ABSTRACT: 207

THE STATUS OF ROOT-KNOT NEMATODE RESEARCH IN SOUTH AFRICA

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Root-knot nematodes are a constraint to soybean production in South Africa. Parasitism of the crop by these sessile, endoparasitic nematodes result in substantial yield losses. Results of a nematode survey that was conducted at 17 localities in local soybean-producing areas during the mid 1900s indicated that twelve plant-parasitic nematode genera and 15 species were associated with the crop. Root knot nematodes (RKN) were the most common genus found, with *Meloidogyne incognita* race 2 and *M. javanica* being the predominant species. The availability of local soybean cultivars resistant to these RKN species is crucial since no synthetic or biologically-derived nematicides have been registered to date locally for use on the crop. Host plant resistance to RKN is thus currently one of the few cost-effective and environmentally-friendly management strategies for reducing yield losses. Subsequent screening of local and foreign soybean genotypes resulted in several cultivars being identified with resistance to the respective RKN species. However, local cultivar LS5995 exhibited a superior level of resistance to *M. incognita* race 2. LS5995 also consistently expressed resistance under microplot and field conditions since it maintained low population densities of the latter species and race. Furthermore, data on quantitative relationships between soybean crop performance and initial population densities of *M. incognita* race 2 showed that LS5995 has a damage threshold level that is 10 times higher than that of a susceptible standard. According to pre- and post-infection and histological studies resistance in cultivar LS5995 manifested in necrosis as well as inhibited development and reproduction of *M. incognita* race 2 and was identified as post-infection antibiosis resistance. Two molecular markers were subsequently linked to *M. incognita* race 2-resistance in cultivar LS5995 and are still used in a marker-assisted breeding programme (MAS). Following crosses of LS5996 and Prima2000, seven S_7 lines were selected for their acceptable agronomical traits as well as the presence of molecular markers linked to *M. incognita* race 2-resistance. Of these lines, one exhibits superior agronomical and *M. incognita* race 2-resistance traits and will be included in the National Cultivar Trials during 2013/2014. However, inclusion of genetically-modified, Round-Up Ready (RR) soybean cultivars with resistance to RKN are needed since more than 85% of the local market are dominated by RR cultivars.

The status of nematode research on soybean in South Africa

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POTCHEFSTROOM CAMPUS

Timeline of nematology research on soybean in SA:
Nematodes associated with soybean

SAPPNS (ARC-PPRI) Ongoing

2012 18 genera 48 spp.

Fourie *et al*. 2001 3 genera & 12 spp. = new records

Kleynhans *et al*. 1996 8 genera & 11 spp.

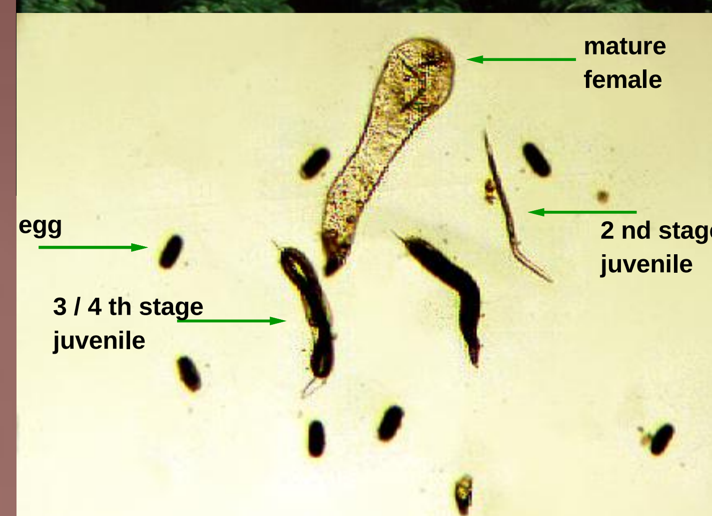
Keetch and Buckley 1984 4 genera & 6 spp.

Coetzee 1968 *M. hapla, M. incoginta, M. javanica*

Van der Linde *et al*. 1959 *Meloidogyne arenaria*

Predominant nematodes in soybean production areas: Official survey

Nematode genus/species	Frequency of occurrence (%)
Meloidogyne spp.	91
Pratylenchus zeae	87
Helicotylenchus dihystera	78
Scutellonema brachyurus	71
P. brachyurus	33

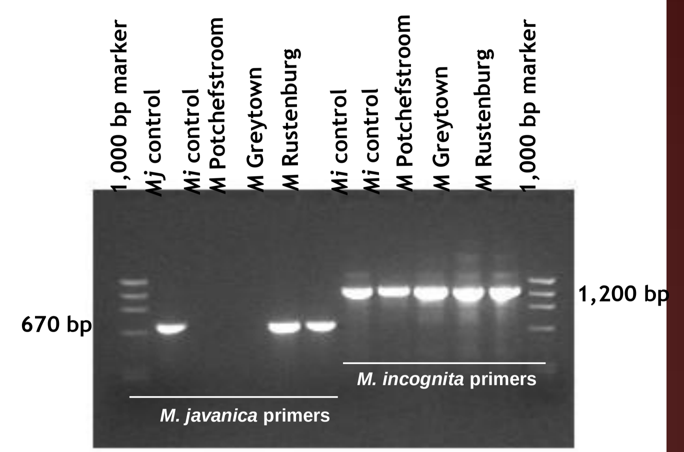


Fourie, H., Mc Donald, A.H. & Loots, G.C. 2001. Plant-parasitic nematodes in field crops in South Africa. 6. Soybean. Nematology, 3:447-454.

Soybean cyst nematode, *H. glycines* not found in SA

Economically important *Meloidogyne* species infecting soybean in SA

Species identification using SCAR-PCR	Yield losses (%)
M. incognita and *M. javanica* Mixture of the two species	12 – 100% (experiments and cultivar trial)



