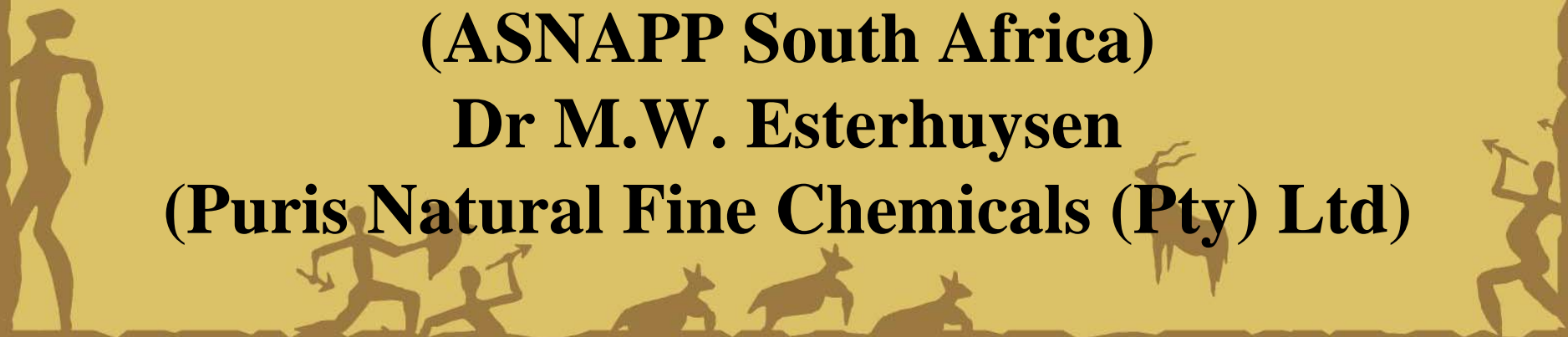


Plant nutritional needs of Buchu (*Agathosma betulina*):

Effect of nutrient solution concentration on plant growth and essential oil quality

Dr Petrus Langenhoven
(ASNAPP South Africa)

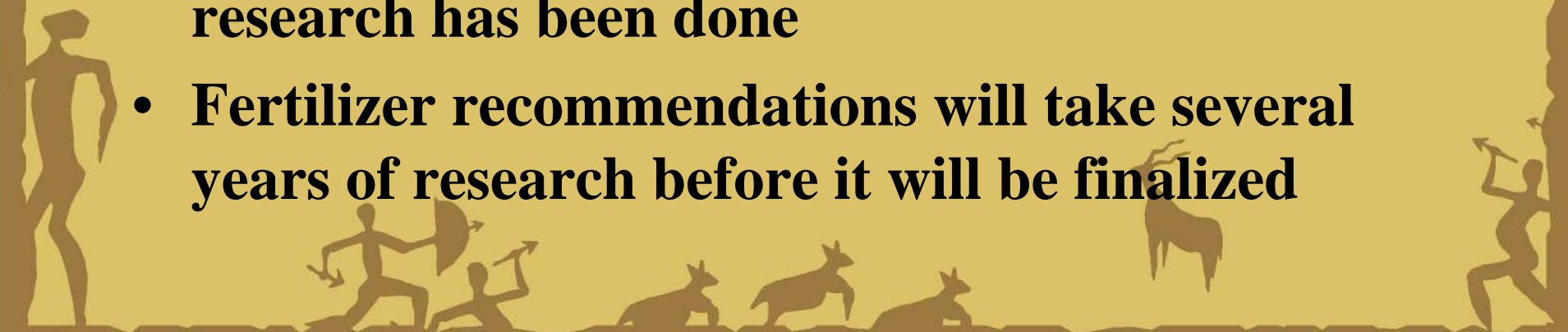
Dr M.W. Esterhuysen
(Puris Natural Fine Chemicals (Pty) Ltd)



Introduction



- **This crop actually needs no introduction**
- **High value and high in demand**
- **However yield can be optimized if fertilizer is applied at optimum levels and at the rite time**
- **No clear guidelines available**
- **Relatively new crop – very little agronomic research has been done**
- **Fertilizer recommendations will take several years of research before it will be finalized**



Material and Method



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- Naturally ventilated greenhouse
- Hydroponic techniques applied to test hypothesis
- Soil-less substrate used – graded sand
- Nutrient solution
- Treatments
 - Five EC (mS.cm^{-1}) levels were used:
 - 0.8
 - 1.1
 - 1.4
 - 1.7
 - 2.0
- Replicates: 5
- Plants per treatment: 10



Material and Method Cont.



