daily with a regular air thermometer.

3.4.5 Weekly chores and monitoring

The following measurements were taken on a weekly basis:

- All pots were weighed weekly and replenished with water to field capacity according to the initial weights. Adjustments were made when the plant mass became a significant factor.
- The average height of plants per pot was measured and recorded.
- 100 ml solution of fertilizer (Supafeed[®]) was added once a week.

3.4.6 Other measurements

Twenty days after application of herbicide the injury caused was assessed for each pot on a scale of 0 to 5 where 0 indicates no herbicide damage and 5 indicates mortality due to herbicide effect. Photographs (Figure 3.14 – 3.18) show the different degrees of damage observed.





Figure 3-14 No injury to any of the seedlings (Injury factor=0)



Figure 3-15 Slight damage visible in chlorosis and deformity of leaves due to herbicide sensitivity (Injury factor=1)



Figure 3-16 Medium damage (further visible signs of chlorosis/leaf deformity) due to herbicide sensitivity (Injury factor=3)



Figure 3-17 Severe damage, plants show signs of senescence (Injury factor=4)



Figure 3-18 All plants have effectively died (Injury factor = 5) (Photos: AS Malan 2008)

Since the effectiveness of herbicides in terms of weed control is an important focus in any herbicide experiment, the pre-emergence and post-emergence herbicide trials included monitoring of weed emergence. As the soil was taken from the Experimental Farm where several weed species occur naturally, it was safe to assume that a particular seedbank of weeds would be represented. This was confirmed by the emergence of different weed species in the control pots of both these trials, as well as consecutive trials.

On day 20 after planting the amount of weed control was recorded by identifying monocotyledons/dicotyledons and counting all weeds in each pot and placing them in two categories namely: grass and broadleaved species. Table 3.5 presents the



broadleaved weed species identified during the trials. Grass species were not individually recorded under species name, merely as grass weeds, but the majority of these were identified as *Urochloa mocambicensis* or bushveld herringbone grass. The weed control factor of each herbicide was then calculated as a percentage of the average weed count for the control. After these two data recordings all weeds were removed and kenaf plants were thinned out to three plants per pot. Weed data are neither presented nor discussed since weed control efficacy is not an objective of this study. According to Act 36 of 1947, manufacturers of the products tested during these trials have to do extensive research in this regard for registration purposes, and therefore weed efficacy was not investigated comprehensively.

Table 3-5 List of weed species that emerged during the trials

Botanical name	Common name
Amaranthus thunbergii	Red pigweed
Amaranthus virivdis	Slender amaranth
Datura stramonium	Common thorn-apple
Tegetes minuta	Tall khaki weed
Portulaca oleracea	Pigweed

3.4.7 Harvesting

Trials were harvested in the fifth week after planting. Prior to harvesting a final recording of plant height was taken. The foliage/stem and root mass were determined for each pot. The soil was washed off from the roots of each of the plants and the top parts removed from the roots with shears. The weights of the fresh top parts were recorded and put into a brown paper bag labeled with the pot's unique number. The roots were dabbed with absorbent paper to remove excess moisture and they were also put into brown paper bags and labeled with the unique number as mentioned before.

These bags were then placed in the drying oven at 65° for 72 hours after which the dry weight of each group of three plants was recorded. The weight of the fresh plants as well as dried roots and plants were divided by the amount of the plants from each pot (which in most cases was three) to determine the average weight per plant. These three parameters were recorded for each sample throughout the project at the end of each experiment.



3.5 Data analysis

Analysis of variance (ANOVA) with the Proc GLM model (Statistical Analysis System (SAS) released in 2008) was used to determine the statistical significance of differences between the different treatment means. Standard deviations (SD) were also calculated. Significance of difference between means was determined at 5% level of probability. The results and conclusions from each experiment are discussed in subsequent chapters.



4.

TRIAL 1: PRE-EMERGENCE HERBICIDE SCREENING

4.1 Introduction

As mentioned in a previous chapter, no herbicides have yet been registered on kenaf (*Hibiscus cannabinus* L.) in South Africa, and the local farmers needed an indication of which pre- and post-emergence herbicides to use with relative safety to the crop. Literature studies indicated a range of herbicides that could possibly be used in the South African context, and after consultation with the agro-chemical companies in this region, as well as the farmers from Winterton area where kenaf is currently being cultivated, a decision was made in terms of which pre-emergence herbicides to use.

The pre-emergence and post-emergence herbicide screening experiments ran concurrently, but will be dealt with in two different chapters. This chapter will only be concerned with the pre-emergence herbicide screening process and its results, while the post-emergence herbicide screening and its results are dealt with in Chapter 5.

4.2 Materials and methods

Since the first experiment commenced during autumn, with temperatures set to drop further towards winter, it was decided to use the glasshouses at Phytotron D at the Hatfield Experimental Farm of the University of Pretoria. These glasshouses provide shelter against sharp temperature spikes with fans cooling the glasshouse during the hottest hours, and it offers some protection against severe drops in temperature at night. Two thermometers were placed inside the glasshouse to measure the average day/night temperature for the duration of the experiment. The average daytime temperature was 24°C and the average nighttime temperature 14°C. The day- and nighttime temperatures in the glasshouse over the entire 5 week period shows a clear drop in temperature as season changes from autumn to winter (Figure 4.1).



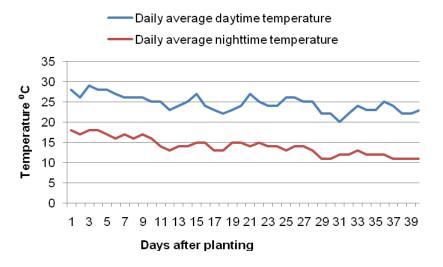


Figure 4-1 The daily average day and night temperatures as measured in a semi-controlled glasshouse during a 5 week growth period

The treatments comprised of 5 herbicides/herbicide mixtures: S-dimethenamid, imazethapyr, fluometuron/prometryn, pendimethalin, S-metolachlor and imazethapyr + S-metolachlor. The herbicides were applied at three different rates: the recommended dosage, double and triple the recommended rates. The control was represented by a zero application rate of herbicide (no herbicide applied). There were four replications of each rate and 24 control pots.

The soil was collected from the Hatfield Experimental Farm. Soil properties are presented in Chapter 3 Section 3.3.1. To ensure that the soil was homogeneous, it was air-dried, sieved and mixed before pots were filled. Ninety-six pots were filled with 1.75 kg soil each. Six seeds of Tainung #2 cultivar were placed on the soil surface in such a manner that they were spaced about 30 mm apart (Figure 4.2).

Seeds were covered with 1.25 kg of soil to ensure a uniform planting depth of 50 mm. All pots were then irrigated with tap water to field capacity (Figure 4.3) which was determined gravimetrically.



Figure 4-2 The planting of the seeds (Photo: AS Malan 2008)



Figure 4-3 Irrigating the pots to field capacity (Photo: AS Malan 2008)



After irrigation of the soil, the herbicide treatment commenced to simulate the application of pre-emergence herbicide treatments at planting. The herbicide dosages were prepared according to the dosages and dilution rates given in Appendix B Table 3.

Harvesting of the plants occurred 40 days after the trial commenced. Emergence was recorded every day of the first week and average plant height per pot was measured weekly. Weeds were counted and removed 20 days after planting. The method of harvesting is described in detail in Chapter 3.4.7.

The data collected over a period 40 days were subjected to statistical analysis. Analysis of variance was done to determine the significance of the effects of herbicide, herbicide rates, as well as their interaction on seedling emergence, fresh and dry mass per plant, plant height and percentage damage caused to kenaf plants (Appendix A). Weed control was also recorded and the data analyzed.

4.3 Results and discussion

4.3.1 Emergence

The percentage seedling emergence as determined prior to the commencement of the trials (in a separate experiment) to establish the viability of the seedlot was 88%. The control in this experiment produced an 84.03% emergence average. When compared with the results in Table 4.1, it is clear that none of the herbicides had a significantly negative effect on kenaf emergence. Between the six herbicides there were no significant differences in terms of seedling emergence (Table 4-1). Dosage also had no significant effect on seedling emergence (Appendix A Table 1 and Appendix C Table 1).

Table 4-1 Emergence of kenaf seed as affected by pre-emergence herbicides

Herbicide	Emergence %
S-dimethenamid	86.10a
imazethapyr	83.33a
fluometuron/prometryn	93.06a
pendimethalin	75.00a
S-metolachlor	83.33a
Control	84.03a

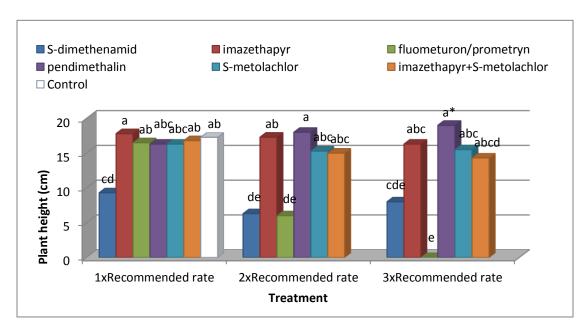
^{*} Treatment means followed with the same letter do not differ significantly from each other.



4.3.2 Plant height

The herbicide x dosage interaction was significant (Appendix A Table 1). Data are presented in Figure 4.4 and a table of the complete dataset can be found in Appendix C Table 2. The recommended rate of S-dimethenamid caused significant height reduction already at the recommended rate of application. S-dimethenamid is declared a seedling growth inhibitor that inhibits the synthesis of very long chain fatty acids. It is absorbed either through the coleoptile when the seedling emerges from the soil or through the roots (Vencill *et al.* 2002). This characteristic clearly led to inhibited growth for the kenaf seedlings and sensitivity of kenaf towards this herbicide was exposed.

Fluometuron was the only other herbicide that differed significantly from the control samples, and this occurred at twice and three times the application rates. Fluometuron is a photosynthesis inhibitor, which blocks CO₂ fixation and production of ATP and NADPH² (Vencill *et al.* 2002). Again, the effect it had on the kenaf seedlings was to inhibit normal growth.



^{*}Treatment means with the same letter does not differ significantly from each other (P<0.001)

Figure 4-4 Significant kenaf plant height differences, 40 DAP, when treated with different preemergence herbicide at different application rates.

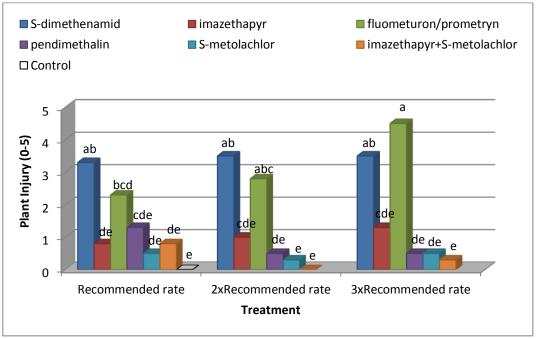
4.3.3 Herbicide injury

Herbicide injury was evaluated 20 days after planting, and the results indicated significant effects for herbicide as well as dosage and the interaction between them (Appendix A Table 1). The results are shown in Appendix C Table 3, and illustrated in Figure 4.5.



It was evident that S-dimethenamid had a pronounced effect on the kenaf seedlings at all three application rates. It showed itself again to be a seedling shoot growth inhibitor which caused visible damage to the young shoots. Fluometuron, the photosynthesis inhibitor, also had significant negative impact on the seedlings. The 3x application rate proved to be lethal for the kenaf seedlings (Figure 4.6).

Of the other herbicides, only imazethapyr at 3x application rate caused damage, albeit less than the previous two herbicides. Imazethapyr is a systemic herbicide of the imidazolinone family, and a branched-chain amino acid inhibitor. After reducing a plant's levels of valine, leucine and isoleucine, protein and DNA synthesis is disrupted (Tecle *et al.* 1993) Although at the normal dosage and even twice the normal dosage it had not affected the kenaf seedlings, the triple dosage obviously did manage to interfere with this synthesis, which manifested as injury (stunting and chlorosis) to the seedlings (Figure 4.7).



^{*} Treatment means with the same letter does not differ significantly from each other (P<0.001)

Figure 4-5 Kenaf seedling damage caused by pre-emergence herbicide treatments on a scale from 0 to 5, 20 DAP (0 = no injury, 5 = senescence of seedlings)





Figure 4-6 Severe injury effect of fluometuron applied at 3x the recommended rate. (Photo: AS Malan 2008)



Figure 4-7 Less severe injury caused by imazethapyr at 3x the recommended rate. (Photo: AS Malan 2008)

The results correlate to some extent with the findings from the previous parameter, and S-dimethenamid and fluometuron again were the most phytotoxic, but this time slight damage was also recorded for pendimethalin at the recommended rate. The fact that the double and triple rates did not, however, cause significant damage, might indicate experimental error.

4.3.4 Weed control: grass species

Application rate was the only factor causing a significant reaction in terms of grass species weed control in kenaf (Appendix A Table 1). However, grass weed control at all application rates was similar and significantly different from the control (Table 4.2). The control of grass weeds by all the herbicides were excellent already from the recommended rate and showed no significant differences between treatments except with the control, which naturally had a zero rating (Appendix C Table 4).

Table 4-2 The average grass weed control in kenaf as affected by application rate

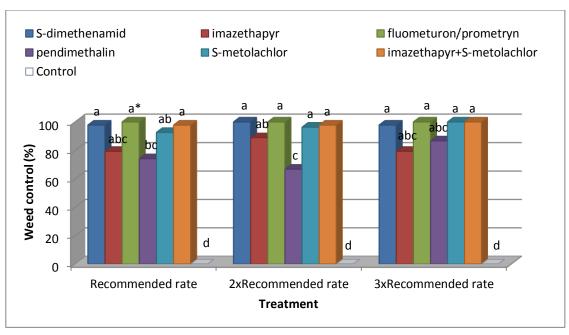
		Dosage		
Control	Recommended rate	Recommended rate x2	Recommended rate x3	
	Effectiveness of weed control (%)			
0b*	100a	99.58a	100a	

^{*} Treatment means with the same letter does not differ significantly from each other (P<0.001)

4.3.5 Weed control: broadleaved species

The herbicide x dosage interaction was significantly different from the control (Appendix A Table 1). Results are shown in Appendix C Table 5 and Figure 4.8. The large difference between average weed count per pot in the control (13.58 weed seedlings) and the treated pots (1.08 weed seedlings) could show the effectiveness of all the herbicides, but since there were no individual weed counts, this could be coincidental.





 $^{^{\}star}$ Treatment means with the same letter does not differ significantly from each other (P<0.001)

Figure 4-8 The effectiveness of broadleaved weed control in kenaf treated with six pre-emergence herbicides at three application rates.

The two herbicides that caused the highest crop injury ratings, and the lowest crop height figures, dimethenamid and fluometuron, also exhibited the best broadleaved weed control. The root growth retardation and photosynthesis inhibition therefore also affected the growth of broadleaved weeds as negatively as it did the kenaf seedlings. The mixture of imazethapyr and metolachlor as well as metolachlor on its own also produced good results in terms of weed control, and was on par with the previously mentioned two herbicides. It is important to note that it did not affect the kenaf negatively thus far. These herbicides have all been registered for the control of broadleaved plant species in various other crops.

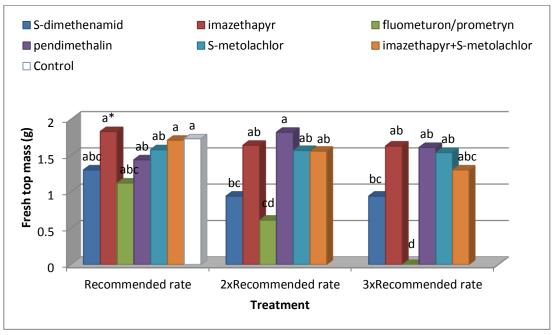
According to Vencill *et al.* (2002), pendimethalin is more of a grass weed inhibitor. Therefore its relatively poor performance in terms of broadleaf weed control is to be expected.

