SUMMARY

Soybean production is hampered worldwide by infection of pests and diseases. Plant-parasitic nematodes are among the economically important pathogens that attack this crop, causing substantial yield losses. Frequently used nematode management strategies to minimize soybean yield losses generally include the use of nematicides, the planting of resistant cultivars and crop rotation. Since nematicides are progressively being withdrawn from world markets, the use of nematode resistant soybean cultivars is a popular and widely applied nematode management strategy at present.

Although a wide range of soybean cultivars is available with resistance to nematodes, these cultivars are not necessarily adapted to climatic conditions occurring in South Africa. Therefore, the present study was undertaken to identify and verify *in vivo* resistance to *Meloidogyne incognita* race 2, and to identify genetic markers associated with this resistance in local soybean germplasm.

In the first part of the present study, 85 local as well as foreign soybean genotypes were evaluated for resistance to *M. incognita* race 2 with the aim to introgress this resistance into local elite material. Two greenhouse experiments were conducted. Nematode variables used were the number of eggs and J2 per root system and per g of roots, the number of egg masses and egg-laying females (ELF) per root system, and the reproduction factor (RF=final egg numbers(Pf)/initial egg numbers(Pi).

RF values were used as the main criterium to select for root-knot nematode resistance since egg production is a more reliable measure for determining resistance. Substantial variation existed among the 85 soybean genotypes with regard to all nematode variables measured. None of these genotypes were classified as immune to *M. incognita* race 2 since egg masses were produced on all genotypes. Twelve cultivars (8 local and 4 foreign) and 21 breeding lines had RF values lower than 1 and were classified as resistant. Cultivar LS5995 had the lowest RF values in both experiments, number of eggs per root system and per g of roots, and differed significantly from the susceptible standard Prima2000 as well as a range of other genotypes This commercial South African soybean cultivar will subsequently be used as the major resistance source in the nematode resistance breeding program.

The second part of the study entailed the verification of the resistance to *M. incognita* race 2 to six resistant soybean genotypes, together with the susceptible cultivar Prima2000, under natural environmental conditions in a microplot experiment, as well as in three 3 field experiments.

Root-knot nematodes (*Meloidogyne. javanica* and *M. incognita*), identified by means of molecular techniques, were predominant in all field localities. *Pratylenchus* spp., Hoplolaimidae and Longidoridae were also present but in low numbers. Significant interactions existed between the seven cultivars and the EDB-fumigated treatment with regard to *M. incognita* race 2 J2 numbers in the soil and roots for the microplot and field experiments. When the data were pooled over the three localities, significant interactions also existed between the seven genotypes and treatments (EDB fumigation *versus* non fumigated plots), genotypes and the three localities, as well as between the treatments and the localities with regard to *M. incognita* race 2 eggs and J2 numbers in the soil and roots samples.

Since the seven soybean genotypes used in the present study belong to different maturity groups and differences in yield among them are caused by differences intrinsic agronomic traits, they were not compared among each other with regard to yield performance. However, the relative performance of each soybean genotype with regard to individual potential yield response to nematicide treatment (EDB fumigation in this case) were assessed by establishing an index for yield (Py=(Yf-Y)100/Y, where Yf=yield in EDB-fumigated soil and Y=yield in non-fumigated soil), followed by calculation of a nematode resistance index (Ri=Py of the resistant cultivar/Py of the susceptible cultivar). Yield and nematode resistance indices were lowest for the resistant genotypes at all localities, except at the Rustenburg field locality where three of these genotypes had higher Ri's than the susceptible cultivar Prima2000.

In contrast with the high reproduction of root-knot nematodes in roots of the susceptible soybean cultivar Prima2000, LS5995 consistently maintaied low populations levels of this pathogen in the microplot and field experiments. This resistance in LS5995 also seemed to be effective against *M. incognita* race 4 (occurring at Rustenburg) as well as against *M. javanica* occurring in combination with *M. incognita* race 2 and 4 at the Greytown and Rustenburg localities, respectively. Since LS5995 always had an Ri index lower than 1 (indicating a high level of resistance) this cultivar probably has the most sustainable *M. incognita* resistance compared to the other resistant genotypes. Yield response was generally dependent on environmental effects, limiting further qualification of a resistant or susceptible soybean cultivar as tolerant, intolerant and hypersensitive.

In the third part of the present study, the effect of initial population density levels of *M. incognita* race 2 on nematode population development and on the yield of a susceptible and resistant soybean cultivar were investigated. Two experiments were conducted: one in a hailnet cage and the other one in microplots. Nematode reproduction was assessed by determining the number of nematode eggs and J2 per root system and per g of roots, number of egg masses and ELF per root system, and RF. The percentage yield reduction was also calculated.

Strong relationships between all nematode variables used in the present study were best described by non-linear equations. Significantly higher numbers of J2, egg masses and ELF were maintained in the roots of Prima2000 compared to LS5995 from Pi=100 (Pi=initial egg and J2 numbers) onwards in both experiments. RF values were inversely related to Pi for both cultivars and were lowest on LS5995 with Prima2000 maintaining significantly higher RF values in both experiments. Furthermore, LS5995 had a damage threshold which is 10 times higher compared to Prima2000, sustained significantly less yield loss and supported significantly lower nematode populations at different initial nematode population levels.

In the fourth part of the present study, the comparative penetration, development and reproduction rate of *M. incognita* race 2 in the roots of the resistant soybean cultivar LS5995 and the susceptible soybean cultivar Prima2000 were investigated in two separate experiments in the greenhouse.