- **Seedlings obtained from Piketberg**
- **Pot size & Growth medium**
 - 5L white pot
 - Grade 1 sand obtained from Consol
- **Irrigation:**
 - Netafim button dripper
 - 6 to 9 x per day
 - 50 to 100ml irrigated per cycle. Drainage % was exceptionally high and therefore the high irrigation volume
- **Nutrient solution**
 - Macro elements – solution as used by Mr Harris
 - Micro nutrients based on Steiner solution
 - Plants received nutrition with every irrigation

Nutrient Solution



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Cations						
	Na^+	NH_4^+	K^+	Ca^{++}	Mg^{++}	H^+
me.l ⁻¹	0.27	0.7	3.31	2.45	1.54	
ppm	6	10	129	49	18	

Anions						
	OH^-	NO_3^-	H_2PO_4^-	$\text{SO}_4^{=}$	Cl^-	HCO_3^-
me.l ⁻¹		5.51	0.7	1.44	0.57	0.14
ppm		77	22	23	20	

Material and Method Cont.



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- **Plant date: 26 September 2003**
- **Harvest dates:**
 - July 2004 (10 months after planting)
 - April 2005 (9 months later)
- **Data collected:**
 - EC/pH values
 - Irrigation water
 - Drainage water
 - Biomass produced
 - Oil yield & quality

Photo: 13 Oct 2003



Material and Method Cont.



- **Distillation:**
 - Clevenger steam extraction
 - Extraction time was 2 hrs from the time steam has started to form
- **Analysis:**
 - Gas chromatography
 - Results are reported on in terms of relative percentages of the total composition, obtained from the integrated peak areas on the chromatogram
 - When diosphenol crystallizes, it is heated to $\sim 50^{\circ}\text{C}$ before the GC analysis is executed

Preliminary Results



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	Cations (me/L)							
	pH	EC	Na ⁺	NH ₄ ⁺	K ⁺	Ca ⁺⁺	Mg ⁺⁺	H ⁺
Municipal water	8.7	0.07	0.21	0.00	0.01	0.25	0.04	
EC = 0.8	6.7	0.76	0.28	0.87	2.78	1.78	1.22	
EC = 1.1	6.6	1.00	0.29	1.29	3.79	2.36	1.61	
EC = 1.4	6.4	1.33	0.33	1.68	5.15	2.97	2.11	
EC = 1.7	6.2	1.60	0.35	2.03	6.06	3.69	2.59	
EC = 2.0	6.2	2.00	0.40	2.63	7.83	4.97	3.11	

Preliminary Results



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	Anions (me/L)					
	OH ⁻	NO ₃ ⁻	H ₂ PO ₄ ⁻	SO ₄ ⁼	Cl ⁻	HCO ₃ ⁻
Municipal water		0.01	0.00	0.01	0.34	0.42
EC = 0.8		4.53	0.45	1.14	0.39	0.50
EC = 1.1		6.61	0.58	1.52	0.40	0.51
EC = 1.4		8.74	0.79	2.05	0.43	0.55
EC = 1.7		10.68	0.92	2.42	0.45	0.54
EC = 2.0		14.42	1.12	2.94	0.53	0.57

Preliminary Results



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Treatment	pH	EC
EC = 0.8 mS.cm ⁻¹	5.7	0.85
EC = 1.1 mS.cm ⁻¹	5.5	1.10
EC = 1.4 mS.cm ⁻¹	5.3	1.40
EC = 1.7 mS.cm ⁻¹	5.4	1.69
EC = 2.0 mS.cm ⁻¹	5.3	1.98

Results: Yield



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	EC (mS.cm ⁻¹)				
	0.8	1.1	1.4	1.7	2.0
Oil yield (v/m%)	0.74	0.74	0.68	0.65	0.69
Fresh weight per plant (g)	538.3	514.6	468.4	355.4	255.4
Volume oil per plant (ml)	3.96	3.80	3.18	2.28	1.90