4.3.6 Kenaf fresh mass: top growth

A significant difference between herbicide and dosage was recorded (Appendix A Table 1 and Appendix C Table 6). No significant growth reductions in this parameter were recorded at the recommended rate for any of the herbicides, as compared to the control (Figure 4.9). The first significant growth reduction was recorded at the 2x recommended rate of both S-dimethenamid and fluometuron. At 3x recommended rate these two herbicides had a negative impact on the top growth, with no growth at all with



fluometuron at this rate. This correlates with the results for herbicide injury and plant height. S-dimethenamid belongs to the chloroacetamide herbicides which inhibit growth and reduce cell division and enlargement (Ashton and Crafts 1981). This would directly impact the weight of the top growth section of affected plants, as is shown by the results. The remaining herbicide treatments did not affect the fresh mass of the kenaf seedling's top growth, even at 3x the recommended rate.



^{*} Treatment means with the same letter does not differ significantly from each other (P<0.001)

Figure 4-9 The fresh top mass of kenaf seedlings as affected by three application rates per herbicide as compared to the control

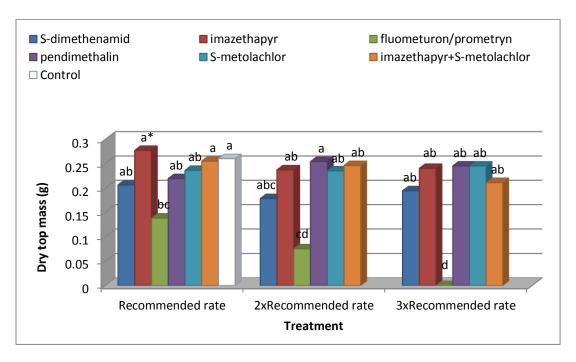
4.3.7 Kenaf dry mass: top growth

A highly significant difference between herbicide and dosage for the dried weights of the top growth of the kenaf plants was recorded (Appendix A Table 1 and Appendix C Table 7). If there was a discrepancy between the top fresh mass and the dried mass, it could have indicated either an experimental error, or that an herbicide had an impact on the fresh metabolism that was not visible in the dried material. The dried mass did, however, correspond with the findings for fresh top growth data, and S-dimethenamid and fluometuron were again shown to be the most phytotoxic (Figure 4.10).

According to Hickey and Krueger (in Ashton and Crafts 1981) chloroacetamide herbicides interfere with primary leaf emergence through the tip of the coleoptile of grass species. Both metolachlor and dimethenamid are herbicides belonging to this family, and yet kenaf showed a marked sensitivity towards dimethenamid, even though not being a monocotyledon. Neither metolachlor on its own or in combination with imazethapyr had



the same effect on the dried top growth of the kenaf seedlings. S-dimethenamid, on the other hand clearly affected the development of the kenaf seedlings negatively (Figure 4.10).



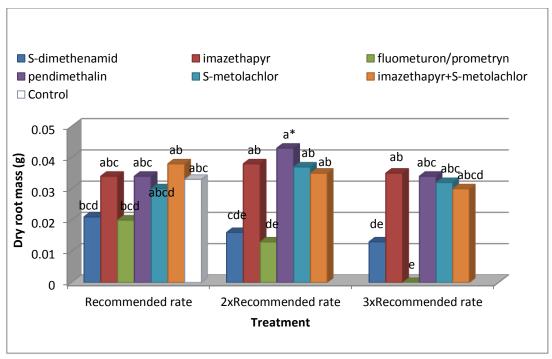
 $^{^{\}star}$ Treatment means with the same letter does not differ significantly from each other (P<0.001)

Figure 4-10 The dry top mass of kenaf seedlings as affected by three application rates per herbicide as compared to the control

4.3.8 Kenaf dry mass: roots

The dried root mass once again proved that there were significant differences between the different herbicide treatments (Appendix A Table 1). These significant differences are illustrated even more clearly when compared with the control (Figure 4.11). The S-dimethenamid and fluometuron at 2x and 3x recommended rates had a severe effect on kenaf root growth as compared to the other herbicides and the control (Appendix C Table 8). The explanation remains that kenaf is not as successful in escaping the effects of these two herbicides, as with the other herbicides.





^{*} Treatment means with the same letter does not differ significantly from each other (P<0.001)

Figure 4-11 The dry root mass of kenaf seedlings as affected by three application rates per herbicide as compared to the control

4.4 Conclusions

In this first trial to determine the sensitivity of kenaf (*Hibiscus cannabinus*) to preemergence herbicides, the following can be concluded. S-dimethenamid and fluometuron are not safe for use in kenaf (Table 4.3). Therefore fluometuron / prometryn would not be included in any of the other trials. It was decided that S-dimethenamid could still be considered at least at the recommended rate, and therefore it remained part of the subsequent trials.

S-dimethenamid, fluometuron, metolachlor and imazethapyr + metolachlor gave good control against grass and broadleaved weeds (Table 4.3). In this trial imazethapyr and pendimethalin was effective on grass weeds only.



Table 4-3 Summary of the pre-emergence herbicide efficacy in kenaf under controlled conditions

Active ingredient	Kenaf safety	Weed control
S-dimethenamid		G/B*
imazethapyr	++	G
fluometuron/prometryn		G/B
pendimethalin	++	G
S-metolachlor	++	G/B
imazethapyr + S- metolachlor	++	G/B

^{*}The letters G, B refer to grasses and broadleaved plants respectively. The injury risk factor is based mainly on crop responses at the 1x herbicide rate and is indicated by either "+" (indicating it is safe to apply on kenaf) or "-"(not safe)



5.

TRIAL 1: POST-EMERGENCE HERBICIDES

5.1 Introduction

Pre-emergence herbicides target the seed of the weed plant *before* and *during* emergence of the seedling. Post-emergence herbicides are used to kill weeds after emergence, when the weed plants have emerged through the soil and are growing. Post-emergence herbicides are designed to be absorbed through the leaves. These are generally applied in the form of sprays and particularly in the agricultural domain they are applied from the air by small aircraft.

These herbicides are applied when weed plants are young and growing vigorously. They are mostly used to control annual, biennial and perennial broadleaved weeds and unless applied carefully, can severely damage any vegetation in the vicinity (Cobb 1992).

The reason, therefore, for applying both pre- and post-emergence herbicides, would naturally be to target species that might have been missed during the first (pre-emergence) application, or if the pre-emergence application only targeted certain species or physiological plant parts and kenaf seedlings/weeds might respond differently to post-emergence herbicides.

When researching the sensitivity of a certain crop towards herbicides, the study would be incomplete if only pre-emergence herbicides were tested. Therefore the logical step was to also include post-emergence herbicides in this study. The same criteria for selecting the pre-emergence herbicides also applied in the selection of post-emergence herbicides and four herbicides were selected. As mentioned in Chapter 2.5.3, the following post-emergence herbicides were used as part of the study:

- bentazone (Basagran[®])
- 2,4-DB
- monosodium methanearsonate (MSMA)
- pyrithiobac sodium (Staple[®])



5.2 Materials and methods

Since Trial 1 (selection of pre-emergence herbicides) and Trial 2 ran concurrently, all methods and materials were the same, the only differences being the following:

- With only four herbicides represented, only 64 pots were required (four herbicides at three rates (recommended rate, 2x recommended rate, and 3x recommended rate) with four replications for each herbicide rate and 16 control pots)
- The herbicides were applied 16 days after planting.
- A surfactant called Allgral 94[®] was used during the application of the four herbicides.
- Monitoring of plant height, herbicide damage and weed control was done two weeks after treatment with post-emergence herbicides (30 days after planting)
- The plants were harvested 50 days after planting, to allow herbicide injury to further reveal itself, or for the kenaf plants to possibly recover from the injury.

For the specific methods and materials that were used, the reader is referred to Chapter 3.3 and 3.4.

The data collected during this part of Trial 1, was similar to that of the pre-emergence herbicide part of the trial. The temperature records were the same, and emergence rate was the same as the control. The emergence rate would not be influenced by any effect the post-emergence herbicides had, but for the sake of data analysis and comparison this had to be included in the data recording. Other data recorded were the following:

An assessment of the possible injuries sustained as a result of herbicide application was done 30 days after planting, and thus two weeks after herbicide application. Harvesting of the kenaf plants following the post-emergence herbicide applications, took place 50 days after planting and 34 days after herbicide application. Before the plants were harvested, the plant heights were measured in cm and recorded as an average per pot. At the same time, a weed count per pot was done and recorded. The data recorded was the herbicide control of grassy weeds and broadleaved weeds as an average per pot. After all data had been recorded, all weeds were removed, and the six kenaf seedlings were thinned out to allow three plants per pot to remain. This lessens competition as an inhibiting factor in the trials.

The same three weight categories were recorded at harvesting as in the pre-emergence herbicides. These are:

• the fresh mass of the above-ground part of the kenaf plants. Each pot's results are an average of the plants removed from it.



- the fresh plant material was then dried for 72 hours at 65° C, and weighed again.
- The dried roots were also weighed and these weights were recorded at the same time as that of the top dried parts.

Statistical results of the data analysis are given in Appendix A Table 2.

5.3 Results and discussion

5.3.1 Emergence

None of the effects were significant (Appendix A Table 2). There were no significant differences between the four herbicides and the control pots in terms of final emerged seedling count. Dosage had no effect on emergence percentage (Appendix C Table 9).

The following table indicates the percentage of seedlings that emerged, averaged over the three treatments (Table 5.1). The percentage of seedling emergence determined prior to the commencement of the trials during establishment of viability, was 88%. The emergence was lower in all the treatments, but according to the statistics there was no significant divergence from the control in this experiment, which had an emergence of 83.3%. Although the emergence was lower than the original seed viability tests, it was consistently so throughout this trial.

Table 5-1 Kenaf seedling emergence

Herbicide	Emergence %
Bentazone	80.56a*
2,4-DB	83.33a
monosodium methanearsonate	86.11a
pyrithiobac sodium	86.11a
Control	83.30a

 $^{^{\}star}$ Treatment means with the same letter does not differ significantly from each other (P<0.001)

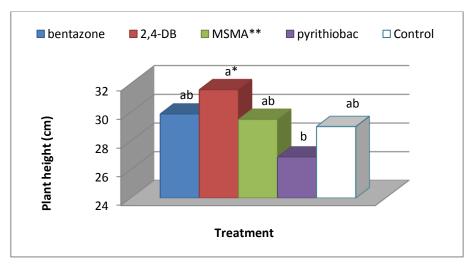
5.3.2 Plant height

There was a significant effect on the plant height in terms of herbicide treatment (Appendix A Table 2). Pyrithiobac sodium affected the plant growth to such an extent that the average plant height of seedlings treated with it differed significantly from the other three herbicides (Figure 5.1). This herbicide was not yet available in South Africa at the time of the trials. It has been developed in northern America by DuPont, and has been registered for safe use with kenaf in the USA. The active ingredient, pyrithiobac, belongs to the Pyrimidinyl(thio)benzoate family (DuPont label), but according to Rao et



al. (1999) the sodium salt currently registered as Staple® does not belong to any particular family because of its unique chemistry. Pyrithiobac sodium belongs to the group of acetolactate synthase (ALS) inhibiting herbicides whose function is to inhibit the synthesis of the essential branched amino acids leucine, iso-leucine and valine. This resulted in affecting growth rate, so that the seedlings treated with pyrithiobac sodium were outgrown by the control and seedlings treated with bentazone, 2,4-DB and MSMA.

Apart from the significant effect of herbicides, there were no further results which indicated that plant height was influenced either by the rate of dosage or the interaction with herbicides (Appendix A Table 2).



^{*} Treatment means with the same letter does not differ significantly from each other (P<0.001)

Figure 5-1 Effect of post-emergence herbicides on plant height of kenaf seedlings

5.3.3 Herbicide injury

There was a highly significant difference (P<.0001) for the main factors (herbicides and dosage) (Appendix A Table 2). The statistical results indicate that none of the post-emergence herbicides performed as well in the selectivity screening as the pre-emergence herbicides did. At least four of the pre-emergence herbicides had almost no detrimental effect on the kenaf seedlings. With the post-emergence herbicides the kenaf seedlings showed a higher average damage score, but here again there was no specific herbicide that caused complete senescence of the plants. It can even be argued that with an average score of between two and four, that the kenaf plants, given enough time and little competition, would be able to recover and still produce a viable harvest.

The herbicide that produced the worst score in terms of kenaf seedling health was pyrithiobac sodium (Figure 5.2). As discussed in the previous section, pyrithiobac sodium

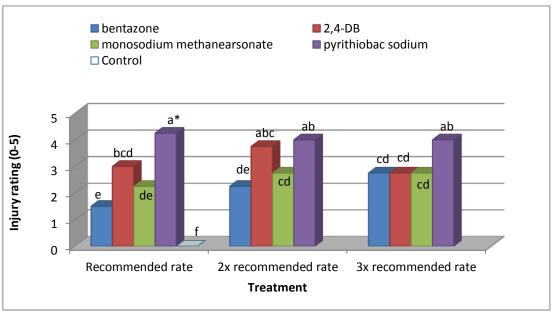
^{**}monosodium methanearsonate



belongs to the group of acetolactate synthase (ALS) inhibiting herbicides whose function is to inhibit the synthesis of the essential branched amino acids leucine, iso-leucine and valine. The inhibition of growth and death of leaf tissue within a week after application clearly indicated inhibition of the biosynthesis process. This directly affected the provision of the vital starting materials for amino acid biosynthesis in the chloroplasts. Several processes are affected in the chloroplast: amino acids derive their nitrogen from glutamate, they are also central to the recycling of nitrogen within the plant, arginine and proline are synthesized from glutamate as well as several other processes which would all heavily affect growth (Cobb 1992).

At the other end of the scale was bentazone, which had only a slight effect on the kenaf seedlings at the recommended application rate. Bentazone is a member of the Benzothiadiazole herbicides which tend to inhibit the photosynthetic flow of electrons in the Photosystem II (PSII) region (Cobb 1992). Since there is a significant difference between the control and seedlings treated by bentazone, there was some injury caused in the PSII region. The same is true for 2,4-DB and MSMA which respectively affected cell wall plasticity and cell membrane desiccation.

Therefore, as far as herbicide screening goes, pyrithiobac sodium had the most adverse effect on the kenaf seedlings, and none of the four post-emergence herbicides (Appendix C Table 10) were as safe as the pre-emergence herbicides were.



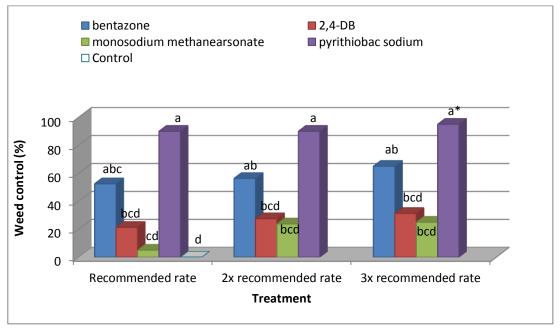
^{*} Treatment means with the same letter does not differ significantly from each other (P<0.001)

Figure 5-2 Kenaf seedling injury caused by post-emergence herbicides at three application rates.



5.3.4. Weed control: grass species

Both the herbicide and dosage main effects were highly significant for control of grass weeds (Appendix A Table 2). Figure 5.3 shows the superior effectiveness of pyrithiobac sodium in controlling grass weeds. It is interesting in the light of the label information which does not claim any grass control by this herbicide. It is also interesting that MSMA is supposed to be both a broadleaved and grass weed control agent, but here it performs the worst in terms of weed control. Neither of the other two herbicides makes any claim to monocotyledon control, but there is clearly a significant difference between 2,4-DB and MSMA on the one hand, and pyrithiobac on the other. It might be said that the pyrithiobac sodium dosage was too high and therefore kenaf seedlings and weeds alike would have been affected (Appendix C Table 11). Since no weed seeds were introduced specifically, this remains speculative.



 $^{^{\}star}$ Treatment means with the same letter does not differ significantly from each other (P<0.001)

Figure 5-3 Grass weed species control in kenaf with different post-emergence herbicides at three application rates.