Second-stage juveniles (J2) of *M. incognita* race 2 initially penetrated roots of both Prima2000 and LS5995 in equal numbers but were significantly lower in roots of LS5995 at 10 days after inoculation (DAI). While J2 numbers increased from 418 to 1,922 in the roots of Prima2000 over a 20 days, J2 numbers in roots of LS5995 only increased from 115 to 370.

Development of *M. incognita* race 2 J2 was significantly affected by soybean cultivar and time, being slowest in the resistant cultivar LS5995 during the 45 day duration period of the development experiment. LS5995 also maintained a significantly higher numbers of sexually differentiated J2 as well as J3 and/or J4 males compared to Prima2000. Furthermore, adult females in the roots of LS5995 produced significantly less eggs per egg mass at 30 and 45 DAI (on average 38 and 155, respectively) compared to Prima2000 (307 and 911, respectively). In LS5995, egg masses contained significantly fewer eggs per egg mass (87.6 and 82.9 %) compared to Prima2000 at 30 and 45 DAI. The same trend was observed with the number of eggs per root system. At 30 and 45 DAI, LS5995 maintained 98.5 and 99.42 % fewer eggs per root system compared to Prima2000. The resistance expressed by LS5995 was identified as typical post-infectional antibiosis.

The primary objectives of the fifth part of the present study were to study and compare giant cell formation in *M. incognita* race 2 infected roots of the resistant soybean cultivar LS5995 and the susceptible Prima2000 at 2, 4, 10, 20, 30 and 45 DAI. Light and transmission electronmicroscopy (TEM) were used to obtain these results. This experiment was conducted in the greenhouse.

Meloidogyne incognita race 2 J2 penetrated roots of both soybean cultivars and migrated intercellularly to the parenchyma cells located in the rest meristem of the vascular cylinder at 2 DAI, with pronounced cellular changes being observed in the roots of both cultivars. From 10 to 45 DAI, giant cell formation in the roots of the resistant cultivar LS5995 differed substantially compared to Prima2000: giant cells in roots of LS5995 were smaller in size and generally also fewer in numbers compared to giant cells in roots of Prima2000. Three different types of irregular giant cells were also observed in the roots of LS5995 at 10 DAI. The first type represents empty as well as advanced degenerated giant cells. The second type of giant cells were characterised by the inclusion of strand-like structures, filling the cytoplasm of such cells, while the thickness of cell walls representing the third type of giant cells varied considerably. While some giant cells exhibited abormall thick cell walls, approximately double the thickness of those formed in Prima2000, others had thin cell walls.

Cell wall ingrowths in giant cells of LS5995 differed substantially from those in Prima2000 since they were fewer in numbers and occurrence, with single, massive cell wall ingrowths frequently being deposited into giant cells in the roots of LS5995. Two additional types of giant cells, not reported before, could be added to the giant cell classification system of Dropkin and Nelson (1960) for soybean: i) the formation of cell wall ingrowths in giant cells in roots of the resistant soybean cultivar LS5995 and ii) the formation of giant cells with abnormally, thick cell walls occurring in LS5995.

The objectives of the sixth part of the present study were i) to determine whether the same QTLs associated with *M. incognita* race 3 resistance in USA soybean cultivars are associated with *M. incognita* race 2 resistance in the local soybean cultivar LS5995, ii) to identify genetic markers for use in marker-assisted selection (MAS) in local material and iii) to identify new QTLs where applicable. This experiment was conducted in a greenhouse. The *M. incognita* race 2 resistant soybean cultivar LS5995 and the susceptible Prima2000 were used as parents in crosses to obtain a F_2 mapping population consisting of 69 F_2 seeds.

Nematode reproduction was assessed by determining the number of nematode eggs and J2 per root system and the root gall index system. Only 5 out of 12 SSR markers were polymorphic between LS5995 and Prima2000 in the region of Satt492 and Satt358 on LG-O, namely Satt259, Satt358, Satt445, Satt487, Satt500, with SSR markers Satt259 and Satt445 located further from the QTL on LG-O.

According to regression analyses significant linkages were evident on LG-M, with markers Satt201 and Satt590 explaining 15.8 % of the variation in *M. incognita* race 2 gall index (indicating the development ability of *M. incognita* race 2). However, linear regression analysis showed a considerable linkage, accounting for 42.3 % of the variation in number of eggs per root system (reflecting the reproduction ability of *M. incognita* race 2). Interval mapping against both of these phenotypic parameters indicated a QTL located between markers Satt201 and Satt590 on LG-M, with a ± 22 cM difference in the location of the two QTLs. The QTL at Satt201 (± 24.4 cM) explained 62.4 % of the variation in gall indexing with a LOD of 3.84. On the other hand, interval mapping with eggs per root system located the QTL very close to Satt201 (± 2.4 cM), with a LOD of 11.31. This QTL explained 80 % of the variation in nematode egg numbers.

Identification of QTLs associated with resistance using both gall index as well as the number of eggs per root system has not been reported before and indicates root galling and reproduction of this nematode in LS5995 is not necessarily related. Frequency distribution of the F_2 progeny using both the nematode variables, gall index and eggs per root system, indicate that resistance to *M. incognita* race 2 was most probably quantitatively inherited, being controlled by a number of recessive genes and not by a single dominant gene.

G:\Maria\Argiewe\Bestuursopsommings\H Fourie\Dr H Fourie-PhD Summary final.doc