Plant growth



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2 weeks after
planting



20 weeks
after planting



30
weeks
after
planting



40
weeks
after
planting



Plant growth



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Results: Oil Quality



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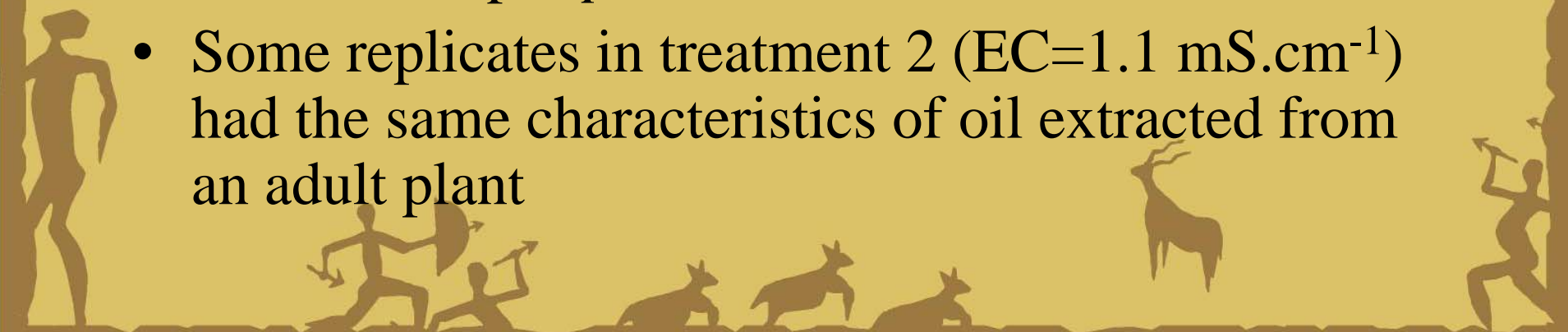
Chemical component	EC (mS.cm ⁻¹)					Accepted range
	0.8	1.1	1.4	1.7	2.0	
Limonene (%)	26.46	24.74	25.32	26.28	24.96	25-30%
Menthone (%)	4.32	4.30	4.10	4.30	3.94	5-10%
Isomenthone (%)	14.20	14.76	13.48	14.10	13.36	15-25%
Pulegone (%)	6.62	12.48	9.26	9.64	11.82	0-5%
psi-Diosphenol (%)	11.48	7.90	10.00	7.42	8.84	8-13%
Diosphenol (%)	13.64	10.38	12.88	11.42	11.00	9-14%
<i>trans</i> -8-Mercapto- <i>p</i> -menthan-3-one (%)	0.50	0.34	0.56	0.58	0.56	0.1-0.4%
<i>cis</i> -8-Mercapto- <i>p</i> -menthan-3-one (%)	6.18	8.24	7.92	7.68	8.56	1.5-2.5%

Oil Quality



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- Analysis shows that oil samples are pure *A. betulina* (Diosphenol high)
- High pulegone concentrations is an indication of plant material age and not hybridization in this case since no *cis/trans*-8-Acetylthio-*p*-mentan-3-one isomers were detected and the ratio of *trans* to *cis*-8-Mercapto-*p*-menthan-3-one isomers was low
- Some replicates in treatment 2 ($EC=1.1 \text{ mS.cm}^{-1}$) had the same characteristics of oil extracted from an adult plant



Oil Quality (19 months)



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Chemical component	EC (mS.cm ⁻¹)					Accepted range	Harvest time
	0.8	1.1	1.4	1.7	2.0		
Isomenthone (%)	14.20	14.76	13.48	14.10	13.36	15-25%	10 months
	21.60	15.40	22.00	20.50	31.00		19 months
Pulegone (%)	6.62	12.48	9.26	9.64	11.82	0-5%	10 months
	6.10	7.50	10.20	9.50	8.80		19 months
psi-diosphenol (%)	11.48	7.90	10.00	7.42	8.84	8-13%	10 months
	9.40	12.50	5.80	8.30	3.90		19 months
Diosphenol (%)	13.64	10.38	12.88	11.42	11.00	9-14%	10 months
	10.90	14.00	6.90	10.00	4.30		19 months

Oil Quality (19 months)