5.3.5. Weed control: broadleaved species

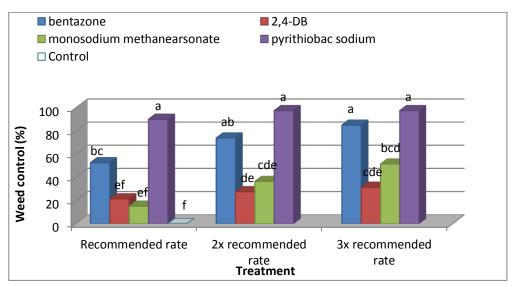
Similar to the previous variable (the control of grass species), there was a highly significant difference between the four herbicide treatments (Appendix A Table 2). 2,4-DB and MSMA both performed relatively poorly in comparison with the performance of pyrithiobac which killed virtually all the broadleaved weed species (Appendix C Table 12).



The results of the effectiveness of bentazone also differed significantly from the underperformers, and killed 85% of the broadleaved weeds at triple the recommended dosage (Figure 5.4). According to Unruh *et al.* (2004) bentazone is a contact herbicide that targets the green tissue which is exposed to the spray, and it is therefore explicitly a broadleaved herbicide. However, under the trial conditions of this study it did not perform particularly well since it only managed to control 53% of the weeds at the recommended rate (Figure 5.4).

Likewise, 2,4-DB is a systemic, foliar-applied herbicide that is mainly used to control broadleaved species, but in this study it had a very poor effect on the broadleaved weeds. The only herbicide that is primarily used in practice on grass species is the monosodium methanearsonate, which was understandably not very effective in controlling broadleaved species.

The dilemma here is clearly that the two broadleaved herbicides which did not affect the weed species also did not harm the kenaf plants, but the pyrithiobac which annihilated all the weed species, was also very harmful to the kenaf seedlings. This probably indicates rather that the dosage could have been too high or too low, rather than a kenaf selectivity for any of the herbicides.



^{*} Treatment means with the same letter does not differ significantly from each other (P<0.001)

Figure 5-4 Broadleaved weed species control in kenaf with post-emergence herbicides at three application rates.

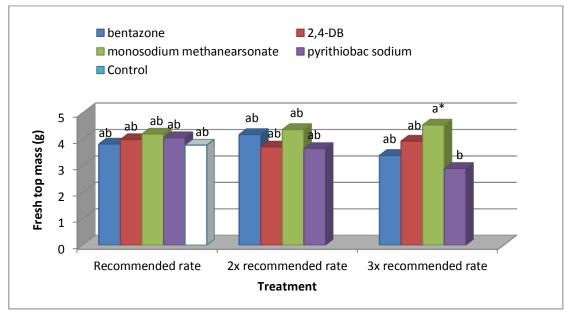
5.3.6. Kenaf fresh mass: top growth

There was a significant difference between dosage and herbicide (Appendix A Table 2). Pyrithiobac sodium at triple the prescribed rate differed significantly from monosodium



methanearsonate at triple the prescribed rate (Figure 5.5). This merely confirms the harmful effect of pyrithiobac sodium on kenaf seedlings when applied at such a high dosage.

The remainder of the herbicides as well as recommended rates of pyrithiobac did not differ significantly from the control or each other (Figure 5.5). The fact that these treated and untreated seedlings produced equal weights (apart from pyrithiobac at 3x the recommended rate) provides proof that these herbicides did not affect the normal development of the kenaf seedlings. This indicates that kenaf managed to escape the toxicity of these herbicides either through developing defense mechanisms or through metabolic transformation (Cobb 1992).



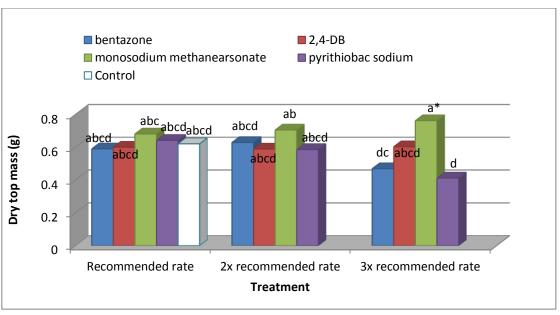
 $^{^{\}star}$ Treatment means with the same letter does not differ significantly from each other (P<0.001)

Figure 5-5 Average fresh mass of kenaf seedling top growth as affected by post-emergence herbicides at three application rates.

5.3.7. Kenaf dry mass: top growth

The results of the dried mass of the aboveground section of the seedlings show that there was a significant difference (Appendix A Table 2) between plants that were treated at triple the recommended rate of monosodium methanearsonate and those that were treated with pyrithiobac sodium and bentazone also at triple the dosage (Figure 5.6). It is however, more notable that there was no significant difference at the recommended rates.





^{*} Treatment means with the same letter does not differ significantly from each other (P<0.001)

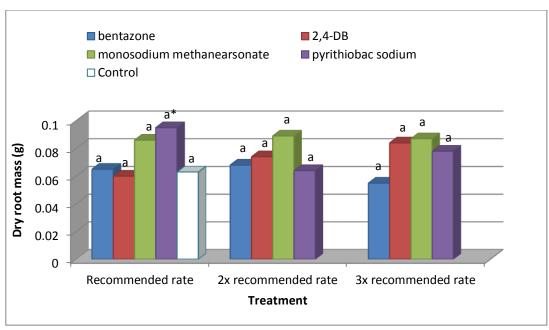
Figure 5-6 The average dried mass of kenaf seedling top growth as affected by post-emergence herbicides applied at three application rates

5.3.8. Kenaf dry mass: roots

The results of the dried root mass indicated a slight significance in differences between the four herbicides and their application rates (Figure 5.7). The double and triple application of MSMA resulted in the highest dry root mass exceeding that of all other treatments. This corresponds with all the previous mass data (fresh and dry top growth), but not with plant height, where kenaf plants treated with MSMA were shorter than those treated with bentazone and 2,4-DB. Since a period of 20 days separated the measurements of plant height from the plant weights, this could indicate a recovery in seedlings treated with MSMA.

The results of the dried root weight of kenaf seedlings correspond with the previous two sections on top weight of plant material in the case of recommended rates. This indicates no discrepancy in performance of the aboveground plant parts and the roots – sometimes symptoms are hidden if only roots are affected by a particular herbicide because its mode of action only targets belowground plant parts.





^{*} Treatment means with the same letter does not differ significantly from each other (P<0.001)

Figure 5-7 The average dry root mass of kenaf seedlings affected by post-emergence herbicides applied at three application rates.

5.4. Conclusions

The results of the post-emergence trial can be summarized in the following points:

- Post-emergence herbicides had only slight negative effects on plant height or on plant mass (tops or shoots)
- Visual injury to kenaf listed from light to severe damage: bentazone < monosodium methanearsonate < 2,4-DB < pyrithiobac sodium
- Pyrithiobac sodium and bentazone could outperform the other two herbicides in weed control, although it should be said that pyrithiobac also affected the kenaf seedlings negatively (Table 5.2).
- Monosodium methanearsonate can be used to control all weeds, while bentazone
 will control broadleaved weeds and nutsedge. 2,4DB and pyrithiobac sodium were
 only effective against broadleaved weeds present in the kenaf pots (Table 5.2).



Table 5-2 Summary of the performance of the post-emergence herbicides for use in kenaf

Product	Active ingredient	Kenaf safety	Weed control
Basagran®	bentazone	- +	B*
2,4-DB	2,4DB	- +	В
MSMA	monosodium methanearsonate	- +	G/B
Staple [®]	pyrithiobac		В

^{*}The letters G, B refer to grasses and broadleaved plants respectively. The injury risk factor is based mainly on crop responses at the 1x herbicide rate and is indicated by either "+" (indicating it is safe to apply on kenaf) or "-"(not safe)



6.

TRIAL 2: INVESTIGATING THE ROLE OF PLANTING DEPTH IN KENAF SENSITIVITY TOWARDS PRE-EMERGENCE HERBICIDES

6.1. Introduction

Pre-emergence herbicides specifically target the emerging weed seedling because it is soil-applied and is therefore absorbed by the roots and coleoptiles. The rationale behind determining the possible influence of planting depth lies in the length of time that the seedling is in contact with the herbicide. Screening safe herbicides therefore implies that even at maximum exposure to the herbicide the crop will not be affected negatively. A seed that has been planted at 5 cm below the soil surface would be in contact with a soil-applied herbicide for a longer period of time than a seed pip that has been planted at a depth of 2.5 cm.

Another consideration is the fact that some of the selective grass killers or graminicides are very volatile in composition and they need to be incorporated into the top 2.5 cm of the soil very rapidly after application for maximum efficacy (Cobb 1992). The chloroacetamides like dimethenamid have a residual effect in the soil, and can kill annual weeds for up to eight weeks after application (Cobb 1992, Vencill *et al.* 2002). Kenaf seeds that have been planted at 5 cm without being affected by a particular residual herbicide would indicate a low sensitivity towards that herbicide. Similarly, kenaf seeds that have been planted at a depth of 2.5 cm and remained unaffected by the graminicide-type of herbicide would indicate low sensitivity towards that herbicide. This is a logical process by which to screen the different types of herbicides.

In this trial it was therefore decided to use the two planting depths, 2.5 cm and 5 cm below soil surface. These are two practical depths generally used in practice on farms and easy to replicate in the field study that should follow the pot experiments. How the two depths were accomplished in the pots has already been described in detail in Chapter 3.4.2.

6.2 Materials and methods

As mentioned above, the general methods and materials that were used throughout the project are described in detail in Chapter 3.4. The finer details that differ from the other trials are the following:



- A total of 120 pots were used.
- 60 pots were planted at a depth of 5 cm and 60 at a depth of 2.5 cm.
- One of the herbicides (fluometuron/prometryn) was eliminated after the previous trial and therefore only five pre-emergence herbicide treatments were used. The herbicide treatments were: S-dimethenamid; imazethapyr; pendimethalin, Smetolachlor and a combination of imazethapyr and metolachlor.
- The herbicides were applied on the day of planting.
- Four rates were applied: zero, one, two and three times recommended rate with zero representing the control.
- Three replications per herbicide treatment combination were planted.

The pots were all placed in a glasshouse that provided limited shelter from cold and heat as well as rain. Results of the statistical data are presented in Appendix A Table 3.

6.3 Results and discussion

6.3.1 Emergence

As could be expected, rate of emergence was clearly influenced by planting depth and all the pots with a planting depth of 2.5 cm emerged faster than the pots with a planting depth of 5 cm. Seedlings planted at 2.5 cm already emerged within three days after planting, while the seedlings planted at 5 cm first emerged from the fifth day after planting. The final seedling count was always taken one week (7 days) after planting. This exceeded the emergence rate of any of the weed seeds present in the pots, which confirms that kenaf is more competitive than most weeds within the first week after germination. A significantly higher final emergence count was recorded for seeds planted at a depth of 2.5 cm than planted at 5 cm (Appendix A Table 3). The emergence count for each planting depth is summarized in Table 6.1, which includes the emergence percentage. According to the statistical data the final emergence count was, however, not affected by herbicide, dosage or any interaction effects (Appendix C Table 13).

Table 6-1 Difference in kenaf emergence between two planting depths

Planting depth (cm)	Emergence count (average number of seedlings per pot)	Emergence percentage (%)
2.5	5.30a*	88.3a
5.0	4.97b	82.8b

^{*} Values with the same letter do not differ significantly from each other



A possible explanation for the lower emergence count at the depth of 5 cm is possibly that seedlings at 5 cm are exposed to the herbicides in the soil for a longer period than those planted at 2.5 cm. Since almost all the herbicides (pendimethalin, metolachlor and pendimethalin) specifically target emerging shoots (Vencill *et al.* 2002), these results appear to confirm the initial hypothesis.

Even though imazethapyr is absorbed slower into the roots than the other herbicides (Vencill *et al.* 2002), there was no clear distinction between the results of the different herbicides either in terms of dosage or planting depth. The outcome therefore was only significantly affected by planting depth itself (Table 6.1).

6.3.2 Plant height

Plant height was measured on the day of harvesting (40 days after planting). From these results it was clear that the growth of plants in terms of height was not affected by planting depth or any specific herbicide (Appendix C Table 14). The only significant effect was that of dosage on plant height (Appendix A Table 23 and Table 6.2) The application rate did indicate that the control and the herbicide at three times recommended dosage differed significantly, and plant height was definitely negatively affected by the high dosage level. It has also shown that the recommended dosage and even twice the rate were not substantially different from the control. This was encouraging in terms of the sensitivity of kenaf growth towards the herbicides under scrutiny, as well as towards the ability of the plants to recover from any negative effects incurred during emergence.

Table 6-2 The significant effects of herbicide dosage on average plant height of kenaf seedlings

Dosage							
Control	Recommended rate	Recommended rate x2	Recommended rate x3				
Average plant height (cm)							
20.17a [*]	19.06ab	19.00ab	17.50b				

 $^{^{\}ast}$ Values with the same letter do not differ significantly from each other

6.3.3 Herbicide injury

Injury rating was done midway through the trial. According to the statistical analysis there was no significant difference between the planting depth and any specific herbicide treatment (Appendix A Table 3). Although there was a significant application rate effect, the only significant difference was between the control and all the herbicide application rates but none among the application rates itself (Table 6.3). Furthermore, the average



injury rating was not unacceptably high either. It can therefore be said that in terms of herbicide damage, there was little to observe, and that the herbicides at either planting depth appeared mostly safe to use in kenaf.

Table 6-3 The average injury rating for kenaf plants as affected by herbicide application rates

Dosage						
Control Recommended rate Recommended rate x2 Recommended rate						
Plant injury (0-5)*						
0b	1.40a**	1.30a	1.40a			

^{*} Injury ratings between 0 and 5 indicated the following levels of injury:

6.3.4 Kenaf fresh mass: top growth

The fresh mass of the top growth of the kenaf plants were recorded on the day of harvesting the trial. Analysis of the data produced no significant difference between the plant mass from the kenaf planted at 5 cm below soil surface and those planted at 2.5 cm below ground (Appendix A Table 2. A graph depicting the real data shows very little distinction between the different planting depths or even the different herbicides (Figure 6.1)

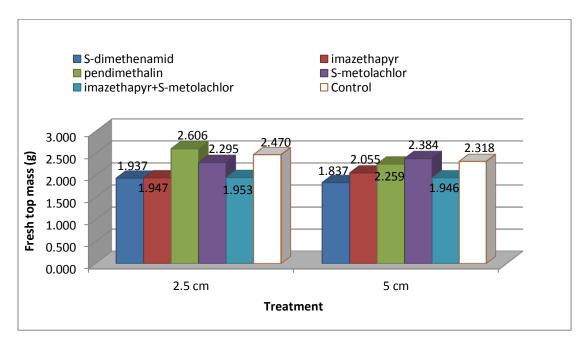


Figure 6-1 Differences in kenaf fresh top growth mass as affected by two planting depths and different herbicide treatments.

^{0 =} no injury visible

^{1 =} slight injury visible

^{2 =} medium injury visible

^{3 =} all plants show medium signs of injury

^{4 =} severe injury visible

^{5 =} senescence of seedlings

^{**} Values with the same letter do not differ significantly from each other



Statistical analysis did, however, indicate significant differences in the main effects of herbicide (Table 6.4) and dosage (Table 6.5). The interaction effect was not significant (Figure 6.3). Results showed significant differences between the herbicide treatments, with kenaf plants treated with pendimethalin showing a substantially higher mass than when treated with either imazethapyr or the mixture of imazethapyr and metolachlor, and a highly significant higher mass than when treated with S-dimethenamid (Table 6.5).