Symptoms:
Root-knot nematode infection

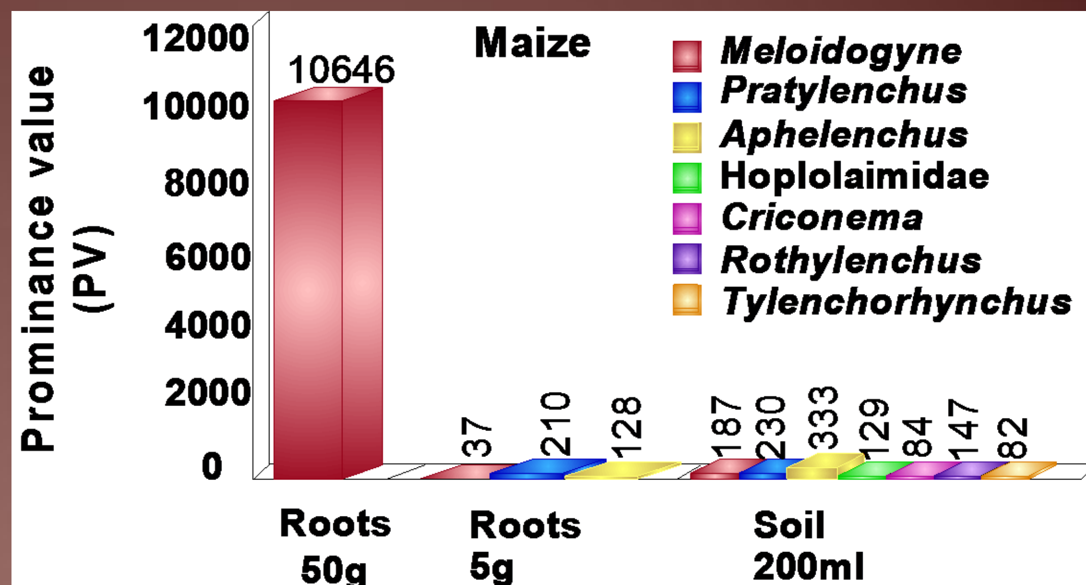

28 January 2013
Diagnostic analysis: NWU
163 334 eggs & J2/50g roots
(P. van Niekerk)


25/04/2007
RKN infected
Uninfected

Yield losses: total destruction

Smit, M.A. & De Beer, G.P. (1998). Report of the national soybean cultivar trials 1998/99. Agricultural Research Council - Oil and Protein Seed Centre, Grain Crops Institute, Agricultural Research Council, Potchefstroom. p. 52.

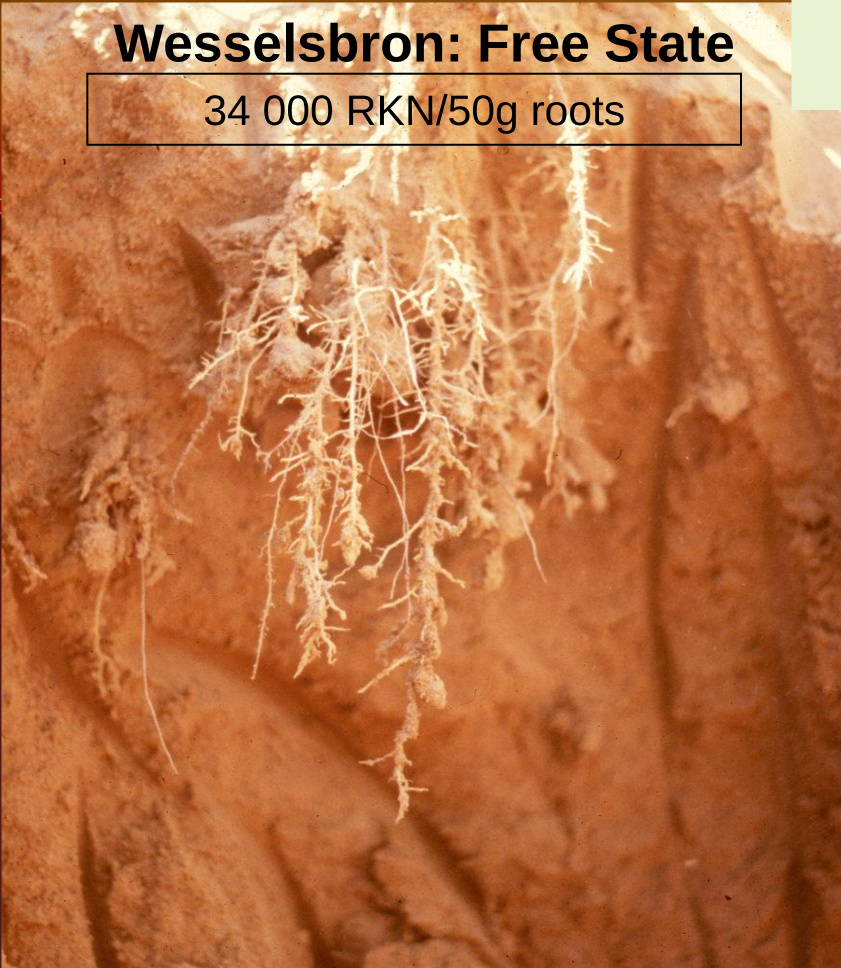
Audit of nematode problems in maize: Diagnostic analyses



Bekker, E. Venter & H. Fourie. 2007. Nematode diagnostic service at the ARC-Grain Crops Institute. 18th NSSA Symposium, Boardwalk Centre, Port Elizabeth, South Africa.

Bekker, S., Mc Donald, A.H., Fourie, H. & Engelbrecht, E. 2011. Prospective extended benefits of plant-nematode diagnostic and advisory services should closer cooperation between laboratories be attainable. 20th NSSA Symposium, Spier Estate, Stellenbosch, South Africa.(

Maize


Wesselsbron: Free State
34 000 RKN/50g roots


Hertzogville: Free State
2011

The root-knot nematode situation in soybean locally = complicated

- At present only one conventional soybean genotype – some level of R to SA populations of *M. incognita*
- All RR cv's available = highly susceptible to RKN
- Increased expansion of soybean plantings into traditional maize production areas
- No nematicide registered on soybean locally
- Crop rotation: complicated - wide host range of RKN
- Producers not always aware of RKN problems

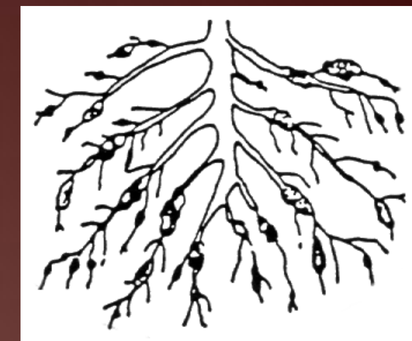
Identification of resistance in cultivars & breeding lines

Venter & Fourie Mbatyoti & Fourie	Ongoing	*M. incoginta* race 2 and *M. javanica*
Fourie *et al.*	2006	*M. incoginta* race 2
Van Biljon	2004	*M. incoginta* races 2 & 4 and *M. javanica*
Fourie *et al.*	1999	*M. incoginta* races 1,2 & 4 and *M. javanica*
Van den Berg & Mc Donald	1991	*M. javanica* and *M. incognita* race 4

Several cv's identified – various levels of resistance to local *Meloidogyne* spp.