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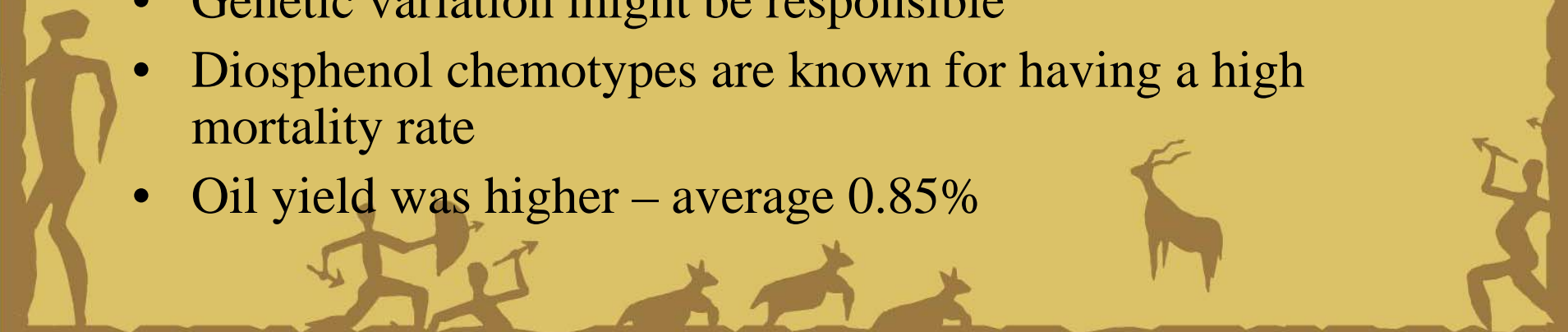
	EC (mS.cm ⁻¹)					Accepted range	Harvest time
Chemical component	0.8	1.1	1.4	1.7	2.0		
<i>trans</i> -8-Mercapto- <i>p</i> - menthan-3-one (%)	0.50	0.34	0.56	0.58	0.56	0.1-0.4%	10 months
	0.40	0.60	0.40	0.40	0.70		19 months
<i>cis</i> -8-Mercapto- <i>p</i> - menthan-3-one (%)	6.18	8.24	7.92	7.68	8.56	1.5-2.5%	10 months
	2.70	3.10	2.80	2.50	5.20		19 months



Oil Quality (19 months)



- Diosphenol was lower with isomenthone higher
- Pulegone slightly lower compared to 1st harvest
- 8-Mercapto-*p*-menthan-3-one concentration also lower compared to first harvest, as a result of plants being older or the vegetative growth stage at that specific time
- Some plants died and as a result it seems that the oil reflects a diosphenol vs. isomenthone chemotype
- Genetic variation might be responsible
- Diosphenol chemotypes are known for having a high mortality rate
- Oil yield was higher – average 0.85%

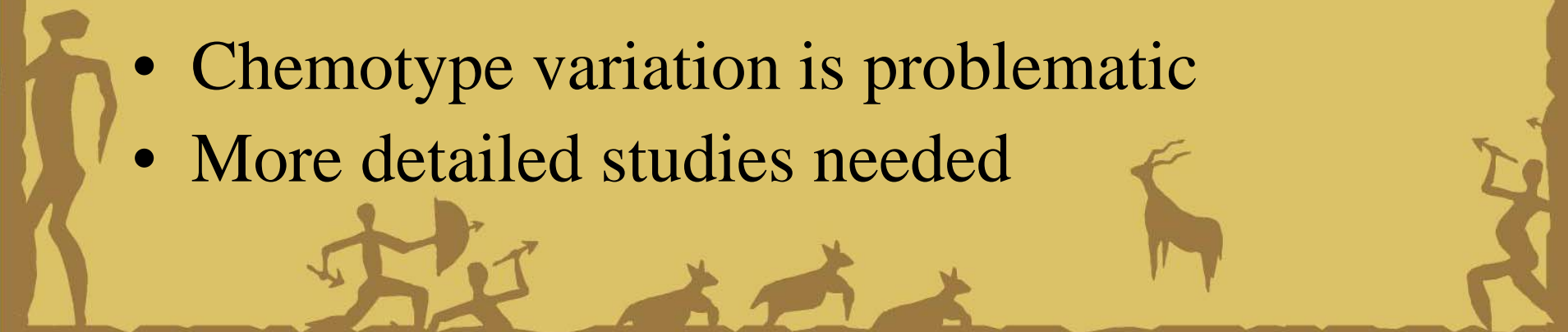


Conclusion



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- EC levels between 0.8 and 1.1 seems to be optimal in terms of biomass accumulation
- Biomass production was exceptionally high (unexpected)
- The oil quality was acceptable, especially at 0.8 mS.cm^{-1}
- Chemotype variation is problematic
- More detailed studies needed



Future Research



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- pH range – in progress
- Nutrient ratio
- Phosphorous tolerance (temperature)
- Time of harvesting in greenhouse setup
- Irrigation scheduling
- etc



Acknowledgements



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