Table 6-4 Average fresh top growth mass of kenaf seedlings as affected by herbicide. (Data averaged over application rate and planting depth)

Herbicide	Average fresh top mass (g)
S-dimethenamid	1.887c*
imazethapyr	2.014bc
pendimethalin	2.432a
S-metolachlor	2.340a
imazethapyr+S-metolachlor	1.950bc
Control	2.394a

^{*} Treatment means with the same letter does not differ significantly from each other (P<0.001)

Dimethenamid belongs to the family of chloroacetamide that has as its main mode of action an inhibition of very long chain fatty acid synthesis (Böger *et al.* in Vencill *et al.* 2002), or so it is thought. The herbicide is absorbed primarily by emerging coleoptiles, but because no significant damage was observed during and after emergence, and no particular injury to the plants midway through the trial, there is no definite answer to the low plant mass of this herbicide. Pendimethalin clearly showed the least negative effect in terms of fresh top growth at harvest.

As far as the significant differences in the application rates are concerned, it is clear that increase in dosage has a directly negative impact on the fresh top mass of the kenaf plants, with the control showing a highly significant difference to the treated plants at twice and triple the recommended rates of herbicides (Table 6.5). It is a positive result to find that there was no significant difference between the control and the herbicides when applied at the recommended rates. Since there were no significant differences in the interaction between the factors of planting depth, herbicide or dosage, no definite conclusions could be drawn regarding the combined factors.

Table 6-5 Average fresh top growth mass of kenaf as affected by application rate. (Data averaged over herbicide treatment and planting depth)

Application rate: average fresh top mass (g)					
Control	Recommended rate	Recommended rate x2	Recommended rate x3		
2.394a*	2.266ab	2.107b	2.001b		

^{*} Treatment means with the same letter does not differ significantly from each other (P<0.001)



6.3.5 Kenaf dry mass: top growth

Pendimethalin-treated kenaf plants weighed the most after being dried, but this time the mass of plants treated with S-dimethenamid, imazethapyr and the combined mixture of imazethapyr and metolachlor produced the lowest mass of kenaf dry top growth (Table 6.6). These statistics confirmed the fact that at least as far as top growth was concerned, that pendimethalin seemed to have been the least harmful to the kenaf plants, and metolachlor equally so.

Table 6-6 Average dry top growth mass of kenaf as affected by herbicide. (Data averaged over application rate and planting depth)

Herbicide	Average dry mass (gram)
S-dimethenamid	0.344ab*
imazethapyr	0.345ab
pendimethalin	0.416a
S-metolachlor	0.395ab
imazethapyr+S-metolachlor	0.339b
Control	0.409a

^{*} Treatment means with the same letter does not differ significantly from each other (P<0.001)

Looking at the herbicide dosage, there was again, as in the case of the fresh top growth, highly significant differences (Appendix A Table 2. The control did not differ substantially from either the recommended rate plants or twice the recommended rate, but three times the recommended rate did produce significantly lower masses (Table 6.7). This more or less correlated with the findings with regards to the fresh top growth in the previous section. It can therefore be concluded that at three times the recommended rate, kenaf plants were sufficiently adversely affected.

Table 6-7 Average dry top growth mass of kenaf as affected by application rate. (Data averaged over herbicide treatment and planting depth)

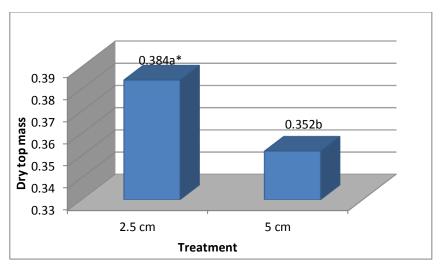
Application rate: average top dry mass (g)						
Control	Recommended rate	Recommended rate x2	Recommended rate x3			
0.409a*	0.389ab	0.364ab	0.349b			

^{*} Treatment means with the same letter does not differ significantly from each other (P<0.001)

Planting depth also showed significant effects when data was averaged over herbicide treatment and application rate. Seedlings planted at 2.5 cm produced a much higher average for the dry top biomass and differed significantly from dry top mass of seedlings



planted at 5 cm. This indicates that plants in the shallow soil had a head start and grew more biomass in the same period of time as plants planted in deeper soil.



^{*} Treatment means with the same letter does not differ significantly from each other (P<0.001)

Figure 6-2 Average dry top growth mass of kenaf as affected by planting depth. (Data averaged over herbicide treatment and application rate)

6.3.6 Kenaf dry mass: roots

There was a slight deviation from the previous two findings when the dry root data were analyzed. Herbicide treatment did not have any significant effect on dry root mass, even if these values differed from the control (Figure 6.3). However, soil depth did indicate an interesting difference (Appendix A Table 3), which was that the root mass from plants planted at 5 cm was greater than from plants planted at 2.5 cm (Table 6.8).

Again it should be noted that the difference between the control and the herbicide treatment at recommended rates was not significant, indicating no serious injury caused by any of the herbicide treatments (at the recommended rate) to the root systems (Figure 6.3).



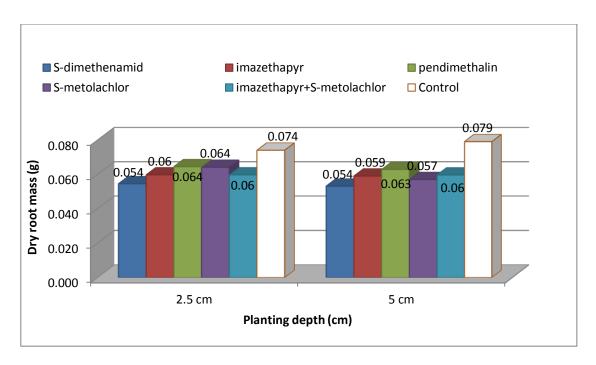


Figure 6-3 Differences in kenaf root mass as affected by different herbicides and two planting depths

Table 6-8 Average dry root mass of kenaf as affected by planting depth. (Data averaged over herbicide treatment and application rate).

Soil Depth 2.5 cm	Soil Depth 5 cm
Dried Root Mass (g)	
0.0626b*	0.0709a

^{*} Treatment means with the same letter does not differ significantly from each other (P<0.001)

One explanation might be that the roots of kenaf plants planted at 5 cm below soil surface might have been too deep to really come into contact with the herbicide. Since the roots grow down into the soil, it could have developed unharmed by the herbicides. Another factor that could cause unhindered root development would be less competition from weeds, since these tend to develop in the top 2 cm of the soil. Another possibility, which confirms findings by Dawson (in Ashton 1981), is that some herbicides affect the subterranean shoot development but not the root system itself. This would occur where the primary site of action is the developing leaf tissue and would explain why the root mass is far less affected than the top mass. However, even the control showed greater weights at 5 cm depth, which indicates that the greater mass might also be explained by stem formation that probably occurred subterraneous.

Results showed significant differences between the different application rates (Table 6.9). The control's roots weighed significantly more than those treated with herbicides at



twice and triple the recommended rates, indicating that there was injury. This indicates that the roots were harmed at higher herbicide application rates.

Table 6-9 Average dry root mass of kenaf as affected by application rate. (Data averaged over herbicide treatment and planting depth)

Application rate: average dry root mass (g)						
Control	Recommended rate	Recommended rate	Recommended rate			
	Recommended rate	x2	х3			
0.074a*	0.069ab	0.065bc	0.059c			

^{*} Treatment means with the same letter does not differ significantly from each other (P<0.001)

6.4 Conclusions

The main focus of this trial had been to determine whether planting depth of kenaf seeds affected the severity of a possible herbicide injury. Only two of the parameters produced statistically significant results that indicated planting depth was a factor to be considered when planting kenaf. However, neither of these parameters showed any significance in interaction with the herbicides.

Emergence was affected negatively at the deeper planting level, which probably did indicate some influence by the herbicides, but there was no significant effect as a result of different herbicide rates. The control showed the same lower percentage emergence at 5 cm planting, and thus it is more complex than blaming the weaker emergence on the herbicide treatments.

The other parameter that was affected significantly was root dry weight, and again, without a clear interaction with the herbicides it cannot be said undisputedly that less herbicide present at the deeper level caused better root development. There is a possibility though, that it had influenced the root injury to a certain extent.

Apart from these findings, the indications are that in terms of herbicide injury, planting depth did not significantly affect development of kenaf seedlings. For purely practical reasons and the possibility of an upper hand in the competition with weeds, the recommended planting depth would be 2.5 cm, but then again, kenaf seedlings have such a fast emergence rate, that there was no significant impact on plant development.



7.

TRIAL 3: THE ROLE OF TEMPERATURE IN THE SENSITIVITY OF KENAF (*HIBISCUS CANNABINUS*) TOWARDS PRE-EMERGENCE HERBICIDES

7.1 Introduction

The history behind the crop failures in the Winterton area where the kenaf had been planted a few seasons earlier indicated that a severe cold front could have contributed substantially to the crop failure. Even though herbicides were thought to be the main cause, there was also a strong possibility that a drop in temperature could have heightened the damaged caused by the herbicides. It was therefore logical to investigate the possible link between herbicide damage and low temperatures.

Metabolism plays a very important role in the selectivity of some herbicides because the plant has developed defense mechanisms such as sulphoxidation, aryl hydroxylation and molecular rearrangement to develop tolerance towards inhibiting compounds (Cobb 1992). Of course, there is a definite correlation between metabolism and temperature, with metabolic activity slowing down at lower temperatures. If a plant has developed tolerance towards an herbicide at optimal temperatures, this would not necessarily indicate the same would be true at extreme temperature levels.

This part of the project therefore focused on creating three temperature regimes whilst excluding other possible external factors such as photo-period, wind, drought or untimely precipitation. The trial was conducted inside three growth chambers with strict temperature controls and with 12 hour cycles of light/darkness.

7.2 Materials and methods

The same methods and materials used in the previous trials were again used here, with the only exception being the use of growth chambers instead of glasshouses, and the amount of samples. The following materials were identical to those of trial 1 and 2:

- Soil and containers with plastic bags
- Kenaf seed: cultivar Tainung II, six seeds per pot, thinned out to three seedlings 20 days after emergence



The same pre-emergence herbicides as those used in Trial 2. They are: S-dimethenamid, imazethapyr, pendimethalin, S-metolachlor and a mixture of imazethapyr and metolachlor.

The methods for planting, monitoring and harvesting were the same throughout this trial, and the data was again analyzed in exactly the same fashion to provide similar sets of results. No data-logger was used to measure soil temperature as in Trial 1.

In addition to these, the following materials and methods were used:

- A total of 135 pots were used, with 45 in each temperature regime.
- Three growth cabinets set at different temperatures were used (Figure 7.1):
 - Growth cabinet I was set at a constant day temperature of 20° C with a night temperature of 10° C (in the data sets this trial was referred to as LOW).
 - O Growth cabinet 2 was initially set at 20° C during the night and 30°C during the day, after 2 days the temperatures were dropped to 5° and 15° C night and day temperatures respectively, to represent a sudden cold spell. This was then sustained for 10 days, after which the original temperatures of 20°/30° were restored for the remainder of the trial (in the data sets this trial was referred to as MED).
 - The third temperature regime was one of 20°/30° C day/night for the entire duration of the trial (in the data sets this trial was referred to as HIGH).
- Day hours were regarded as 06h00 until 18h00, with nighttime being 18h00 to 6h00.
 The lights in the cabinets therefore were set to go out at 18h00 and come back on at 06h00.
- Three application rates were used: zero (control), one and two times the recommended dosage. For each herbicide rate, there were three replications.



Figure 7-1 The kenaf containing pots arranged inside the medium temperature range growth chamber (Photo: AS Malan 2008)



7.3 Results and discussion

7.3.1 Emergence

Growth chambers 2 and 3 both started off with the same warm daily temperatures, with chamber 1 having the lower temperature range. Since the metabolism of the plants in chamber 1 were lower because of the cooler temperatures, it came as no surprise when the data analysis showed a significant difference in emergence between the low temperature range and that of the medium and high temperature ranges (Table 7.1). The minimum significant difference for the effect of temperature was 0.55, indicating that emergence in chambers 2 and 3 did not differ significantly from each other.

Table 7-1 Kenaf seedling emergence as affected by temperature (Data averaged over herbicide treatment and application rate).

Temperature range					
Med	High				
Amount of seedlings per po	ot				
4.9a	5.0a				
	Med Amount of seedlings per po				

^{*} Treatment means with the same letter does not differ significantly from each other (P<0.001)

None of the other factors (dosage or herbicide) played a significant role in the emergence of the seedlings; neither did any possible interactions between the three factors deliver any significant results. It is clear therefore that the cooler conditions affected the amount of seedling emergence negatively. Because none of the herbicides showed significantly different results and there was no clear distinction between the zero application rate (control) and the other two rates, it might indicate that herbicides were not responsible for the findings in the field, but merely low temperatures affecting the seedling metabolism and activity. For the rate of emergence, a graph was drawn from the raw data as the plants emerged and again, it was clear that cooler temperatures affected the rate of emergence (Figure 7.2).



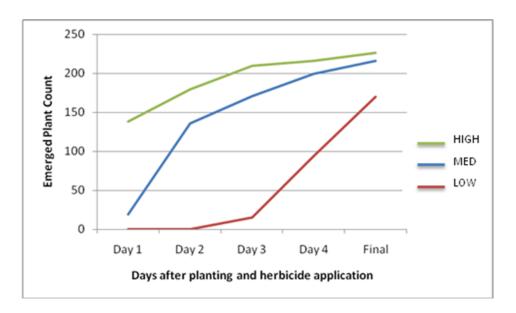


Figure 7-2 Kenaf seedling mergence rate as affected by temperature (Data averaged over herbicide treatment and application rate. Count = total amount of seedlings emerged)

7.3.2 Plant height

There was a highly significant result in the interaction between all three factors: dosage, herbicide and temperature regime (Table 7.2). The two lower temperature zones produced almost one set of plant heights, where the high temperature zone had a significantly higher growth rate. In the LOW temperature regime, herbicide and application rate delivered no noticeable differences from the control, which implies that the low temperatures had a detrimental effect on all the plants, regardless of treatment.

The MED range also produced no distinction between herbicide and dosage, and delivered only slightly improved outcomes for plant height. The HIGH zone was without any doubt the best temperature range at which to grow kenaf. Again there were no significant differences except between metolachlor at recommended rate and dimethenamid at twice the recommended rate, as well as between metolachlor and the combination of imazethapyr and metolachlor, both at the recommended rate. It could be in the latter instance that the addition of imazethapyr made the difference, but then imazethapyr on its own it did not fare a lot worse than metolachlor on its own.



Table 7-2 Kenaf plant height as affected by temperature, herbicide treatment and application rate.

	Temperature					Herb				
Herbicide		Low		Med			High			
Herbicide	Dosage					Mean – C*				
	Control	X1	X2	Control	X1	X2	Control	X1	X2	
A***	11.0e**	9.3e	9.3e	13.0e	14.3de	9.3e	24.3abc	24.0abc	21.0cdc	14.5
В	11.3e	11.0e	10.3e	12.3e	13.3e	12.7e	24.0abc	28.7ab	25.0abc	16.8
С	11.5e	10.3e	12.3e	11.3e	11.7e	14.0de	27.0abc	27.3abc	28.7ab	17.4
D	10.3e	10.7e	10.3e	14.3de	11.7e	10.3e	24.7abc	30.3a	26.7abc	16.7
E	10.3e	11.7e	9.3e	13.3e	11.7e	12.3e	27.0abc	22.0bc	26.7abc	15.6
Dos x Temp Mean	10.71	10.6	11.0	12.87	12.53	11.73	25.4	26.47	25.6	

^{*}Herbicide mean without control

E: imazethapyr + S-metolachlor

7.3.3 Herbicide injury

The statistical analysis of the data for herbicide injury indicated that the only significant effects were the interaction between dosage and temperature (Table 7.3) as well as herbicide and dosage (Figure 7.3).

Table 7-3 Kenaf seedling injury rating as affected by temperature and dosage. (Data averaged over herbicide treatments)

		Tomp Moon			
Temperature	Control	Control X1 X2		Temp Mean (without control)	
	Injury Rating (0 – 5)**			(without control)	
Low	0.0b*	1.9a	2.0a	1.95	
Med	0.0b	1.1ab	1.9a	1.5	
High	0.0b	0.5ab	0.5ab	0.5	
Dosage Mean	0.0	1.2	1.5		

 $^{^{\}star}$ Treatment means with the same letter does not differ significantly from each other (P<0.001)

- 0 = no injury visible
- 1 = slight injury visible
- 2 = medium injury visible
- 3 = all plants show medium signs of injury
- 4 = severe injury visible
- 5 = senescence of seedlings

Considering the two dosage means, there was a distinct increase in injury rating both with the increase in dosage and temperature range. The optimal condition therefore would be to apply herbicides at the recommended rate at temperatures higher than

^{**}Values followed by the same letter do not differ significantly from each other.