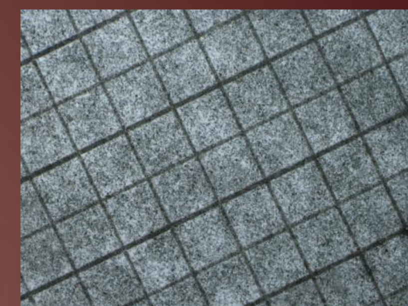
Only Egret still commercially available

Parameters used to identify resistant genotypes



Gall-rating indices

Egg-mass indices &
egg and J2 / root system (Pf)

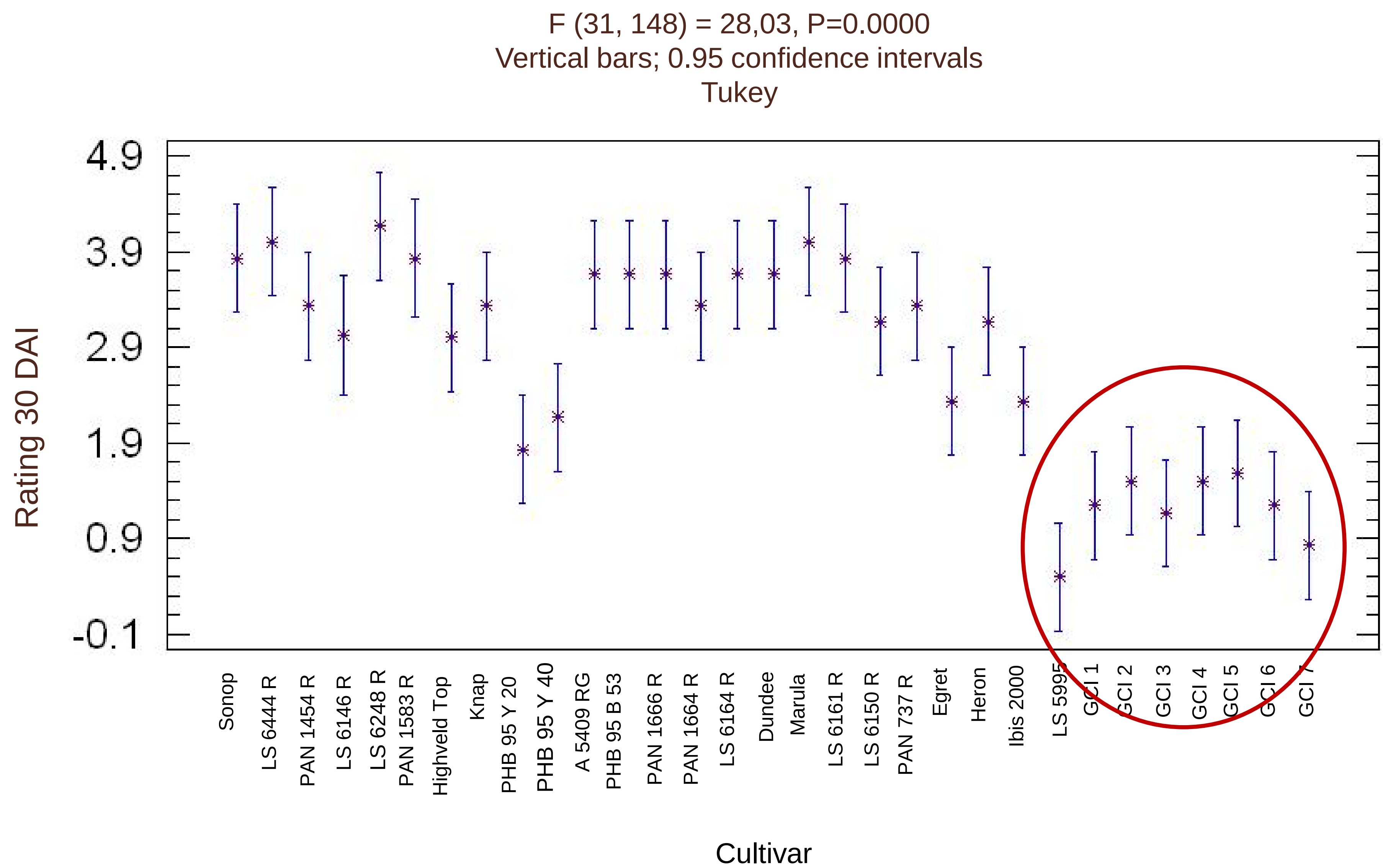


Rf values (Pf/Pi)

RR values (Pf of R cv / Pf of S cv) x 100

Fourie, H., Mc Donald, A.H. & Loots, G.C. 1999. Host suitability of South African commercial soybean cultivars to two root-knot nematode species. *African Plant Protection* (5) 2: 119 – 124.

Fourie, H., Mc Donald, A.H. & De Waele, D. 2006. Host suitability of South African and foreign soybean cultivars to *Meloidogyne incognita* race 2. SA Plant and Soil, 23 (2): 132-137.

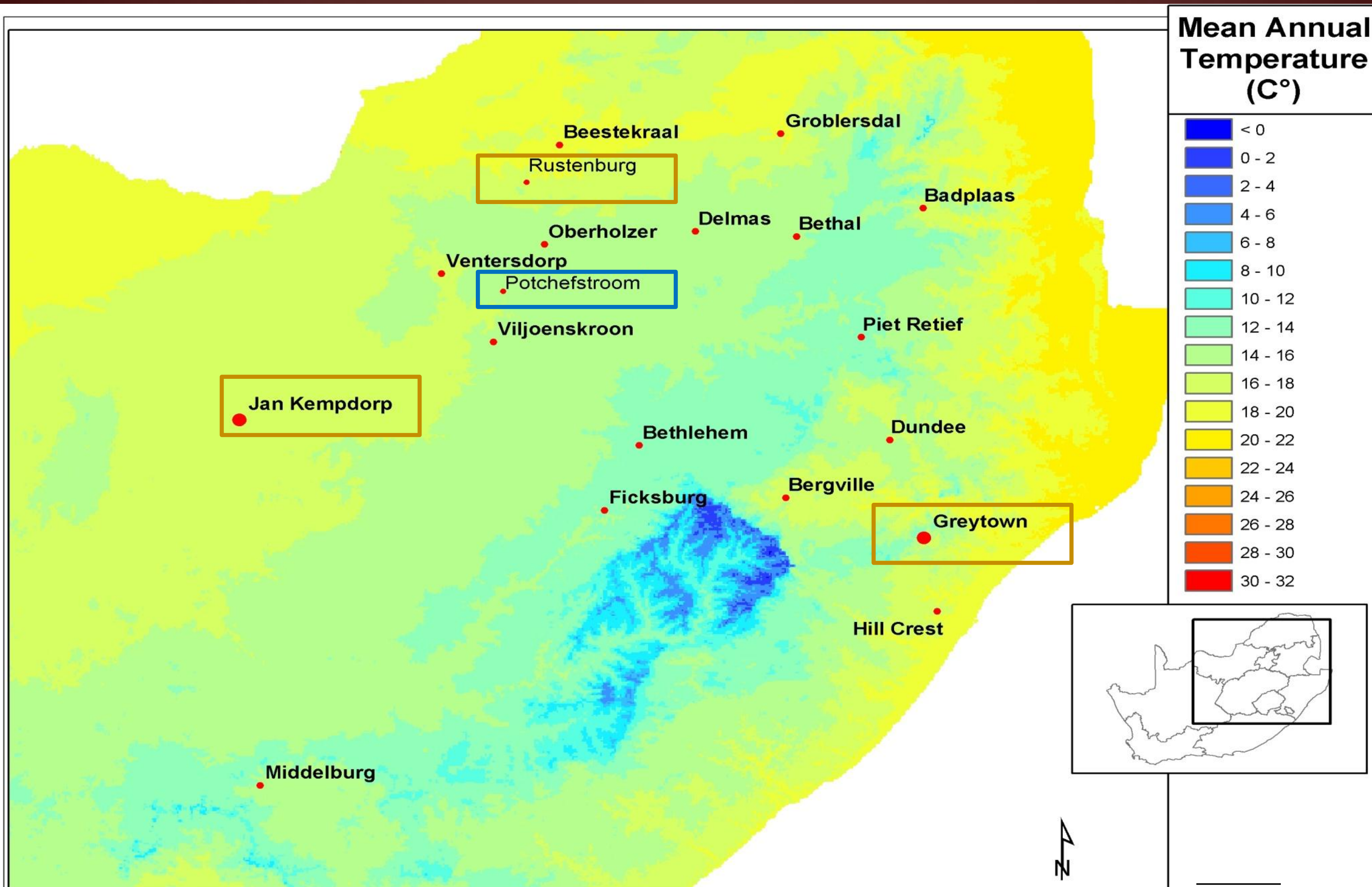

F (31, 148) = 28,03, P=0.0000
Vertical bars; 0.95 confidence intervals
Tukey
Rating 30 DAI
4.9
3.9
2.9
1.9
0.9
-0.1
Sonop
LS 6444 R
PAN 1454 R
LS 6146 R
LS 6248 R
PAN 1583 R
Highveld Top
Knap
PHB 95 Y 20
PHB 95 Y 40
A 5409 RG
PHB 95 B 53
PAN 1666 R
PAN 1664 R
LS 6164 R
Dundee
Marula
LS 6161 R
LS 6150 R
PAN 737 R
Egret
Heron
Ibis 2000
LS 5995
GCI 1
GCI 2
GCI 3
GCI 4
GCI 5
GCI 6
GCI 7
Cultivar

Verification of resistance

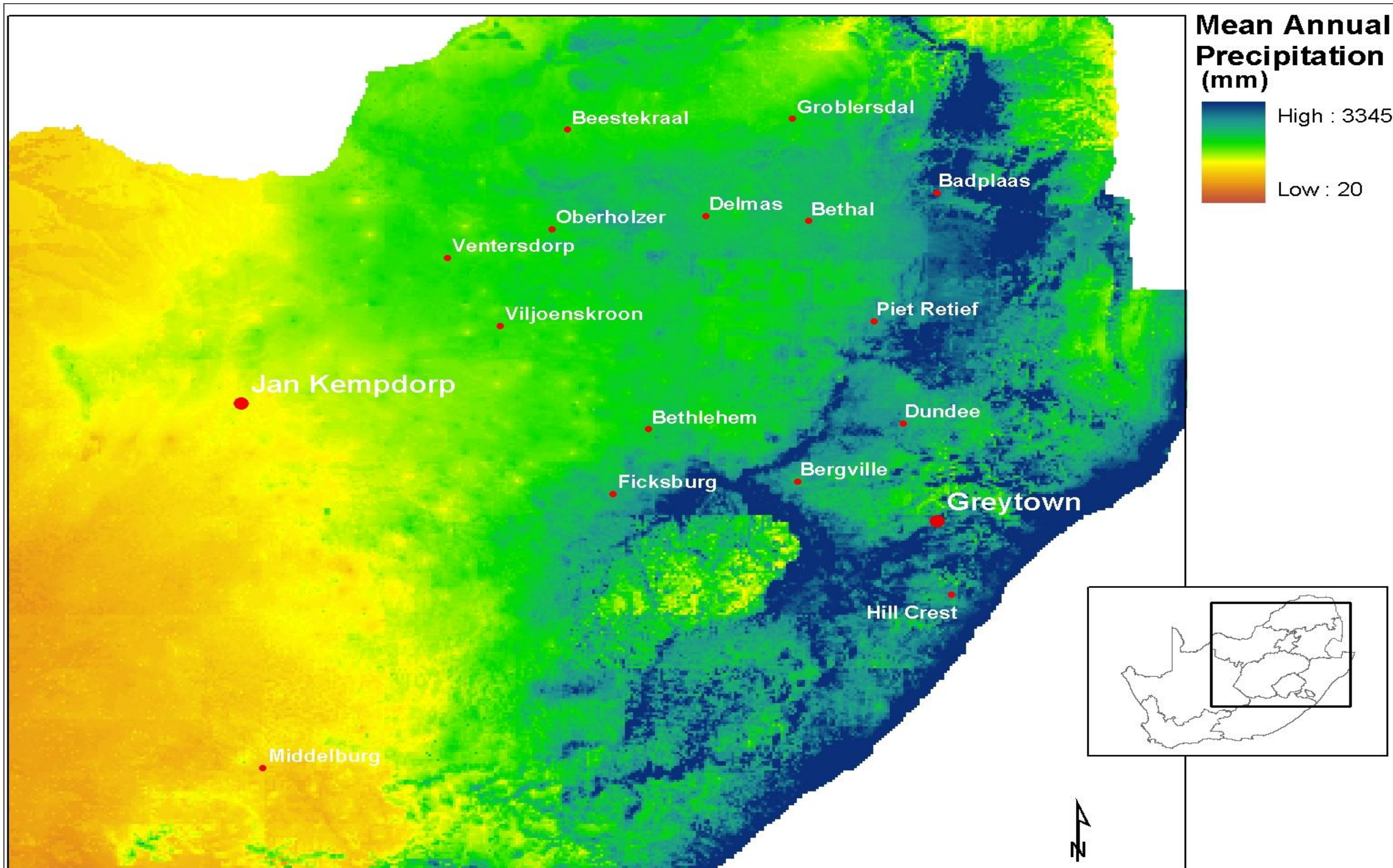
- **Microplot and field experiments**

H. Fourie, A.H. Mc Donald and D. De Waele. *In vivo* host and yield responses of *Meloidogyne incognita*-resistant and susceptible soybean genotypes. International Journal of Pest Management (accepted in January 2013).

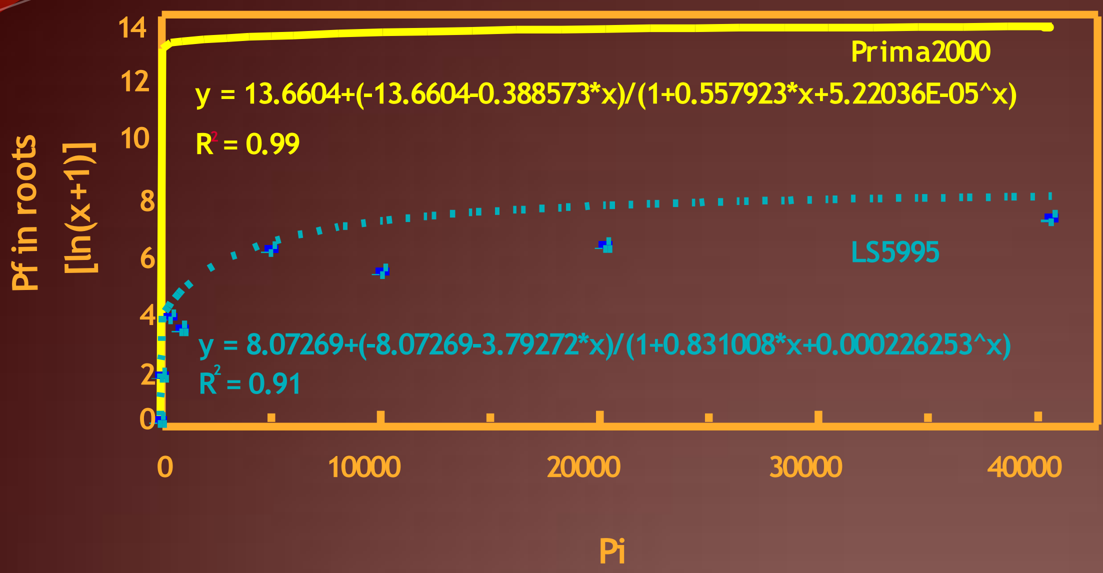
Orientation: Temperature

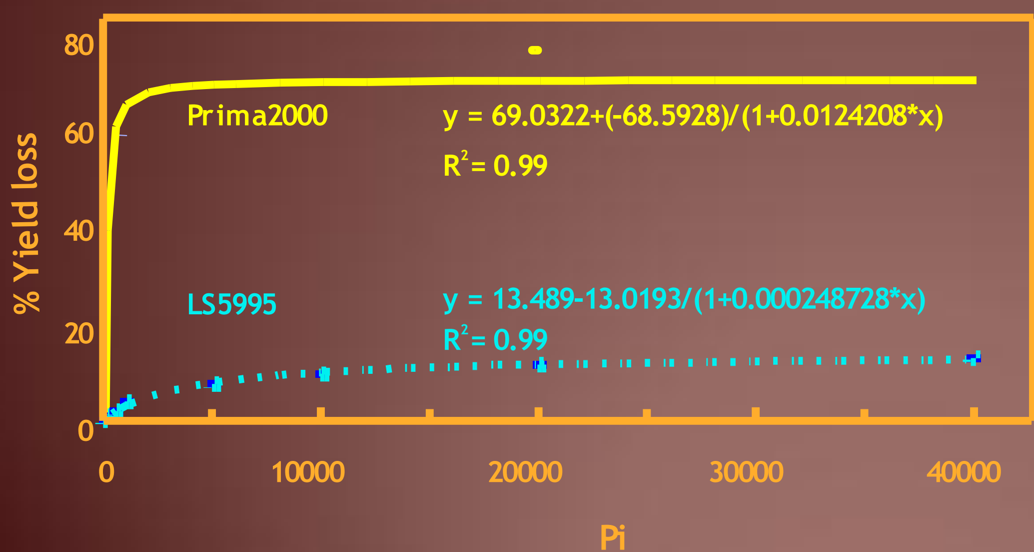


Orientation: Rainfall



Microplot (semi-natural)





Demonstrated the monetary benefit when using a resistant cultivar

=

damage threshold level 10x higher than that of susceptible cv

Fourie, H., Mc Donald, A.H.. & De Waele, D. 2010. Relationships between initial population densities of *Meloidogyne incognita* race 2 and nematode population development in terms of variable soybean resistance. *Journal of Nematology* 42(1): 55-61.

Field (Natural)

Soybean genotype	Maturity Group (MG) (days to harvest maturity)	First growing season	Second growing season
D82-3298 (Resistant)	VII (150)	√	√
LS5995 (Resistant standard – Fourie *et al*. 2006)	VI (137)	√	√
PAN660 (Resistant)	VI (135)	√	√
Potties (Resistant)	VII (143)	√	√
TXS 98/10/53 (Resistant)	VI (135)	√	√
Egret (Resistant)	VII (147)	-	√
Prima (Susceptible standard – Fourie *et al*. 1999)	V (135)	√	-
Prima2000 (Susceptible standard – Fourie *et al*. 2006)	V (136)	-	√

Field sites

Localities	pH (H_2O)	% sand	% silt	% clay	% organic matter	Rainfall (mm)	Irrigation (mm)
First season:							
Potchefstroom (microplot)	6.25	86.6	2.5	10.9	0.2	634	238
Greytown (field)*	4.20	42.1	30.2	24.5	3.2	606.4	0
Jan Kempdorp (field)	5.86	98.2	2.1	6.6	0.6	620	600
Second season:							
Potchefstroom (microplot)	6.55	93.6	1.9	3.9	0.6	585.3	225
Potchefstroom (field)	6.28	93	2	4.5	0.5	634	375
Greytown (field)*	4.44	19.7	36.6	42.7	1.0	526.8	0
Rustenburg (field)	6.51	64.9	11.1	23.2	0.8	592.0	180

Meloidogyne spp. population levels

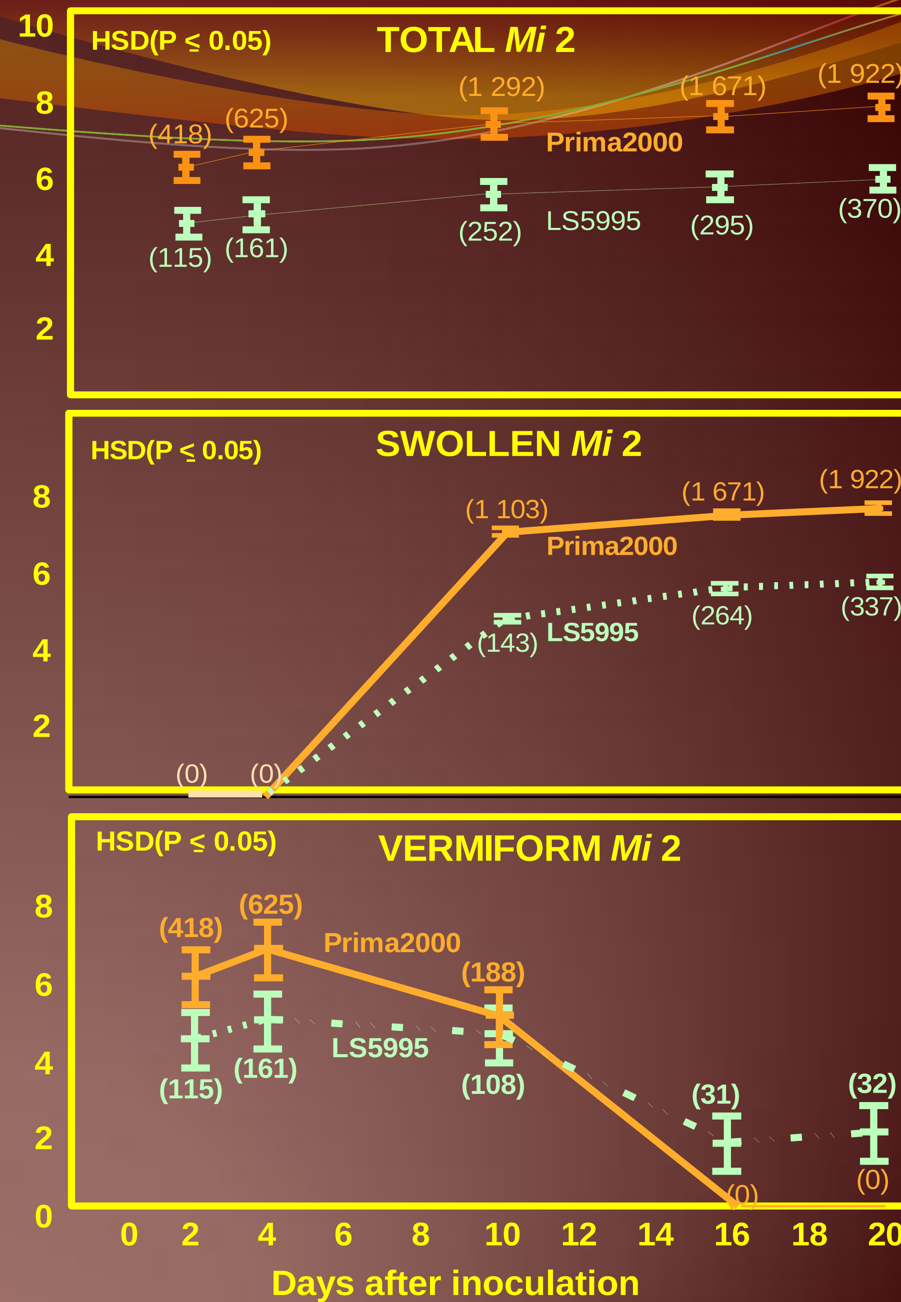
Genotypes	Potchefstroom			Rustenburg			Greytown		
	Eggs & J2/50 g roots	RR values[3] (per root system)	J2/200 ml soil	Eggs & J2/50 g roots	RR values[3] (per root system)	J2/200 ml soil	Eggs & J2/50 g roots	RR values[3] (per root system)	J2/200 ml soil
LS5995	6.6 a (981)	0.1	0 a (0)	0.4 a (2)	0.08	0.5 a (4)	1.3 a (31)	0.1	0.9 a (21)
PAN660	8.4 a (5 684)	0.7	2.1 a (49)	0.6 a (4)	0.1	0 a (0)	3.7 a (164)	0.1	1.4 a (16)
Potties	10.1 b (28 139)	3	0 a (0)	1.8 a (14)	0.5	0 a (0)	4.8 a (3 449)	0.2	0.9 a (28)
Prima /Prima2000[2]	13.4 c (868 770)	100	8.6 b (5 862)	6.9 b (2 578)	100	5.2 c (280)	11.9 b (176 578)	100	9.5 b (15 957)
D82-3298[1]	7.5 a (2 200)	0.3	0 a (0)	0 a (0)	0	0 a (0)	3.5 a (249)	0.6	1.9 a (91)
TXS98/10/93	8 a (3 748)	0.4	0 a (0)	2.9 a (443)	17	0.7 a (14)	2.9 a (443)	0.8	2.3 a (135)
Egret	7.9 a (6 234)	0.7	0.9 a (35)	0.3 a (1)	0.03	1.5 b (131)	1 a 26	0.8	1.8 a (123)
Genotype x Treatment: F-ratio P value	***25.62 ≤0.05***	***- -***	***5.78 ≤0.05***	***8.42 ≤0.05***	***- -***	***4.52 ≤0.05***	***17.58 ≤0.05***	***- -***	***0.58 ≥0.05***

Yield loss (%)

Genotype	Greytown	Potchefstroom	Rustenburg
LS5995[1]	13	7	1
PAN660	10	-3	-10
Potties	-5	13	-13
Prima / Prima2000[2]	-20	-52	-8
D82-3298	-10	-7	-14
TXS98/10/53	7	-15	-1
Egret	-3	-21	-7

Penetration, development and reproduction of *M. incognita* on susceptible and resistant soybean genotypes

Penetration:



H. Fourie, A.H. Mc Donald, D. De Waele and A. Jordaan. Comparative cellular responses in resistant and susceptible soybean cultivars infected with *Meloidogyne incongita*. Nematology (accepted in January 2013).

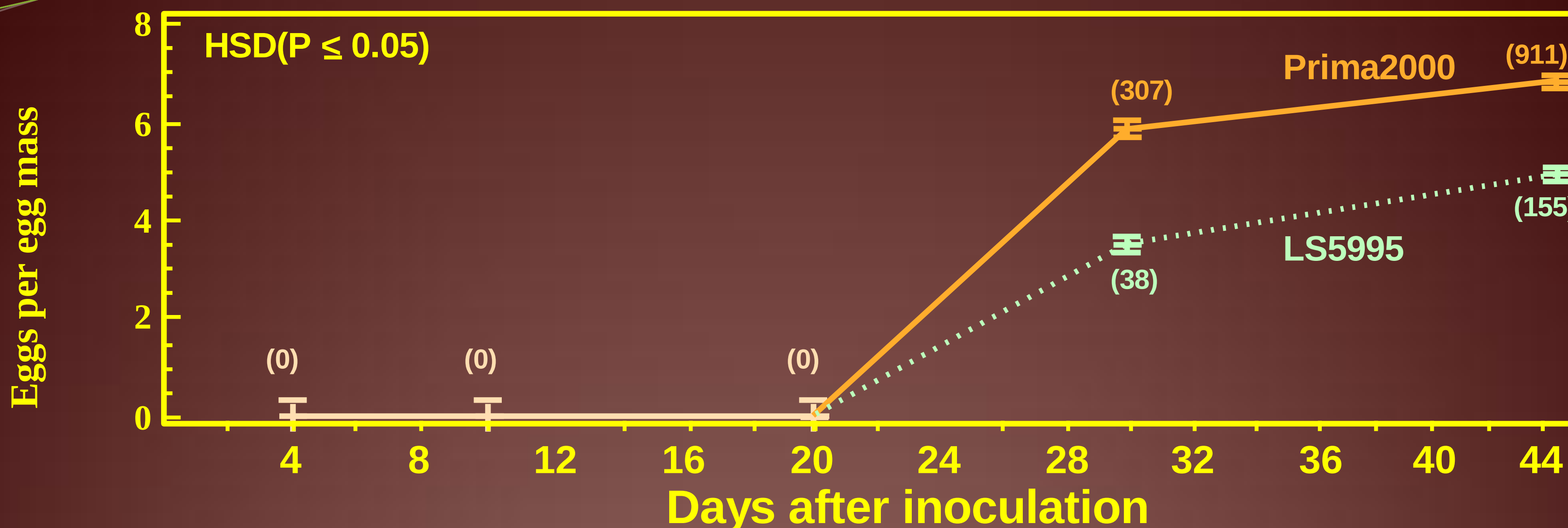
Development:

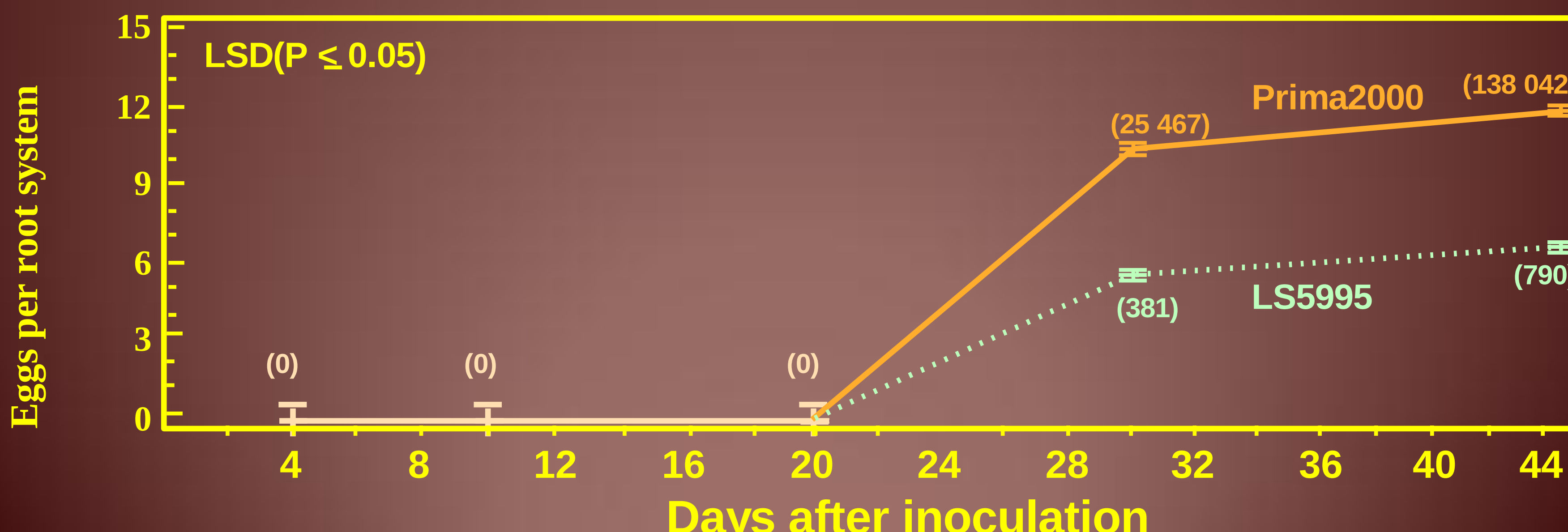
Sexually undifferentiated individuals at
10 days after inoculation

Resistant cultivar

Susceptible cultivar

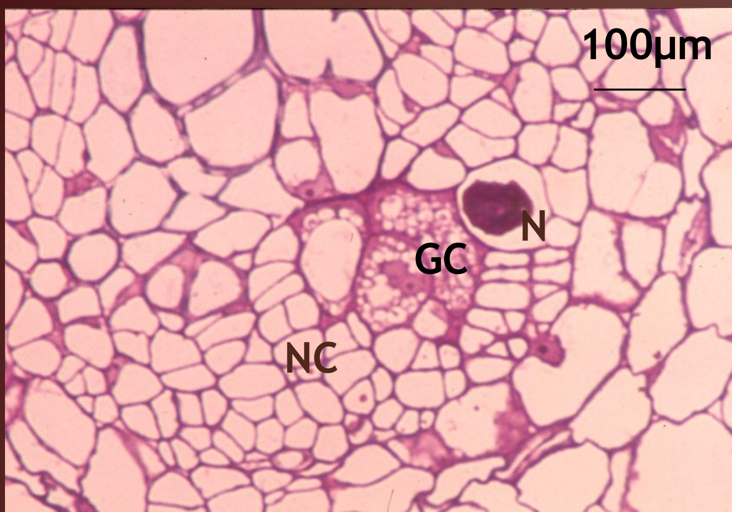
Reproduction:



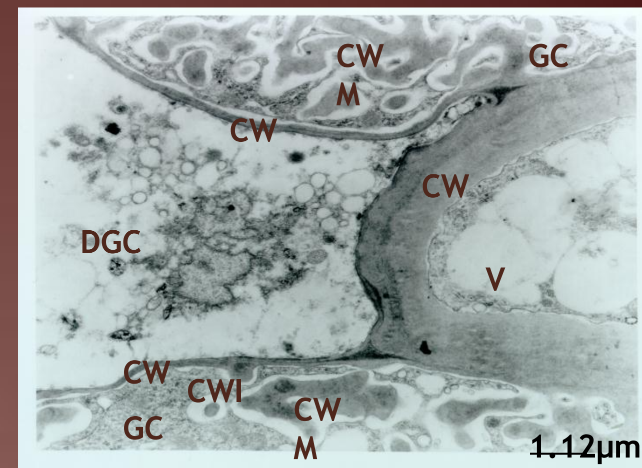


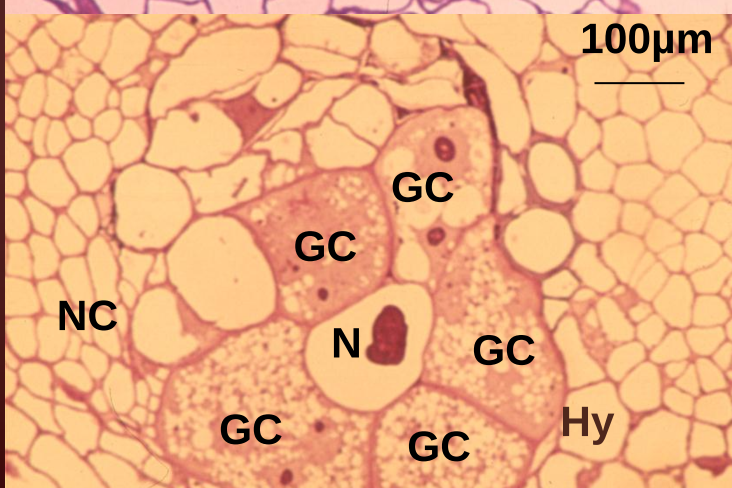
Cellular responses of resistant and susceptible soybean cultivars infected with *M. incognita*

10 Days after inoculation

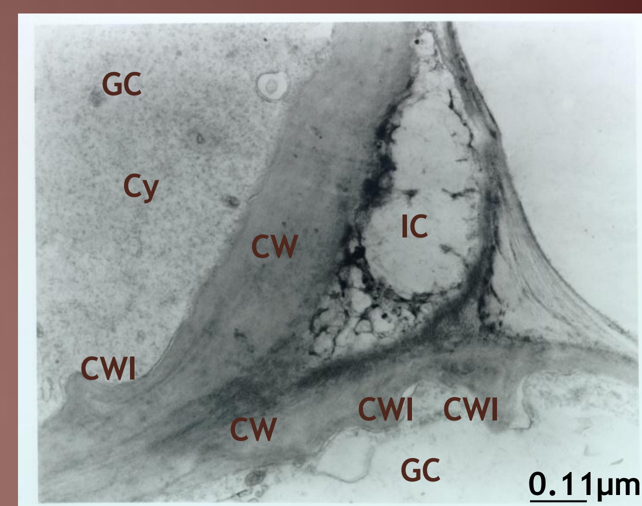


Resistant cv





Susceptible cv



Identification and verification of genetic markers associated with *M. incognita* resistance in soybean

M. incognita	% Variation in gall index	% Variation in final population levels	*M. javanica*	% Variation in gall index
Satt201 - LG-M	-	80	E-ACC/M-CTC2, SOJA6	41
Satt590 - LG-M	62.4		E-AAC/M-CAT1, SOJA7	42
Satt358 - LG-O	31.7		Both on LG-F	
			2001/9498 (PRF)	

Fourie, H., Mienie, C.M.S., Mc Donald, A.H. & De Waele, D. 2008. Identification of genetic markers associated with *Meloidogyne incognita* race 2 resistance In soybean (*Glycine max L. Merr)*. Nematology, 10:651-661.

Mienie, C.M.S., **Fourie, H.,** Smit, M.A., Van Staden, J & Botha, F.C. 2002. Identification of AFLP markers in soybean linked to resistance to *Meloidogyne javanica* and conversion to sequence characterized amplified regions (SCARS). Plant Growth Regulation, 37:157-166.

Cultivar	LG-M		LG-O	RF-value	Host suitability[1]
	Satt201	Satt590	Satt358		
1. LS5995	+	+	+	0.01	R
2. PAN 660	+	-	+	0.2	R
3. Potties	+	-	+	0.2	R
4. PI 96354	-	-	+	0.2	R
5. Egret	H	-	+	0.08	R
6. LS 599	-	+	-	0.08	R
7. Forrest	+	-	+	1.3	S
8. A 5409 RG	-	+	-	5.5	S
9. AG 5601	+	-	-	5.1	S
10. AG 5603	+	-	-	2.9	S
11. AG 6101	+	-	-	1.8	S
12. Amstel	+	-	-	3.1	S
13. CRN 5550	-	+	-	4.6	S
14. Maruti	-	-	+	3.2	S
15. Knap	+	-	-	27.6	S
16. LS 555	+	-	-	11.3	S
17. PAN 564	+	-	-	2.1	S
18. PAN 626	+	-	-	12.3	S
19. SNK 500	+	-	-	2.4	S
20. Stork	-	-	+	1.6	S
21. Dumela	-	-	-	27.7	S
22. Highveld Top	H	-	-	9.4	S
23. LS 666	-	-	-	0.6	R
24. LS 677	-	-	-	5.2	R
25. LS 678	-	-	-	2.8	S
26. Marula	-	-	-	6.3	S
27. PAN 809	-	-	-	2.8	S
28. Prima 2000	-	-	-	33.6	S
29. SCS 1	-	-	-	6.6	S
30. Dundee	-	-	-	Not available[2]	S
31. Ibis	-	-	-	Not available[2]	S

Genotype	Ranking order	Yield (t/ha)	Moisture content	Satt201	Satt590	Satt487	Satt358
LS 555 (check)	1	3.68	10.7	+	-	-	-
LS5995 x SNK500 1	2	3.54	10.7	+	+	+	+
LS5995 x Prima2000 1	3	3.43	11.5	+	+	+	+
LS 677 (check)	4	3.13	11.7	-	-	-	-
LS 678 (check)	5	3.13	11.8	-	-	-	-
LS5995 x Prima2000 2	6	3.09	12.8	+	+	+	+
LS5995 x Prima2000 3	7	3.01	10.9	+	+	-	+
LS5995 x Prima2000 4	8	3.00	11.3	+	+	+	+
LS5995 x Prima2000 5	9	2.92	11.5	+	+	+	+
LS5995 x SNK500 2	10	2.90	12.1	+	+	+	+
LS5995 x Prima2000 6	11	2.84	11.5	+	+	+	+
LS5995 x SNK500 3	12	2.84	11.6	+	+	+	+
LS5995 x SNK500 4	13	2.83	11.8	+	+	+	+
LS5995 x SNK500 5	14	2.77	11.5	+	+	+	+
LS5995 x SNK500 6	15	2.73	10.5	+	-	+	-
LS5995 x Prima2000 7	16	2.71	11.1	+	+	-	-
LS5995 x SNK500 7	17	2.69	11.7	+	+	+	+
LS5995 x SNK500 8	18	2.68	12.1	+	+	+	-
LS5995 x SNK500 9	19	2.67	12.4	+	-	+	+

Evaluation of nematicides for use on soybean in South Africa: *M. javanica*

Commenced during the late 1990's (Fourie and Mc Donald, 2001)

Nematicides evaluated included EDB®, aldicarb and chlorpirifos as well as two biological products containing *Paecelomyces lilacinus* and *Bacillus* spp.

Treatments fumigated with EDB® consistently resulted in the lowest *M. javanica* population levels and highest yields, followed by the aldicarb and terbufos treatments

The bionematicide treatments did, however, not always differ significantly from the untreated control or other treatments in terms of their efficacy

Evaluation of nematicides for use on soybean in South Africa: *M. incognita*

Conducted under field conditions where high infestation levels of *M. incognita* were present (Fourie and Mc Donald, 2007)

14 nematicide treatments were included and represented various dosages of aldicarb, abamectin, cadusafos, oxagran, oxamyl and terbufos.

Significant differences in efficacy of nematicides existed for the number of egg and J2 counts 50 g/roots at all localities.

Only oxamyl SL, terbufos GR and abamectin/seed treatments resulted in a significant reduction of *M. incognita* numbers in 50 g roots and generally showed a higher income/ha compared to the untreated control.

Future prospects

- Release of RKN-resistant cultivar(s) by ARC-GCI
- Quicker, effective methods of identifying RKN resistant cultivars
- Continuous audit of nematode assemblages in soybean-production areas (conventional vs. genetically-modified plantings)
- Audit of “soil health” in terms of nematodes
- Interdisciplinary and -institutional collaboration (locally and abroad) to make soybean production sustainable in the presence of nematodes

Marketing

NSSA will host 6ICN in
Cape Town in May 2014

IAPPS – pamphlets at reception

ACKNOWLEDGEMENTS

- NRF, OPDT and ARC-GCI
- Everyone attending
- NWU