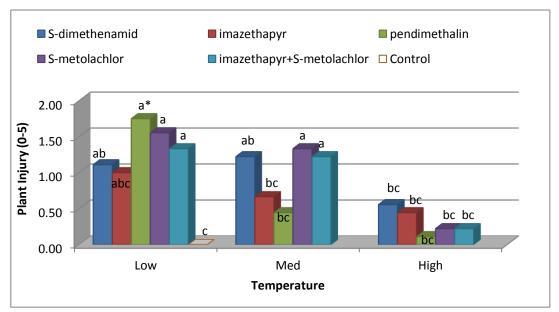
^{***}A: S-dimethenamid B: imazethapyr C: pendimethalin D: S-metolachlor

^{**} Injury ratings between 0 and 5 indicated the following levels of injury:



10°/20° C for night / daytime temperatures. The fact that there was also a significant difference between the medium temperature range at twice the recommended rate and the optimal high range, shows that the drop in temperature had definitely also affected the plant's sensitivity towards herbicides at a low temperature. Dodge (1989) found that differential metabolism plays a deciding role in determining selectivity of many herbicides. He also warned that metabolism could relate to selectivity of a particular herbicide in certain crop/weed situations, where in other cases factors such as uptake and translocation may be just as important.

Apart from the significant results in the interaction between dosage and temperature, there was also a significant difference between temperature and herbicide (Figure 7.3 and Appendix C Table 15). The trial showed significant evidence that temperature and the combinations of temperature and herbicide and temperature and dosage affected the growth of kenaf seedlings detrimentally as far as low temperatures are concerned.



^{*} Treatment means with the same letter does not differ significantly from each other (P<0.001)

Figure 7-3 Kenaf seedling injury rating as affected by temperature and herbicide

7.3.4 Kenaf fresh mass: top growth

The most dramatic outcome of the statistical results for Trial 3 was the interaction between the temperature range, application rate and the herbicide treatments (Appendix A Table 4). There were highly significant differences between some of these parameters individually and also a highly significant effect of the interaction of the three (Table 7.4).



Table 7-4 Significant differences in the interaction of herbicide treatment, application and temperature range on fresh top mass of kenaf seedlings

	Temperature									
l lawhiaida	Low				Med		High		Herb	
Herbicide					Dosage					Mean*
	Control	X1	X2	Control	X1	X2	Control	X1	X2	- C
S-	1.03	0.75jk	0.89jk	1.36	1.27	0.91jk	3.12ab	2.22ab	1.74cd	1.30
dimethenamid	jk**	0.7 Oj.k	o.oojit	fghijk	hijk	0.0.1	0.1200	cdefgh	efghij	
imazethapyr	0.95jk	0.92jk	0.78jk	1.36	1.42	1.25	2.28ab	2.51	2.43	1.55
шагешаруі	บ.ออเห	0.92jK	0.7 OJK	fghijk	fghijk	hijk	cdefg	abcd	abcde	
pendimethalin	0.66k	0.84jk	1.07jk	1.44	1.32	1.66de	2.19bc	2.53	2.69	1.69
pendinemann	U.OOK	U.04JK	1.07jK	efghijk	fghijk	fghijk	defghi	abcd	abc	
S-metolachlor	0.86jk	0.83jk	0.97jk	1.31 ghijk	1.10jk	1.20ijk	2.32 abcdef	2.76ab	2.82ab	1.61
imazethapyr+S -metolachlor	0.87jk	0.94jk	0.72k	1.05jk	1.16jk	1.33 fghijk	2.98ab	2.23ab cdefgh	3.21a*	1.60
Dos Mean	0.874	0.856	0.886	1.304	1.254	1.27	2.578	2.45	2.578	
Temp Mean		0.872			1.276			2.535		

^{*}Herbicide mean without control

Results indicated a significantly higher fresh top mass for kenaf seedlings growing in the HIGH temperature treatment. The average weight of 2.54 g for all the plants growing in this temperature range clearly shows a significantly higher mass than the medium range where the temperature was the same except for the 10 days midway through the trial when the average temperature was 10 °C. Nevertheless, the medium range cabinet also had a substantially higher mass average than the low temperature range cabinet. This is clearly confirmation that temperature has a significant effect on the growth rate of kenaf plants.

The temperature/herbicide/dosage interaction was significant and showed that again temperature was the determining factor in the plants' growth rates, as the top mass weights at individual dosages did not differ significantly within one temperature treatment, but did differ highly significantly from dosage rates in different temperature treatments. Especially plants in the HIGH temperature range had a significantly higher growth rate, and therefore bigger fresh plant weight than those in the other two chambers, regardless of whether these were the control or treated plants.

As was evident earlier in section 7.3.2 where height was measured, S-dimethenamid had the worst effect on the kenaf plants, with the combined mixture performing best and pendimethalin very close behind. This fact was confirmed by the fresh weight of plants treated with dimethenamid, which was substantially lower than the remainder of the herbicides.

^{**}Treatment means with the same letter does not differ significantly from each other (P<0.001)



7.3.5 Kenaf dry mass: top growth

There was a significant difference in the temperature, herbicide and dosage (P value 0.0346), which further confirms the findings of the fresh mass results of the top growth of the kenaf plants (Table 7.5).

Table 7-5 Significant differences in the interaction of herbicide treatment, application and temperature range on dry top mass of kenaf seedlings

	Temperature									
Hambiaida	Low			Med		High			Herb	
Herbicide	Dosage						Mean - C*			
	Control	X1	X2	Control	X1	X2	Control	X1	X2	C
S- dimethenam id	0.197ef	0.15f	0.186ef	0.209ef	0.218ef	0.186ef	0.501 a**	0.386 abcd	0.319 bcde	0.241
imazethapyr	0.187ef	0.187ef	0.163ef	0.229 def	0.235 def	0.236 def	0.389 abcd	0.458 ab	0.408 abc	0.281
pendimethal in	0.132f	0.171ef	0.213ef	0.231 def	0.216ef	0.283 cdef	0.481a	0.48ab	0.444 abc	0.301
S- metolachlor	0.18ef	0.183ef	0.21ef	0.218ef	0.21ef	0.224ef	0.398 abc	0.501a	0.449 ab	0.294
imazethapyr +S- metolachlor	0.21ef	0.222ef	0.177ef	0.205ef	0.206ef	0.225ef	0.425 abc	0.4abc	0.483a	0.286
Dos Mean	0.182	0.183	0.190	0.218	0.217	0.228	0.439	0.445	0.421	
Temp Mean		0.185			0.221			0.435		

^{*}Herbicide mean without control

7.3.6 Kenaf dry mass: roots

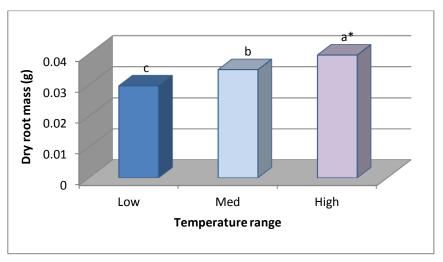
As in the previous two sections, temperature range produced significant differences (Appendix A Table 4 and Figure 7.4). Again the HIGH temperature range produced root mass that weighed the most, with MED range following and the LOW temperature range affecting plant performance most negatively. Once more this confirms the statistic findings that the kenaf plants simply grew better at the higher temperature range of 20°/30°C. Even the MED range with its drop in temperature produced bigger plants with a greater root mass than the plants that grew in the cool chamber (Appendix C Table 16).

Another unrelated, but still significant result was that of the herbicide effect (Figure 7.5). Similarly, the root mass confirmed the previous findings (fresh top growth), with S-dimethenamid affecting the plants' growth most, then almost similarly followed

^{**}Treatment means with the same letter does not differ significantly from each other (P<0.001)

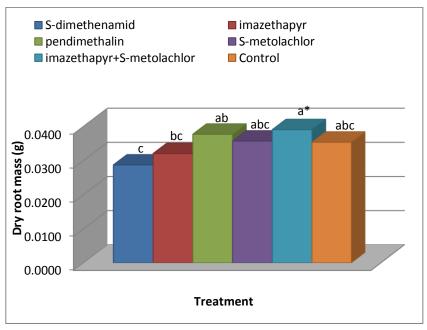


imazethapyr. Again the combined mixture of metolachlor and imazethapyr performed best with pendimethalin almost as well (Appendix C Table 16). There appeared to be no statistically significant results from the interaction between the three parameters of dosage, temperature and herbicide.



^{*}Treatment means with the same letter does not differ significantly from each other (P<0.001)

Figure 7-4 Significant differences in the effect of temperature range on dry root mass (Data averaged over herbicide treatment and dosage)



Treatment means with the same letter does not differ significantly from each other (P<0.001)

Figure 7-5 Significant effects of herbicide treatment on kenaf dry root mass (Data averaged over dosage and temperature)



7.4 Conclusions

This part of the project delivered highly significant results in terms of outcomes for farming practices. It was probably also as a result of a direct problem that farmers dealt with in the field. There no longer exists any doubt that temperature (specifically the range between day and night) plays a vital role in the growth processes of kenaf plants. This is of course nothing new as literature has often pointed out that kenaf was originally found in warm humid sub-tropical Africa (Ustinova 1938 in Dempsey 1975, LeMahieu *et al.* 1991).

In summary, virtually all the factors that had been monitored produced significant results connected with temperature. Every instance confirmed the fact that the kenaf plants preferred the highest temperature range and performed best at this range. The herbicides had the least effect within this range because plants were more robust and could effectively resist herbicide injury better.

The herbicides that had the least effect on kenaf were again the combined mixture of imazethapyr and metolachlor, with pendimethalin performing equally well. The herbicide that affected kenaf most negatively in this trial was S-dimethenamid with imazethapyr only somewhat less so.



8.

TRIAL 4: INVESTIGATING THE ROLE OF APPLICATION TIMING IN HERBICIDE SENSITIVITY OF KENAF

8.1 Introduction

Pre-emergence herbicides target the seed of the weed plant before and during emergence of the seedling, as well as during the vulnerable early stages of development. Post-emergence herbicides are used to kill weeds after emergence, when the plants are growing vigorously, and when all the physiological functions of the plants are at optimum levels of performance. Post-emergence herbicides are designed to be absorbed through the leaves and hence these are generally applied in the form of sprays, and particularly in the agricultural domain, from the air by aeroplanes in the case of large areas.

Soil-applied herbicides, or generally known as pre-emergence herbicides are mainly concerned with the relative positions and growth rates of the weed and the crop. In Trial 2 the role of planting depth was investigated in the sensitivity of kenaf towards herbicides. In this chapter the timing of herbicide treatment as an important contributor to herbicide selectivity was the focus of the experiment. The effect that application timing could have on the kenaf seedling was researched under controlled conditions. Selectivity usually results from a complex interaction of several factors (Cobb, 1992), and an effort was made to isolate the timing factor in kenaf's sensitivity towards herbicides.

The assumption was that the seed is moistened and therefore softened by the initial water supplement on the day of planting (day zero). The absorption of herbicide in combination with water by the newly planted seed was the basic premise of this trial. Particularly the possible vulnerability of the kenaf seed had to be investigated. The application of pre-emergence herbicides on the day of planting formed part of this trial for the purposes of comparison, even though this same procedure was followed during the previous trials.

Since kenaf has shown itself to be a very competitive seedling, with emergence within two to three days under optimal conditions, the effect of pre-emergence herbicides on the young seedling immediately following sprouting from the safety of the seed cover (±day two) and its initial emergence from the soil (±day four) had to be investigated as well.



Several factors play a role in application timing. As soon as the herbicide is applied to the top soil, it mainly forms a layer on top of the soil and in the first few millimeters below the surface. With the addition of water filling the soil to field capacity, some of the herbicide is leached deeper into the soil to reach the planting level of 50 mm. Several possible scenarios of exposure to the herbicide by the seed and newly emerged seedling become possible. The premise is that initially, the longer the seedling is exposed to the herbicide in the soil, the higher the probability of contact with the herbicide. This gives the herbicide more opportunity to find receptors within the plant cells to "do its worst". On the other hand, should the herbicide be applied four days after planting, it might come into contact with the seedling at a stage when it is most vulnerable. The newly developed primary leaves readily absorb the moisture accessible in the soil, and thus the herbicide is taken up into the plant cells of the leaves to affect the metabolic processes if the plant is sensitive to the particular herbicide. Of course, the purpose of this study is to screen which herbicides can be used with safety on kenaf, hence it is vital to find the application period most suitable to harm weeds but not the kenaf crop.

8.2 Materials and methods

The same general materials and methods were used during this trial as for the previous trials and the detailed description is found in Chapter 3. The following materials deviated from the previous trials:

- A total of 90 pots were used.
- This trial was conducted in a temperature controlled glasshouse with a temperature regime of 17° C at night and a maximum of 28° C during the daytime.
- The same four pre-emergence herbicides were used as in Trials 2 and 3. They are: S-dimethenamid; imazethapyr; pendimethalin; S-metolachlor and a mixture of imazethapyr and S-metolachlor.
- Only two rates were used: zero (representing the control) and the recommended dosage.
- There were three replications for each herbicide rate.
- Three different application timings were scheduled:
 - On the day of planting (day zero)
 - Two days after planting and
 - Four days after planting



8.3 Results and discussion

The same parameters were measured as in all the previous trials. The table containing the results from the Analysis of Variance tests is found in the Appendix A Table 5.

8.3.1 Emergence

There were no significant differences between either the control or the herbicides at recommended rates, or any significant differences between the three application timings (Appendix A Table 5 and Table 8.1). The coefficient of variance was 18.07 which indicated a fairly low experimental error. It can be safely stated that between the five treatments, seedling emergence was not affected whether the herbicides were applied on the day of planting, or two days, or four days after planting of the seeds. Seedling emergence is also similar for all five treatments when applied at the recommended rate.

Table 8-1 The final average count of emerged kenaf seedlings per pot as a percentage

Application	Herbicide	Control	Recommended rate
timing		Eme	rgence count (%)
	S-dimethenamid	66.67	77.8
	imazethapyr	83.33	100.0
0 days after	pendimethalin	72.22	94.4
planting	S-metolachlor	83.33	77.8
	imazethapyr+S-metolachlor	83.33	88.9
	Dosage mean	77.8	87.8
	S-dimethenamid	72.1	83.3
	imazethapyr	72.2	88.9
2 days after	pendimethalin	72.2	83.3
planting	S-metolachlor	72.2	77.8
	imazethapyr+S-metolachlor	72.2	66.7
	Dosage mean	72.2	80.0
	S-dimethenamid	66.7	83.3
	imazethapyr	83.3	94.4
4 days after	pendimethalin	88.9	83.3
planting	S-metolachlor	83.3	77.8
	imazethapyr+S-metolachlor	94.4	88.9
	Dosage mean	83.3	85.6



8.3.2 Plant height

Plant height, measured just before harvesting, indicated no significant differences between the different herbicides and the control, or between the different application timings. (Table 8.2) The average plant height measured was 11.09 cm and the coefficient of variance was 12.86. The growth of the kenaf plants were thus not affected by applying herbicides, either on the day of planting, or two, or four days after planting.

Table 8-2 The average plant height for each treatment and application timing compared to the control

Herbicide	Aj	Herbicide		
-	Day 0	Day 2	Day 4	mean
-	Ken	af plant height (d	cm)	_
S-dimethenamid	10.7a*	11.0a	12.0a	11.2
Imazethapyr	12.0a	11.0a	10.0a	11.0
Pendimethalin	13.0a	10.3a	13.0a	12.1
S-metolachlor	10.0a	10.7a	11.7a	10.8
imazethapyr+S-metolachlor	13.7a	10.3a	11.0a	11.7
Control	11.08a	10.92a	10.34a	10.8

^{*} Treatment means with the same letter does not differ significantly from each other

8.3.3 Herbicide injury

The statistical analysis indicated a significant amount of plant injury to kenaf plants treated with herbicide at the recommended rate when compared to the control (Table 8.3). There was no significant difference between the three application timings or between the five herbicide treatments.

Table 8-3 Injury rating (0-5)** of kenaf plants as affect by herbicide application rate.

Control	Control Recommended rate					
	Injury Rating (0 - 5)**					
0.00a*	2.16b					

^{*} Treatment means with the same letter does not differ significantly from each other

- 0 = no injury visible
- 1 = slight injury visible
- 2 = medium injury visible
- 3 = all plants show medium signs of injury
- 4 = severe injury visible
- 5= senescence of seedlings

^{**} Injury ratings between 0 and 5 indicated the following levels of injury:



8.3.4 Kenaf fresh mass: top growth

The statistical analysis of the fresh top growth weight indicated a difference between the application timing and dosage. The minimum significant difference was 0.117 g (Appendix A Table 5). It is clear that there was no difference between the herbicides and control when applied on the day of planting, but there was a difference between the control and the herbicides both when herbicides were applied two days after planting, and four days after planting of the kenaf seeds (Table 8.4). This could indicate that there was growth retardation, but the confusing factor is that the mass of the plants that were treated four days after planting was higher than the untreated control. This could indicate an experimental error, even though the coefficient of variance (19.39) might not reflect this.

Table 8-4 Significant differences in kenaf seedling fresh top mass due to the interaction between application timing and dosage

	Dosage				
Application timing	Control	Recommended rate			
	Fresh top mass (g)				
0 days	1.176a [*]	1.174a			
2 days	1.221a	1.002b			
4 days	1.111ab	1.228a			
Dosage Mean	1.174	1.135			

^{*} Treatment means with the same letter does not differ significantly from each other

8.3.5 Kenaf dry mass: top growth

According to the analysis of variance tests, there were no significant differences in the interaction between the three parameters after the fresh top growth had been dried. This confirms that the slight difference obtained in fresh top growth mass, could have been due to an experimental error, as indicated in 8.3.4. In this case the coefficient of variance was 15.45, indicating very little chance of gross experimental error. It would appear that the top growth was not severely affected by the application of herbicides at any of the three times: zero, two or four days after planting.

8.3.6 Kenaf dry mass: roots

The results of the analysis of variance indicated no significant difference between the dried roots of the control or the kenaf plants. There was also no significant difference



between any of the five treatments in terms of root weight (Appendix A Table 5). Root growth was therefore not affected by the timing of herbicide application, or the herbicides themselves (Table 8.5).

Table 8-5 No significant differences in the effects of any of the parameters that could affect kenaf dry root mass

		Herbicide					
Herbicide	Day 0	Day 2	Day 4	– mean			
	D	Dry root mass (gram)					
S-dimethenamid	0.017a	0.021a	0.026a	0.021			
imazethapyr	0.028a	0.025a	0.029a	0.027			
pendimethalin	0.034a	0.026a	0.029a	0.030			
S-metolachlor	0.027a	0.026a	0.025a	0.026			
imazethapyr+S-	0.021a	0.027a	0.028a	0.025			
metolachlor							
Control	0.023a	0.027a	0.027a				

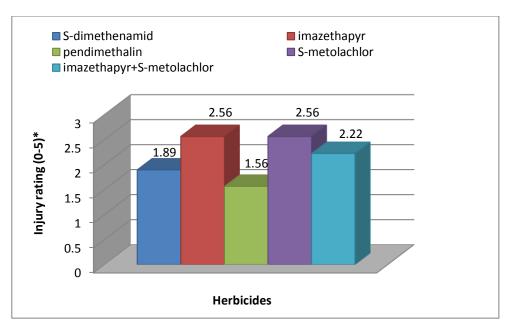
8.4 Conclusions

None of the parameters measured in Trial 4 gave any indication of a significant difference between herbicide application timings, whether applied on the day of seed planting, or two, or four days after planting. The most important question raised before the trial, was whether kenaf itself would be affected by the application of pre-emergence herbicides at any of three application times. After the initial screening process the four safest herbicides were chosen for the remainder of the project, and this trial merely confirmed that although some damage was observed (an average of 1.093 which was still far below mortality), the pre-emergence herbicides proved themselves fairly safe for use on kenaf.

From the results demonstrated in Figure 8.3, the safest herbicides emerging from this trial were pendimethalin and S-dimethenamid. The combination of imazethapyr and S-metolachlor appeared to be safer than the individual herbicides, but the differences were not significant.

This trial, however, indicated that none of the herbicides affected kenaf significantly when applied at any of the three application timings. The conclusion would be that either of the four herbicides, and probably a combination of some, can be applied at any date between the day of planting and the actual emergence of the kenaf plants.





^{*} Injury ratings between 0 and 5 indicated the following levels of injury:

- 0 = no injury visible
- 1 = slight injury visible
- 2 = medium injury visible
- 3 = all plants show medium signs of injury
- 4 = severe injury visible
- 5= senescence of seedlings

Figure 8-1 The relative safety of pre-emergence herbicides used in Trial 4 on kenaf seedling injury



9.

SUMMARY

9.1 Summary

Weed science is a fairly young discipline that has developed due to an overpopulated earth. With the current human population figures at between 4 and 5 billion, every arable hectare is required to provide people with an adequate diet as well as clothing and space for living and recreation. It is in this scenario where weeds have become superfluous, and as the population figure increases, even non-essential crops could potentially become "weeds" (Ashton and Crafts 1981).

Among plant species there are of course no natural discrimination between wanted and unwanted, simply more or less successful, or in ecological terms, pioneer or climax species as far as their role in the ecological process goes. It is due to the need for every available square meter of soil for crop production that the practice of chemically treating unwanted plant species developed. Numerous disciplines, including botany, plant physiology, biochemistry, microbiology, soil science, organic chemistry and toxicology, have contributed towards the development of herbicides.

One crop that has managed to turn its status from unwanted weed species to viable fibrous crop is *Hibiscus cannabinus* or kenaf. For this previous "weed" to now succeed as crop, certain herbicides have to be isolated as safe for use to ensure that kenaf now succeeds against other "weeds". For every ecosystem on the earth there are of course different limiting factors for a particular plant species. In South Africa, optimal environmental conditions had to be found where this new crop could be cultivated with success. Limiting factors would include temperature, precipitation, soil, pests and inherent physiology. This was one step in a process. Another was determining the plant competition once this environment had been determined. Part of this process was determining the herbicides that would eradicate the competition in favour of kenaf.

Herbicides are classified according to their mode of action. According to Ashton this refers to the entire sequence of events from introduction of the herbicide into the environment to the death of the targeted plant species (Ashton and Crafts 1981). Due to the fact that weed science is such a relatively new field, and only fragmentary knowledge about the great array of commonly used herbicides exist, a huge part of crop production remains to be based on experimental discovery of "safe" herbicides per crop.



This document was the outcome of such an experimental stage in the introduction of a crop to a new cropping area in South Africa. Kenaf is a plant species that became desirable as crop in southern Africa for its fibres specifically in the automotive industry. After some research a cropping area was selected by the company that speared the introduction. Initial crop failures prompted the company to conduct investigations into possible herbicide misuse. The fact that no herbicide has officially been tested in South Africa prompted the series of experiments described in this document.

A range of herbicides were selected for the experimental phase. They were mainly selected based on previous successful use especially in the United States, but also several other parts of the world. Herbicides that had proved safe with related crop species such as cotton (*Gossypium* ssp) were also considered in the selection of these experimental herbicides.

The herbicides had to represent the range of biochemical mechanisms that could affect a plant's development. These include photosynthesis, respiration, RNA synthesis, protein synthesis, lipid synthesis and many other reactions.

Selectivity refers to the fact that, under a given set of conditions, certain species of plants (weeds) are killed or seriously injured, when simultaneously; the desired plant species (crop) is not injured. Herbicides induce numerous responses in plants, from inhibiting growth to drastic morphological aberrations. They are able to alter cell division, cell enlargement, tissue differentiation and cellular and tissue deterioration. The visible symptoms include growth inhibitions, epinasty, formative effects, foliar chlorosis, albinism, necrosis, reduced cuticle formation as well as organelle and membrane modifications (Ashton and Crafts 1981). All herbicides inhibit growth, and some with rapid contact action will kill the cell tissue before an inhibition of growth can be observed. Others might simulate growth initially, but before death a phase of growth inhibition does occur as well (Ashton and Crafts 1981). It is this retardation of growth that will give plants unaffected by the herbicides the competitive edge.

Another dimension in the use of herbicides is the optimal period of application. Research have shown that, based on rates of absorption and amount taken up, the cuticle of young roots are more permeable than that of leaves (Dodge 1989). For this reason, preemergence herbicides are often the preferred choice of herbicide. Five pre-emergence and four post-emergence herbicides were selected for screening.

In the screening process to determine the safest herbicides for use in kenaf under South African conditions, several factors had to be considered. As a starting point, both pre-



emergence herbicides and post-emergence herbicides had to be researched. The selection of the herbicides depended on three criteria. Herbicides researched elsewhere in the world on kenaf were an obvious choice, as was the herbicide used previously by the farmers in the Winterton area (example - imazethapyr). Availability of the herbicides also played a role since it only made sense to research herbicides that were cost effective and readily available to farmers. The only exception was the use of Staple[®], which is a product used with great success in the US on kenaf, and it was provided at no charge by Du Pont. A third dimension was to use herbicides outside these mentioned, to determine whether other herbicides are available in South Africa that would be as effective as those previously researched, and perhaps less costly. The final selection of pre-emergence herbicides was S-dimethenamid, imazethapyr, fluometuron/prometryn, pendimethalin, S-metolachlor and a combination of imazethapyr and S-metolachlor. The post-emergence herbicides that were included in the trials were bentazone, 2,4-DB, monosodium methanearsonate and pyrithiobac sodium.

The mode of action of the selected herbicides covered a wide range and these are summarized in Table 3 of Appendix B. These include photosynthesis inhibitors, branched chain amino acid inhibitors, inhibitors of the biosynthesis of several plant components like fatty acids, lipids, proteins, isoprenoids and flavonoids, inhibitors of acetolactate synthase ALS and the systemic and organic herbicides.

After the initial screening of the nine herbicides, the focus shifted to include the influence of external factors that could affect herbicide action. The depth at which kenaf seeds might be most vulnerable was investigated. Application timing after seed planting was also investigated as well as the possible detrimental effect of temperature. The results of these trials are summarized below.

9.2 Conclusion

9.2.1 Herbicide selectivity

In the screening process of safe herbicides only one pre-emergence herbicide proved itself totally unacceptable. This was the herbicide Cotogard®, of the Phenylurea family with its main ingredients fluometuron (a urea-derivative) and prometuron (triazine). The variables where fluometuron/prometryn showed the most significant differences from other herbicides, was plant height, injury rating, dried top weight and dried root weight. In each of these variables the kenaf seedlings were killed by either twice or three times the



recommended rate and severely damaged by the recommended rate. The herbicide is an inhibitor of photosynthesis at the photosystem II part of mostly cotolydenous plant species. Death occurs from oxydation of proteins and lipids (Langham *et al.* 2007). Although rapid metabolism is an important means of selectivity in tolerant plant species, kenaf was unable to overcome the toxic inhibition of photosynthesis.

Although S-dimethenamid also showed significant differences from the safer herbicides, it did not perform significantly worse at the prescribed rate, and it was decided to retain it as part of the consecutive trials. The remainder of the pre-emergence herbicides (imazethapyr, pendimethalin and S-metolachlor) showed no significantly detrimental effect on kenaf seedlings and can be recommended as safe for kenaf during the subsequent field trials. It also showed that the cocktail of imazethapyr and metolachlor could no longer be held responsible for the crop failures in Winterton.

In the screening trial of post-emergence herbicides, the newcomer to the South African scene, pyrithiobac sodium, produced some significant results which could be construed as unfit for use on kenaf. It did not, however, cause any crop mortality and only affected the kenaf seedlings severely at three times the prescribed dosage. Although it would still require more research, an important recommendation would be to lower the recommended rate somewhat to prevent significant inhibition of acetolactate synthase ALS, which is its primary mode of action. There was no outright favourite amongst the remainder of the post emergence herbicides (bentazone, 2,4-DB and monosodium methanearsonate).

9.2.2 Influence of soil depth

The first of the external factors that was researched, was planting depth. Only two parameters showed significant results for planting depth, and these were rate of emergence and dried root mass. The emergence rate did not show a correlation with any particular herbicide and this indicates that planting kenaf at a depth of 2.5 cm simply gives it an added advantage to weed seedling emergence. Again with dried root mass there appeared to be no interaction between herbicide and planting depth, but it can be argued that herbicide effect might have had a more deleterious impact on the roots of seedlings planted at the level just below the soil surface, since the root mass of seedlings planted 5 cm below the surface was greater than those planted at 2.5 cm. It is, however, also possible that the greater mass figures for seeds planted at 5 cm below soil surface can be attributed to belowground shoot development.



9.2.3 Influence of temperature

The possibility that temperature could influence herbicide injury had to be researched because of crop failures by kenaf farmers. Although imazethapyr in particular was blamed for these failures by the local farmers, it coincided with a sudden cold front which could have aggravated/caused the damage to the kenaf seedlings.

There are three components present in the response of a plant to any herbicide or growth regulator. These three are the genetic physiology of the plant itself, the growth stage and the environmental conditions (Muzik, 1976). Although selectivity is always relative, when additional stress, such as herbicide treatment is applied under non-optimal environmental conditions, then the outcome is certain to be more deleterious than under optimal conditions (Muzik, 1976)

In the trial where the combined effect of temperature (climate) and herbicides were evaluated, every variable confirmed the fact that the kenaf plants preferred the highest temperature range and performed best at this range. The herbicides probably had the least effect within this range because plants were more robust and could effectively resist herbicide injury better. It is clear from this trial that kenaf seedlings perform better at higher temperature ranges.

9.2.4 Influence of application timing of pre-emergence herbicides

In the final trial where the interaction of the time of application with herbicides was investigated, no preferred timing window could be determined. There were no significant differences between any of the variables between the three different application times. None of the pre-emergence herbicides can be considered more or less lethal whether applied on the day of planting or any period prior to emergence.

9.2.5 Recommendations

As far as herbicide application is concerned, it is clear that stringent field tests are required before a final verdict can be reached about the selectivity of kenaf for potential herbicides. At this stage, it appears that pre-emergence herbicides have less negative effects on kenaf, and kenaf exhibits least sensitivity for imazethapyr, pendimethalin and S-metolachlor. Contrary to what farmers had surmised, the combination of imazethapyr and S-metolachlor did not appear to negatively affect kenaf.



Although the post-emergence herbicides did not perform as well in interaction with kenaf as the pre-emergence herbicides, a combination of some of these with pre-emergence herbicides should not be ruled out during the field trials.

Planting depth caused very few significant results; except for clearly advantaging kenaf seedlings in comparison to weed seedlings when planted at 2.5 cm. Soil depth of 2.5 cm would thus be the recommended depth at which to place the kenaf seeds.

The most significant outcome of the herbicide trials is the fact that temperature is a limiting factor both during the emergence stage, as well as during the initial period of seedling growth. No significant interaction with any particular herbicide was recorded, and it can therefore be deducted that temperature below 20°C negatively impacts the emergence and growth rate of kenaf seedlings. At low temperatures kenaf is also less competitive and sudden drops in temperature during the initial stages of development will lower crop production significantly. A practical recommendation therefore would be to avoid planting kenaf in areas/periods that has a high risk of low temperatures during the planting season.



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APPENDIX A ANALYSIS OF VARIANCE

Appendix A Table 1 ANOVA data of Trial 1: The effect of pre-emergence herbicides on kenaf

Variable	R-Square	Coefficient of Variance	Root MSE*	Mean	Source	LSD**	PR > F
Emorgoneo					Herbicide	NS***	0.1540
Emergence (final count)	0.317312	17.58421	0.89727	5.104167	Dosage	NS	0.6835
(IIIIai Court)					Herb x dosage	NS	0.1024
Kenaf plant					Herbicide	3.2506	<.0001
·	0.752254	21.71924	3.140241	14.45833	Dosage	2.3842	<.0001
height					Herb x dosage	8.3972	<.0001
Konof plant					Herbicide	0.7691	<.0001
Kenaf plant	0.813590	65.44057	0.743023	1.135417	Dosage	0.5641	<.0001
injury					Herb x dosage	1.9869	<.0001
Weed control					Herbicide	NS	0.4241
	0.999582	1.362720	1.020621	74.89583	Dosage	0.7749	<.0001
Grasses					Herb x dosage	NS	0.4649
Weed control					Herbicide	10.996	<.0001
Broadleaved	0.950918	15.81091	10.62296	67.18750	Dosage	8.0653	<.0001
Dioauleaveu					Herb x dosage	28.41	0.0025
					Herbicide	0.2927	<.0001
Fresh top mass	0.755705	19.67479	0.282801	1.437375	Dosage	0.2147	<.0001
-					Herb x dosage	0.7562	<.0001
					Herbicide	0.0435	<.0001
Dry top mass	.0755150	19.22719	0.042019	0.218542	Dosage	0.0319	<.0001
					Herb x dosage	0.1124	<.0001
					Herbicide	0.0071	<.0001
Dry root mass	0.740718	23.47015	0.006831	0.029104	Dosage	0.0052	0.0002
					Herb x dosage	0.0183	<.0001

^{*}Root MSE = Root Mean Squared Error;

^{**}LSD = Least significant difference;

^{***}NS = Non significant



Appendix A Table 2 ANOVA data of Trial 1: The effect of post-emergence herbicides on kenaf

Variable	R-Square	Coefficient of Variance	Root MSE*	Mean	Source	LSD**	PR > F
Emorgonco					Herbicide	NS***	0.8252
Emergence (final count)	0.284650	15.71318	0.790569	5.031250	Dosage	NS	0.6397
(IIIIai Courit)					Herb x dosage	NS	0.0863
Kenaf plant					Herbicide	4.026	0.0286
height	0.402966	14.52716	4.278702	29.45313	Dosage	NS	0.0530
Height					Herb x dosage	NS	0.1457
Kenaf plant					Herbicide	0.4504	<.0001
•	0.926667	21.27616	0.478714	2.250000	Dosage	0.4504	<.0001
injury					Herb x dosage	1.2229	<.0001
Weed control					Herbicide	17.643	<.0001
Grasses	0.806309	51.50215	18.75000	36.40625	Dosage	17.643	<.0001
Grasses					Herb x dosage	47.898	0.0031
Weed control					Herbicide	8.8473	<.0001
Broadleaved	0.949837	22.16483	9.402737	42.42188	Dosage	8.8474	<.0001
Dioauleaveu					Herb x dosage	24.02	<.0001
Freeh ton					Herbicide	NS	0.1288
Fresh top	0.430525	15.12885	0.585042	3.867063	Dosage	NS	0.3380
mass					Herb x dosage	1.4945	0.0066
					Herbicide	0.0855	0.0103
Dry top mass	0.558365	14.92952	0.090816	0.608297	Dosage	NS	0.1255
					Herb x dosage	0.232	0.0002
					Herbicide	NS	0.1943
Dry root mass	0.338839	25.43539	0.019367	0.076141	Dosage	NS	0.9364
					Herb x dosage	0.0495	0.0438

^{*}Root MSE = Root Mean Squared Error;

^{**}LSD = Least significant difference;

^{***}NS = Non significant



Appendix A Table 3 ANOVA data of Trial 2: The effect of soil depth and pre-emergence herbicide treatment on kenaf

Variable	R-Square	Coefficient of Variance	Root MSE*	Mean	Source	LSD**	PR > F
					Depth	0.3003	0.0301
					Herbicide	NS***	0.3300
Emergence					Dosage	NS	0.7372
(final count)	0.377845	16.10337	0.826640	5.133333	Depth x dosage	NS	0.3936
(miar count)					Herb x dosage	NS	0.3011
					Depth x herb	NS	0.8323
					Depth x herb x dos	NS	0.1152
					Depth	NS	0.3790
					Herbicide	NS 4.0770	0.1201
Kenaf plant	0.000050	40.00044	0.470557	40.00000	Dosage	1.6778	0.0011
height	0.386258	13.08041	2.476557	18.93333	Depth x dosage	NS	0.9303
					Herb x dosage	NS NC	0.5606
					Depth x herb	NS NS	0.3380 0.7253
					Depth x herb x dos Depth	NS NS	0.7253
					Herbicide	NS NS	0.3302
					Dosage	0.7239	<.0001
Kenaf plant	0.471425	102.5749	1.068488	1.041667	Depth x dosage	0.7239 NS	0.6611
injury	0.47 1425	102.3743	1.000+00	1.041001	Herb x dosage	NS	0.5993
					Depth x herb	NS	0.2901
					Depth x herb x dos	NS	0.6065
					Depth Depth	NS	0.9531
					Herbicide	0.34	0.0004
					Dosage	0.2859	0.0029
Fresh top	0.444583	19.25352	0.422026	2.191942	Depth x dosage	NS	0.6371
mass					Herb x dosage	NS	0.6702
					Depth x herb	NS	0.4069
					Depth x herb x dos	NS	0.5794
					Depth	0.0284	0.0396
					Herbicide	0.063	0.0057
					Dosage	0.053	0.0216
Dry top mass	0.436728	20.69950	0.078196	0.377767	Depth x dosage	NS	0.8973
					Herb x dosage	NS	0.4509
					Depth x herb	NS	0.3645
					Depth x herb x dos	NS	0.2855
					Depth	0.0049	0.0011
					Herbicide	NS	0.6494
					Dosage	0.0092	0.0008
Dry root mass	0.394734	20.28404	0.013535	0.066725	Depth x dosage	NS	0.7371
					Herb x dosage	NS	0.7307
					Depth x herb	NS	0.6551
					Depth x herb x dos	NS	0.8137

^{*}Root MSE = Root Mean Squared Error;

^{**}LSD = Least significant difference;

^{***}NS = Non significant



Appendix A Table 4 ANOVA data of Trial 3: The effect of temperature and pre-emergence herbicide treatment on kenaf

Variable	R-Square	Coefficient of Variance	Root MSE*	Mean	Source	MSD**	PR > F
Emergence	0.442103	23.95153	1.093905	4.567164	Temperature Herbicide Dosage Temp x Dos Herb x dosage Temp x herb Tempxherbxdos	0.5518 NS*** NS NS NS NS NS	<.0001 0.8111 0.2465 0.8823 0.6654 0.5031 0.4511
Kenaf plant height	0.941978	12.99434	2.126610	16.36567	Temperature Herbicide Dosage Temp x Dos Herb x dosage Temp x herb Tempxherbxdos	1.0727 1.6187 NS NS 12.355 NS 7.1364	<.0001 0.0067 0.6432 0.4416 0.0073 0.0898 0.0104
Kenaf plant injury	0.798334	64.239620.	0.560898	0.873134	Temperature Herbicide Dosage Temp x Dos Herb x dosage Temp x herb Tempxherbxdos	0.2829 NS 0.2829 1.8822 NS 1.5358 NS	<.0001 0.1004 <.0001 <.0001 0.2925 0.0158 0.0603
Fresh top mass	0.903785	19.15925	0.300337	1.567582	Temperature Herbicide Dosage Temp x Dos Herb x dosage Temp x herb Tempxherbxdos	0.1515 NS NS NS 1.3444 0.5757 1.0079	<.0001 0.4845 0.5112 0.9359 0.0002 0.0440 0.0069
Dry top mass	0.896387	17.05673	0.048021	0.281537	Temperature Herbicide Dosage Temp x Dos Herb x dosage Temp x herb Tempxherbxdos	0.0242 NS NS NS 0.2083 NS 0.1611	<.0001 0.1352 0.9809 0.5486 0.0351 0.3368 0.0346
Dry mass roots	0.549239	20.19715	0.007033	0.034821	Temperature Herbicide Dosage Temp x Dos Herb x dosage Temp x herb Tempxherbxdos	0.0035 0.0054 NS NS NS NS NS	<.0001 0.0012 0.5574 0.4977 0.3011 0.3233 0.2050

^{*}Root MSE = Root Mean Squared Error;

^{**}LSD = Least significant difference;

^{***}NS = Non significant



Appendix A Table 5 ANOVA data of Trial 4: The effect of application timing and pre-emergence herbicide treatment on kenaf

Variable	R-Square	Coefficient of Variance	Root MSE*	Mean	Source	MSD**	PR > F
					Time	NS***	0.0539
					Herbicide	NS	0.1067
					Dosage	NS	0.0551
Emergence	0.360231	18.07445	0.881383	4.876404	Timexdos	NS	0.4447
					Herbxdos	NS	0.3528
					TimexHerb	NS	0.6684
					Timexherbxdos	NS	0.9174
					Time	NS	0.1940
					Herbicide	NS	0.3022
Kenaf plant					Dosage	NS	0.0971
height	0.432036	12.85990	1.426148	11.08989	Timexdos	NS	0.1936
ricigitt					Herbxdos	NS	0.3812
					TimexHerb	NS	0.1736
					Timexherbxdos	NS	0.1328
					Time	NS	0.3237
					Herbicide	NS	0.2288
Kenaf plant					Dosage	0.3221	<.0001
injury	0.778185	69.65170	0.759125	1.089888	Timexdos	NS	0.3237
ii ijui y					Herbxdos	NS	0.2288
					TimexHerb	NS	0.7049
					Timexherbxdos	NS	0.7049
					Time	NS	0.4878
					Herbicide	NS	0.1048
Fresh top					Dosage	NS	0.4746
mass	0.375488	19.38761	0.223848	1.154596	Timexdos	0.117	0.0185
111055					Herbxdos	NS	0.5438
					TimexHerb	NS	0.8356
					Timexherbxdos	NS	0.2895
					Time	NS	0.1585
					Herbicide	NS	0.1599
					Dosage	NS	0.5895
Dry top mass	0.460766	15.45167	0.029773	0.192685	Timexdos	NS	0.1929
					Herbxdos	NS	0.0832
					TimexHerb	NS	0.3536
					Timexherbxdos	NS	0.0574
					Time	NS	0.1590
					Herbicide	NS	0.2343
Dry mass					Dosage	NS	0.8811
Dry mass	0.363851	21.70487	0.005565	0.025640	Timexdos	NS	0.4315
roots					Herbxdos	NS	0.1356
					TimexHerb	NS	0.9120
					Timexherbxdos	NS	0.1646

^{*}Root MSE = Root Mean Squared Error;

^{**}LSD = Least significant difference;

^{***}NS = Non significant

APPENDIX B

SUMMARY OF THE MODE OF ACTION OF HERBICIDES

Appendix B Table 1 The mode of action of pre-emergence herbicides used in Trial 1, 2, 3 and 4

Active ingredient	Trade name	Chemical family	Mode of action
S-dimethenamid	Frontier- Optima [®]	Chloroacetamide	Absorbed via the coleoptile the moment the young seedling breaks through the soil. It is also taken up through the roots of annual one-seed or two seed lobed weeds. Biochemistry: it is not yet that clearly understood, but the main belief is that the herbicide induces an inhibition of very long chain fatty acid synthesis.
imazethapyr	Pursuit [®] Hammer [®]	Imidazolinone	It is a systemic herbicide, absorbed by the roots and foliage, with translocation in the xylem and phloem, and accumulation in the meristematic regions. Biochemistry: Imazethapyr is a branched chain amino acid inhibitor. It therefore reduces the plant's levels of valine, leucine and isoleucine, which leads to a disruption of protein and DNA synthesis. Selectivity in soy beans and peanuts is attributed to rapid detoxification via hydroxylation and glycosylation (Tecle et al.1993).
fluometuron (urea-derivative) prometryn (triazine)	Cotogard [®]	Phenylurea, substituted urea, or urea	Belongs to Group C herbicides and is an inhibitor of photosynthesis at the photosystem II part of mostly cotolydenous plant species. Death occurs from oxydation of proteins and lipids. (Langham <i>et al.</i> 2007) It is readily absorbed by roots after soil application and translocated via the apoplast (including xylem) to the shoots. It is not as well absorbed when applied to the foliage and is not properly translocated from the treated leaf via the phloem. Biochemistry: fluometuron undergoes successive <i>N</i> -demethylation as the primary detoxification process in plants. Competitive or subsequent hydroxylation may occur allowing the formation of sugar conjugates. Rapid metabolism is an important means of selectivity in tolerant plant species
pendimethalin	Prowl [®] Stomp [®]	Dinitroaniline	When applied as a pre-emergence solution it is absorbed by the roots and coleoptiles where most of the kenaf plant injury is done. The mitosis of the cells at the ends of the roots is affected.

Active ingredient	Trade name	Chemical family	Mode of action
metolachlor	Dual II Magnum [®]	Chloroacetamide	Inhibits the biosynthesis of several plant components such as fatty acids, lipids, proteins, isoprenoids, and flavonoids. It is absorbed by emerging shoots (grass coleoptile, broadleaved hypocotyl or epicotyl). Some root absorption occurs also. Plants beyond the seedling stage can absorb into roots and translocate to the shoots through the xylem and phloem and can accumulate in the vegetative parts and less in the reproductive parts. However, it is phytotoxic only to emerging weed seedlings. Biochemistry: it is a chloroacetanilide herbicide that inhibits the biosynthesis of several plant components like fatty acids, lipids, proteins, isoprenoids and flavonoids. The conjugation of acetyl coenzyme A seems to be involved (Vencill et al. 2002)

From: http://www.hracglobal.com/Publications/ClassificationofHerbicideModeofAction/tabid/222/Default.aspx

Appendix B Table 2 The mode of action of post-emergence herbicides used in Trial 1

Active ingredient	Trade name	Chemical family	Mode of action
Bentazone	Basagran [®]	Benzothiadiazole	PSII inhibitor because it inhibits the photosynthetic photosystem II. PSII inhibitors act by preventing the transfer of electrons during photosynthesis. The inhibition blocks photosynthesis, the fixation of CO ² and the production of ATP or NADPH. Plant death is caused by the production of free radical species which are able to initiate lipid peroxidation, and eventually cell death. (Silverman <i>et al.</i> 2004) Basically the metabolism of the plants is severely affected (LeBaron and Gressel 1982, Corbett 1994). Biochemistry: bentazone is a salt of organic acids
2,4-DB	2,4-DB	Phenoxybutyric	Systemic herbicide that is translocated in the plant's vascular system. This is the vehicle of transport also for the plant's nutrients and water. Systemic herbicides act slower, generally over a period of days (Unruh <i>et al.</i> 2004). <u>Biochemistry:</u> it is not yet fully understood but it appears that these compounds affect cell wall plasticity nucleic acid metabolism. 2,4-DB must first be converted to 2,4-D which acidifies the cell wall by stimulating a membrane-bound ATPase proton pump. The reduction in apoplasmic pH induces cell elongation by increasing the activity of enzymes responsible for cell wall loosening (Vencill <i>et al.</i> 2002).
monosodium methanearsonate	MSMA [®]	Organic arsenical	Not well understood. The rapid desiccation indicates cell membrane destruction <u>Biochemistry:</u> the herbicide is readily absorbed by the foliage and translocated in the symplast, as well as the apoplast. There is little translocation to the shoots following root absorption from nutrient solution (Vencill <i>et al.</i> 2002)
pyrithiobac sodium	Staple [®]	Pyrimidinylthiobenzoic acid	Inhibition of acetolactate synthase ALS (acetohydroxyacid synthase AHAS). Fairly new herbicide

From: http://www.hracglobal.com/Publications/ClassificationofHerbicideModeofAction/tabid/222/Default.aspx

Appendix B Table 3 Dilution table for the pre-emergence herbicides used in Trial 1, 2, 3 and 4

Product	Prescribed Rate/ha	Rate/m²	Dilute with H ² O	Step 1	=	Step 2	=	Step 3	=
Herbicide A: S-dimethenamid	750 ml	0.075ml	7.5ml/100ml		0.75ml/ 100ml		0.075ml Add 40ml H ² O and apply		
Herbicide B: imazethapyr	200ml	0.02ml	20ml/100ml		2ml/100ml		0.2ml/ 100ml		0.02ml Add 40ml H²O and apply
Herbicide C: fluometuron/ prometryn	21	0.2ml	20ml/100ml	Take 10ml of solution	2ml/ 100ml	Take 10ml of solution	0.2ml Add 40ml H ² O and Apply	Take 10ml of solution	
Herbicide D: pendimethalin	21	0.2ml	20ml/100ml	and add 90ml of H ² O	2ml/ 100ml	and add 90ml of H ² O	0.2ml Add 40ml H ² O and Apply	and add 90ml of H ² O	
Herbicide E: S-metolachlor	500ml	0.05ml	5ml/100ml		0.5ml/ 100ml		0.05ml Add 40ml H²O and Apply		
Herbicide F: imazethapyr + S-metolachlor	200ml+ 500ml	0.02ml+ 0.05ml	2ml/100ml+ 5ml/100ml		0.2ml/ 100ml + 0.5 ml/ 100ml		0.02ml +0.05ml Add 40ml H ² O and apply		

Appendix B Table 4 Dilution table used for post-emergence herbicides used in Trial 1

Product	Prescribed Rate/ha	Rate/m²	Dilute with H ² O	Step 1	=	Step 2	=	Step 3	=
Herbicide A: bentazone	21	0.2ml	20ml/100ml		2ml/100ml		0.2ml Add 40ml H²O and apply		
Herbicide B: 2,4 DB	80ml	0.08ml	80ml/100ml	Take 10ml of solution	8ml/100ml	Take 10ml of solution	0.8ml/100ml	Take 10ml of solution	0.08ml Add 40ml H ² O and apply
Herbicide C: monosodium methanearsonate	31	0.3ml	30ml/100ml	and add 90ml of H²O	3ml/100ml	and add 90ml of H²O	0.3ml Add 40ml H²O and apply	and add 90ml of H²O	
Herbicide D: pyrithiobac sodium	60g	0.006g	6g/100ml		0.6g/100ml		0.06g Add 40ml H²O and apply		0.006g Add 40ml H²O and apply



APPENDIX C SUPPLEMENTARY TABLES

Appendix C Table 1 Trial 1: Kenaf seedling emergence count expressed as a percentage

		Dosage		
	Recommended	Recommended	Recommended	•
	rate	rate x2	rate x3	Herbicide
Herbicide		Emergence (%)		mean
S-dimethenamid	83.3	87.5	87.5	86.1
imazethapyr	87.5	75.0	87.5	83.3
fluometuron/prometryn	91.7	95.8	91.7	93.1
pendimethalin	87.5	62.5	75.0	75.0
S-metolachlor	75.0	83.3	87.5	81.9
imazethapyr+S- metolachlor	83.3	95.8	95.8	91.7
Dosage mean	84.7	83.3	87.5	85.2
Control	84.02			

Appendix C Table 2 Trial 1: Effects of pre-emergence herbicides on kenaf plant height

		Dosage						
	Recommended	Recommended	Recommended					
	rate	rate x2	rate x3	Herbicide				
Herbicide		Plant height (cm)		mean				
S-dimethenamid	9.3bcd	6.3de	8.0cde	7.87				
imazethapyr	17.8a*	17.3ab	16.3abc	17.13				
fluometuron/prometryn	16.5ab	6.0de	0.0e	7.50				
pendimethalin	16.3abc	18.0a	19.0a	17.77				
S-metolachlor	16.3abc	15.3abc	15.5abc	15.70				
imazethapyr+S- metolachlor	16.8ab	15.0abc	14.3abcd	15.37				
Dosage mean	15.50	12.98	12.18	_				

^{*} Treatment means with the same letter does not differ significantly from each other (P<0.001)

Appendix C Table 3 Trial 1: Effects of pre-emergence herbicides on plant health

		Dosage		
	Recommended	Recommended	Recommended	Herbicide
	rate	rate x2	rate x3	mean
Herbicide		Plant injury(0-5*)		
S-dimethenamid	3.3ab	3.5ab	3.5ab	3.43
imazethapyr	0.8de	1cde	1.3cde	1.03
fluometuron/prometryn	2.3bcd	2.8abc	4.5a**	3.20
pendimethalin	1.3cde	0.5de	0.5de	0.77
S-metolachlor	0.5de	0.3e	0.5de	0.43
imazethapyr+S- metolachlor	0.8de	0e	0.3e	0.37
Dosage mean	1.5	1.35	1.77	

^{*} Injury ratings between 0 and 5 indicated the following levels of injury:

- 0 = no injury visible
- 1 = slight injury visible
- 2 = medium injury visible
- 3 = all plants show medium signs of injury
- 4 = severe injury visible

Appendix C Table 4 Trail 1: Weed control for grass species in kenaf

Herbicide	Recommended	Recommended	Recommended	Herbicide
Herbiciae	rate	rate x2	rate x3	mean
	Control	of grass weed sp	ecies %	
S-dimethenamid	100a	100a	100a	100
imazethapyr	100a	97.6a	100a	99.2
fluometuron/prometryn	100a	100a	100a	100
pendimethalin	100a	100a	100a	100
S-metolachlor	100a	100a	100a	100
imazethapyr+S-	1000	100a	1000	100
metolachlor	100a	100a	100a	100
Dosage mean	100	99.6	100	

⁵⁼ senescence of seedlings

**Treatment means with the same letter does not differ significantly from each other (P<0.001)



Appendix C Table 5 Trail 1: Weed control for broadleaved species in kenaf

		Dosage		
Herbicide	Recommended	Recommended	Recommended	Herbicide
пеныстае	rate	rate x2	rate x3	mean
	Control of	oroadleaved weed	l species %	
S-dimethenamid	97.8a*	100a	97.8a	98.5
imazethapyr	79.4abc	89.0ab	79.4abc	82.6
fluometuron/prometryn	100a	100a	100a	100
pendimethalin	74.2bc	66.9c	86.7abc	76
S-metolachlor	92.6ab	96.3a	100a	96.3
imazethapyr+S-	97.8a	07.00	1000	00 5
metolachlor	91.0a	97.8a	100a	98.5
Dosage mean	90.3	91.7	94	

^{*} Treatment means with the same letter does not differ significantly from each other (P<0.001)

Appendix C Table 6 Trial 1: The interaction between herbicide and dosage for kenaf fresh top growth mass

Herbicide	Recommended	Recommended	Recommended	Herbicide
пегысіче	rate	rate x2	rate x3	mean
	Mass	of fresh top grow	th (g)	
S-dimethenamid	1.3abc	0.94bc	0.94bc	1.06
imazethapyr	1.83a*	1.64ab	1.63ab	1.7
fluometuron/prometryn	1.12abc	0.61cd	0d	0.58
pendimethalin	1.44ab	1.82a	1.61ab	1.62
S-metolachlor	1.58ab	1.57ab	1.54ab	1.56
imazethapyr+S-	1.71a	1 FGob	1.3abc	1 50
metolachlor	1./1a	1.56ab	1.3abc	1.52
Dosage mean	1.50	1.36	1.17	

^{*} Treatment means with the same letter does not differ significantly from each other (P<0.001)

Appendix C Table 7 Trial 1: The interaction between herbicide and dosage for kenaf dry top growth mass

		Dosage				
Herbicide	Recommended	Recommended	Recommended	Herbicide		
пенысіце	rate	rate x2	rate x3	mean		
	Mas	Mass of dry top growth (g)				
S-dimethenamid	0.206ab	0.178abc	0.194ab	0.193		
imazethapyr	0.277a*	0.237ab	0.24ab	0.25		
fluometuron/prometryn	0.138bc	0.075cd	0d	0.071		
pendimethalin	0.219ab	0.254a	0.245ab	0.239		
S-metolachlor	0.236ab	0.235ab	0.245ab	0.238		
imazethapyr+S- metolachlor	0.255a	0.246ab	0.211ab	0.237		
Dosage mean	0.222	0.204	0.189			

^{*} Treatment means with the same letter does not differ significantly from each other (P<0.001)



Appendix C Table 8 Trial 1: The interaction between herbicide and dosage for kenaf dry root mass

Herbicide	Recommended	Recommended	Recommended	Herbicide
i lei bicide	rate	rate x2	rate x3	mean
	N	lass of dry roots (g)	
S-dimethenamid	0.021bcd	0.016cde	0.013de	0.017
imazethapyr	0.0343ab	0.038ab	0.035ab	0.036
fluometuron/prometryn	0.02bcd	0.013de	0e	0.011
pendimethalin	0.034abc	0.043a*	0.034abc	0.037
S-metolachlor	0.029abcd	0.037ab	0.032abc	0.033
imazethapyr+S-	0.038ab	0.035ab	0.03abcd	0.034
metolachlor	0.036ab	0.035ab	0.03abca	0.034
Dosage mean	0.030	0.030	0.024	

^{*} Treatment means with the same letter does not differ significantly from each other (P<0.001)

Appendix C Table 9 Trial 1: The emergence count expressed as a percentage

		Dosage		
	Recommended	Recommended	Recommended	•
	rate	rate x2	rate x3	Herbicide
Herbicide		Emergence (%)		mean
bentazone	79.2	70.8	91.7	80.6
2,4D-B	75.0	91.7	83.3	83.3
monosodium				
methanearsonate	91.7	75.0	91.7	86.1
pyrithiobac sodium	83.3	91.7	83.3	86.1
Dosage mean	82.3	82.3	87.5	
Control	83.3			

Appendix C Table 10 Trial 1: Significant differences in the interaction of post-emergence herbicide x dosage on kenaf plant health

		Dosage				
Herbicide	Recommended	Recommended	Recommended	Herbicide		
пегысіце	rate	rate x2	rate x3	mean		
	-	Plant injury (0-5)*				
bentazone	1.5e	2.3de	2.8cd	2.2		
2,4D-B	3bcd	3.8abc	2.8cd	3.2		
monosodium methanearsonate	2.3de	2.8cd	2.8cd	2.6		
pyrithiobac sodium	4.3a	4.0ba	4.0ba	4.1		
Dosage mean	2.8	3.2	3.1			

^{*} Injury ratings between 0 and 5 indicated the following levels of injury:

^{0 =} no injury visible

^{1 =} slight injury visible

^{2 =} medium injury visible

^{3 =} all plants show medium signs of injury

^{4 =} severe injury visible

⁵⁼ senescence of seedlings



Appendix C Table 11 Trial 1: Significant differences in the post-emergence herbicide ${\bf x}$ dosage interaction on grass weed control in kenaf

		Dosage			
Herbicide	Recommended	Recommended	Recommended	Herbicide	
пеннице	rate	rate x2	rate x3	mean	
	Effective	eness of weed cor	ntrol (%)		
bentazone	52.5abc	56.3ab	65.0ab	57.9	
2,4D-B	21.3bcd	27.5bcd	31.3bcd	26.7	
monosodium methanearsonate	5.0cd	23.8bcd	25.0bcd	17.9	
pyrithiobac sodium	90.0a*	90.0a	95.0a	91.7	
Dosage mean	42.2	49.4	54.1		

^{*} Treatment means with the same letter does not differ significantly from each other (P<0.001)

Appendix C Table 12 Trial 1: Significant differences in the herbicide x dosage interaction on broadleaved weed control in kenaf

		Dosage			
Herbicide	Recommended	Recommended	Recommended	Herbicide	
nerbicide	rate	rate x2	rate x3	mean	
	Effectiv	eness of weed cor	ntrol (%)		
bentazone	52.5bc	73.8ab	85.0a	70.4	
2,4D-B	21.3ef	27.5de	31.3ced	26.7	
monosodium methanearsonate	15.0ef	36.3ced	51.3bcd	34.2	
pyrithiobac sodium	90.0a*	97.5a	97.5a	95.0	
Dosage mean	44.7	58.8	66.3		

^{*} Treatment means with the same letter does not differ significantly from each other (P<0.001)



Appendix C Table 13 Trial 2: Kenaf seedling emergence count expressed as a percentage

			Dosage		
		Recommended	Recommended	Recommended	
Soil		rate	rate x2	rate x3	Herbicide
depth	Herbicide		Emergence (%)		mean
	S-dimethenamid	94.5	88.8	83.3	88.9
	imazethapyr	66.7	100.0	100.0	88.9
	pendimethalin	83.3	83.3	88.8	85.2
2.5 cm	S-metolachlor	88.8	94.5	94.5	92.6
2.3 CIII	imazethapyr+S- metolachlor	94.5	83.3	100.0	92.6
	Dosage mean	94.5	88.8	83.3	88.9
	Control	83.3			
	S-dimethenamid	94.5	77.8	72.2	81.5
	imazethapyr	94.5	77.8	88.8	87.1
	pendimethalin	72.2	83.3	77.8	77.8
5 cm	S-metolachlor	72.2	77.8	88.8	79.6
5 CM	imazethapyr+S- metolachlor	83.3	100.0	77.8	87.1
	Dosage mean	94.5	77.8	72.2	81.5
	Control	88.0			

Appendix C Table 14 Trial 2: Average kenaf plant height

			Dosage		
		Recommended	Recommended	Recommended	
Soil		rate	rate x2	rate x3	Herbicide
depth	Herbicide		Plant Height (cm)		mean
	S-dimethenamid	18.0	17.0	17.3	17.4
	imazethapyr	17.7	19.3	17.3	18.1
	pendimethalin	20.7	20.7	20.0	20.5
2.5 cm	S-metolachlor	19.3	20.0	16.0	18.4
2.5 (111	imazethapyr+S-	40.0	17.7	16.7	17.5
	metolachlor	18.0	17.7	10.7	17.5
	Dosage mean	18.7	18.9	17.5	
	Control	20.2			
	S-dimethenamid	18.7	18.0	17.7	18.1
	imazethapyr	19.3	19.3	17.0	18.5
	pendimethalin	18.3	21.7	19.0	19.7
5 cm	S-metolachlor	21.0	18.3	20.3	19.9
5 CIII	imazethapyr+S-	19.7	18.0	16.3	18.0
	metolachlor	19.7	16.0	10.3	16.0
	Dosage mean	19.4	19.1	18.1	
	Control	20.1			



Appendix C Table 15 Trial 3: Kenaf seedling injury rating as affected by temperature and herbicide. (Data averaged over application rates)

	Temperature			I I a what at all a	
Herbicide	Low	Med	High	Herbicide - mean	
	Injury Rating (0 – 5)**				
-dimethenamid	1.67ab	1.83ab	0.83bc	1.44	
imazethapyr	1.50abc	1.00bc	0.67bc	1.06	
pendimethalin	2.33a*	0.67bc	0.17bc	1.06	
S-metolachlor	2.33a	2.00ab	0.33bc	1.56	
imazethapyr+ S- metolachlor	2.00ab	1.83ab	0.33bc	1.39	
Temp Mean	1.97	1.47	0.47		
Control	0.0c				

^{*} Treatment means with the same letter does not differ significantly from each other (P<0.001)

- 0 = no injury visible
- 1 = slight injury visible
- 2 = medium injury visible
- 3 = all plants show medium signs of injury
- 4 = severe injury visible
- 5= senescence of seedlings

Appendix C Table 16 Trial 3: significant effects of herbicide treatment on kenaf dry root mass

Herbicide		— Herbicide mean	
ricibiolac	Dry root mass (gram)		
S-dimethenamid		0.0287c	
imazethapyr		0.0320bc	
pendimethalin		0.0377ab	
S-metolachlor		0.0357ab	
imazethapyr+S-metolachlor		0.0390a*	
Control		0.0353abc	

 $^{^{\}star}$ Treatment means with the same letter does not differ significantly from each other (P<0.001)

^{**} Injury ratings between 0 and 5 indicated the following levels of injury: