MAIZE TRUST BIANNUAL REPORT 2013/14
(1 October 2013 to 31 March 2014)

CULTIVAR ADAPTATION

DETAILS

PROJECT NUMBER		M101/10
PROJECT TITLE		Evaluation of medium- and long-season maize hybrids for different production systems
PROJECT MANAGER		SH Ma'ali
CO-WORKER(S)	Internal	D De V Bruwer, DJ Muller, W Jansen, JL Snijman
	External	Seed industry, cooperatives, GSA, farmers, PDA.
PROJECT STATUS		Continue
DURATION		01/04/1999 to 31/03/2015

ACTIONS TAKEN TO DATE

The maize production area is divided into western and eastern regions for more accurate evaluation and recommendation purposes. Private seed companies nominated 50 medium and long-season maize cultivars for evaluation at 70 localities in the eastern and western production regions under dry land conditions and 7 localities for disease study purposes for a total of 77 trials. Evaluations included incidence of diseases, some agronomical traits and grain yield. Sixty one trials were planted by collaborators and 16 trials by ARC-GCI.

PROGRESS MADE

Seventy one of 77 trials in the western and eastern areas were planted. Each trial consisted of 50 entries, with three replicates. An annual meeting was held with seed companies where the results were discussed and approved before being published. The Maize National Cultivar Trial Reports for the 2012/13 growing season and for multi-seasons (2010/11 - 2012/13) have been published and the publications distributed to farmers and interested organizations. These reports are also available on the ARC-GCI website. Results for the MIG of 2013/14 season were completed and published earlier than usual for the farmer's benefit. One comprehensive popular article about maize cultivar recommendations was published in the Grain SA in March 2014. All of the trials were visited and inspected by ARC-GCI staff.

RESULTS ACHIEVED TO DATE

Trials for the 2013/14 production season were established. Results are not available since trials are not yet harvested.

PROBLEMS ENCOUNTERED

None.

DETAILS

PROJECT NUMBER		M101/11
PROJECT TITLE		Evaluation of short- and ultra-short-season maize hybrids under irrigation
PROJECT MANAGER		SH Maali
CO-WORKER(S)	Internal	D De V Bruwer, DJ Muller, W Jansen, JL Snijman
	External	Seed Industry, farmers, cooperatives, GSA.
PROJECT STATUS		Continue
DURATION		01/04/2007 to 31/03/2015

ACTIONS TAKEN TO DATE

By the end of each season, maize producers need to decide which cultivars they want to plant during the following season. Since the introduction of short- and ultra-short-season maize hybrids it was realised that these cultivars perform quite differently from existing medium- and long-season cultivars. Short- and ultra-short-season cultivars are usually planted at much higher densities compared to the other cultivars and they are mostly cultivated under conditions of sufficient water supply or under irrigation. Twenty trials were planted under irrigation in the eastern and western areas in order to evaluate the performance of 24 short- and ultra-short-season maize hybrids. In addition, seven trials were evaluated for disease incidence.

PROGRESS MADE

Nineteen short growing season trials were planted under irrigation, of which each consisted of 24 entries with three replicates. An annual meeting was held with seed companies where the results were discussed and approved before being published. The Maize National Cultivar Trial Reports for the 2012/2013 growing season and for multi-seasons (2010/11 - 2012/13) have been published and distributed to farmers and interested organizations. These reports are also available on the ARC-GCI website. Results for the MIG of 2013/14 growing season were completed and submitted for publication. One detailed popular article about last season results has been published in Grain SA. Trials were visited and inspected by the ARC-GCI staff.

RESULTS ACHIEVED TO DATE

Trials for the 2013/14 production season have been established. Results are not available since trials are not yet harvested.

PROBLEMS ENCOUNTERED

None.

CROP MODELLING

DETAILS

PROJECT NUMBER		M103/16
PROJECT TITLE		Climate and genotype effects for maize grain yield
PROJECT MANAGER		W Durand
CO-WORKER(S)	Internal	DL du Toit, TL Mathobisa, MA Prinsloo
	External	ARC-ISCW
PROJECT STATUS		Continue
DURATION		01/04/2011 to 31/03/2014

ACTIONS TAKEN TO DATE

Using all available databases and data sources, additional data relating especially to climate of the data compiled in the cultivar trial publications, were sourced and assembled. This required sifting through large amounts of raw data and manipulating it using software such as ArcGIS that can handle databases not able to be manipulated in Access. Information on variety type of white and yellow maize and seed companies were sourced from the Plant Variety Journals. The dominant soil type was identified by overlaying site locations in a GIS environment and the soil texture was derived from the ACRU-user guidelines that allow linkages to other soil information.

PROGRESS MADE

Forty publications containing trial data for commercial maize cultivars from 1989 to the 2009/10 season have been scanned, imported into an Excel format and quality checked. All tables have been transposed and normalised and imported into an Access database. All trial locations have been geo-referenced to the nearest town. Cultivars have been identified, as far as possible, to be either white or yellow, hybrid and GMO, and holders of breeding rights. Effort is being made to link the data to soils and climate information to enable calculation of water use efficiencies.

RESULTS ACHIEVED TO DATE

All the data is in an Access database containing 85 000 records. This represents the yields of 490 cultivars planted at 280 locations. A first pass analysis confirms that there has been significant increase in yields that can be attributed to improved plant genetics, most probably due to the introduction of GMO's. Rather than linking daily climate data, monthly data was explored. Very few sites have detailed soil profile descriptions. Thus only general texture classes derived from the captured soil profile descriptions could be linked and those sites that did not have this were assigned the dominant soil profile texture class according to the land type. Some options of zoning options for cultivar trial use were explored.

PROBLEMS ENCOUNTERED

Earlier problems relating to the OCR software have been resolved.

WEED SCIENCE

DETAILS

PROJECT NUMBER		M111/13
PROJECT TITLE		Improved grass control systems in maize
PROJECT MANAGER		E Hugo
CO-WORKER(S)	Internal	MM van der Walt, KE Ramatseng, S Tsamai
	External	University of Pretoria
PROJECT STATUS		Continue
DURATION		31/03/2011 to 31/03/2016

ACTIONS TAKEN TO DATE

During the October 2013 to March 2014 time-frame, the following actions were taken:

Glasshouse trials to determine aggressivity of *Digitaria nuda* and *D. sanguinalis* was completed successfully. *Digitaria nuda* and *D. sanguinalis* seedlings were cultivated in glasshouse to be used for molecular identification of these grass species.

One field trial was planted to establish different *D. nuda* infestation levels to verify the hyperbolic regression model (yield loss model) determined in glasshouse trials. Collaboration with Dr Suzette Bezuidenhout, Dapart,ent of Agriculture, was established to repeat competition field trials with *D. sanguinalis* at Cedara Experimental farm in KwaZulu Natal.

PROGRESS MADE

Competitive indices i.e. competitive ratio (CR), aggressivity index (AI), relative yield (RY), relative yield total (RYT) and relative crowding coefficient (RCC) were calculated for dry mass of root, shoot and total biomass of both *D. nuda* and *D. sanguinalis*.

Young leaves (seven to eight weeks old) from *D. sanguinalis* and *D. nuda* were harvested and genomic DNA extractions were performed using the CTAB method. Sixteen primer sets were evaluated and PCR products were initially viewed on agarose and polyacrylamide gels. Polymorphisms was then measured based on the banding patterns observed.

Field trial is being maintained and data are being recorded.

RESULTS ACHIEVED TO DATE

Water stress decreased the number of tillers and panicles of large crabgrass (*D. sanguinalis)* significantly in monoculture and treatment combinations. Total biomass of naked crabgrass (*D. nuda*) in monoculture was greater compared to large crabgrass. Seed mass of large crabgrass was significantly (> 58%) higher in monoculture and treatment combinations. The AI was positive for naked crabgrass with regard to root mass and positive for large crabgrass with regard to shoot mass. Naked crabgrass was more competitive in the wet soil profile (CR = 1.88), while large crabgrass was more competitive in the dry soil profile (CR = 2.02). When in full competition (2N:2S) naked crabgrass and large crabgrass were equally competitive with regard to root, shoot and total biomass. The RY and RYT values were close to one, indicating that both species are making the same demands for resources. The RCC values also did not differ between species for all biomass parameters. Strong competition between naked crabgrass and large crabgrass existed when grown in mixtures, whereas equal competitiveness was observed when planted in equal proportions. The aggressivity and competitiveness of naked crabgrass manifested in the root system, as opposed to the shoots of large crabgrass.

The RAPD markers were able to amplify regions of the genomic DNA of both grass species. From these amplification products, differences and similarities which exist between the two species could be characterised. The differences in the bands were not as clearly visible when agarose gels were used during electrophoresis and were replaced with polyacrylamide gels.

PhD thesis of E Hugo has been submitted for examination. One scientific article was accepted in South African Journal of Botany. One oral and 2 Poster presentations were made at the Combined Congress held in January 2014.

PROBLEMS ENCOUNTERED

Due to irregular rainfall early in season not all the infestation levels of *D. nuda* could be established and maintained. Trial will be repeated.

ENTOMOLOGY

DETAILS

PROJECT NUMBER		M131/14
PROJECT TITLE		Deployment of Bt technology for control of the African stem borer, *Busseola fusca*
PROJECT MANAGER		A Erasmus
CO-WORKER(S)	Internal	SF Grobler, UM du Plessis, LL Ramonyane, MM du Toit, HS Nthangeni, G Makokoe
	External	Seed Industry, Farmers, North West University
PROJECT STATUS		Continue
DURATION		01/04/2008 to 31/03/2017

ACTIONS TAKEN TO DATE

Results of "Refuge in a Bag", Bt/Herbicide tolerant stacked (Bt/HT) and resistant levels of *Busseola fusca* trials were finalized. Data were analysed and potential research areas were identified to prepare for new growing seasons trials.

Trials resulted out of the previous growing season: (a) *Busseola fusca* populations were collected from Delmas, Lichtenburg, Bothaville, Bethlehem, Petrusburg, Vaalharts, Grootpan and Brits to evaluate resistance levels against Bt maize commercialized in South Africa. (b) Field trial to evaluate the effect of stacked Bt/HT maize cultivars and the application of glyphosate on *B*. *fusca* and *Chilo partellus* was planted. Photosynthesis was recorded during the duration of the trial.

Field evaluation of experimental Bt-hybrids containing the Bt-gene alone or stacked with the Roundup Ready gene was conducted (Pannar, Scandinawiedrift) when five week old maize plants were inoculated with *Busseola fusca* first instars. A field trial was conducted in which 11 entries with different experimental Bt-genotypes (Pioneer) were evaluated for control of *B*. *fusca* larvae and *C*. *partellus* when maize plants were inoculated in the whorls.

PROGRESS MADE

- Erasmus, A., 2013. Wat weet ons van die Afrika-stamboorder, *Busseola fusca*? *SA Graan/Grain*, Vol. 15 (12): 20 - 23. December 2013.
- Erasmus, A. & Van den Berg, J., 2014. Effect of Bt maize expressing Cry1Ab toxin on non-target Coleoptera and Lepidoptera pests of maize in South Africa. *African Entomology*, Vol 22(1). March 2014.
- Erasmus, A., 2014. Genetic Engineering. Potchefstroom College of Agriculture. Dept. of Education Agriculture Teachers' Workshop. Potchefstroom. 26 February 2014.
- Erasmus, A., 2014. Entomology (Maize and Biotechnology). Tshwane University of Technology Visit to ARC-GCI. 27 February 2014.
- Marais, J., 2013. Migration patterns and survival of *Busseola fusca* larvae in maize plantings with different ratios of Bt and non-Bt seed. Master's thesis. North West University, Potchefstroom campus. November 2013. (Erasmus, A - Supervisor).
- Rudman, J., 2013. Stem borer response to Bt and herbicide tolerant maize. Honours report. North West University, Potchefstroom campus. November 2013. (Erasmus, A - Supervisor).
- Huyser, E., 2013. Screening *Busseola fusca* populations for resistance to commercialised Bt maize events. Honours report. North West University, Potchefstroom campus. November 2013. (Erasmus, A - Supervisor).

RESULTS ACHIEVED TO DATE

During the "Refuge in the Bag" field trial the incidence of damaged ears, stem damage and damaged internodes per stem were recorded and relationships between these variables determined by means of correlation analyses. A review was conducted in order to identify and discuss similarities and differences between the high-dose/refuge and seed mixture strategies. This was done to determine which strategy would be the most appropriate insect resistance management (IRM) strategy against *B*. *fusca*. The rate of survival and migration of *B*. *fusca* larvae was significantly higher in the plots with maize expressing Cry1Ab and control plots, than in plots with the pyramid Bt event (Fig. 1). Older larvae exhibited improved growth and survival in the laboratory experiment when they were transferred from non-Bt to Bt plants (Fig. 2). Positive correlations were found between early and late season damage, although some weaker than others. Plants of the pyramid Bt event suffered less late-season damage than those of the single-gene event. Since the increase in number of damaged maize plants over time is associated with migration of older and larger larvae, the observed tendencies may indicate that the assumed high-dose does not kill larvae above a

certain developmental stage. The high-dose refuge strategy seems to be the better option for delaying resistance development.

For the Bt/HT and glyphosate effect trial, the larval survival of *B*. *fusca* on the Bt/HT stack treated with glyphosate was slightly lower than the Bt/HT stack not treated with glyphosate, and differed significantly. However the mean mass of the *B*. *fusca* larvae that fed on the Bt/HT stack treated with glyphosate was lower than the mean mass of the larvae that fed on the untreated Bt/HT stack treatment and did not differ significantly from the untreated Bt/HT. No survival of *C*. *partellus* was recorded on treatments that contain the Bt gene. Larval survival of *C*. *partellus* on HT maize treated with glyphosate was high and differed significantly from the control and untreated HT treatment. However, the mean mass of *C*. *partellus* was significantly higher on HT maize treated with glyphosate compared to the control and HT not treated with glyphosate. Larval survival of both species is illustrated in Figure 3. No effect of glyphosate applications on survival of stem borers was therefore observed. This study will be repeated under field conditions with more cultivars in the 2014/15 season.

Results for screening *B*. *fusca* resistance levels of populations collected from Venda, Vaalharts and Ventersdorp were analysed. The larval survival observed from the different populations varied from 20% up to 60% respectively on the maize expressing Cry1Ab proteins (Fig. 4). There was no survival recorded on the pyramid event (Fig. 4)). Larval mass for some populations was significantly higher on the non-Bt iso-hybrid compared to the single gene event. This study provides results for all African countries where Bt maize will be introduced in future. Methodology on collection of baseline data is being developed and base line data generated for future evaluation of resistance levels of *B*. *fusca* to different Bt maize events in South Africa.

PROBLEMS ENCOUNTERED

Heavy rains influenced field trial planted for spray applications that was inoculated with *B*. *fusca* first instars. Hail storm destroyed Bt/Herbicide tolerant field trial. This field trial will be replanted in next growing season 2014/15.

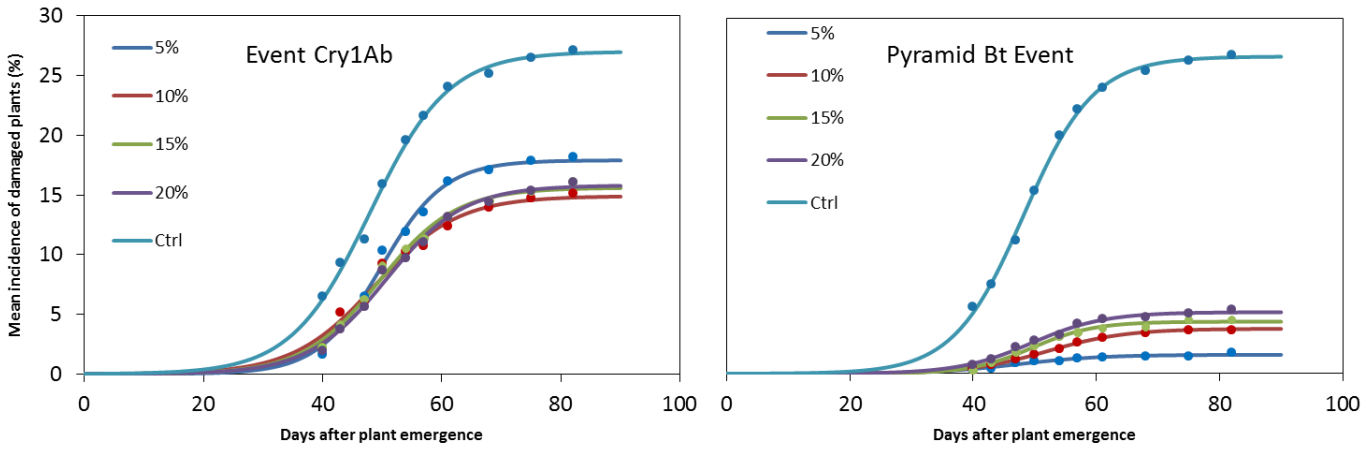

Figure 1. Mean incidence of damaged plants at different times after infestation by *B*. *fusca* of maize plants in plots planted to different ratios of the two events and non-Bt maize seed.

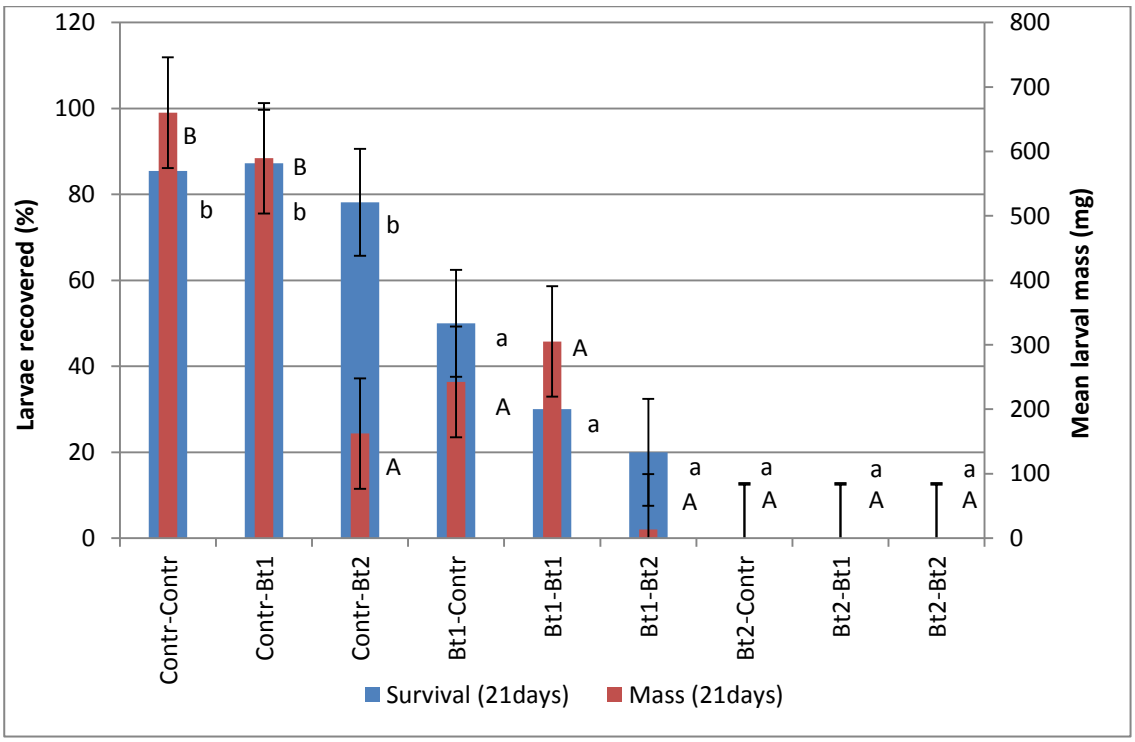

Figure 2. Survival and mass of twenty one day-old larvae on maize whorl treatments. Bars with the same higher and lower case letters do not differ significantly for mass and survival. Error bars indicate standard errors (SE). Bt1 = Cry1Ab event and Bt2 = Pyramid Bt event.

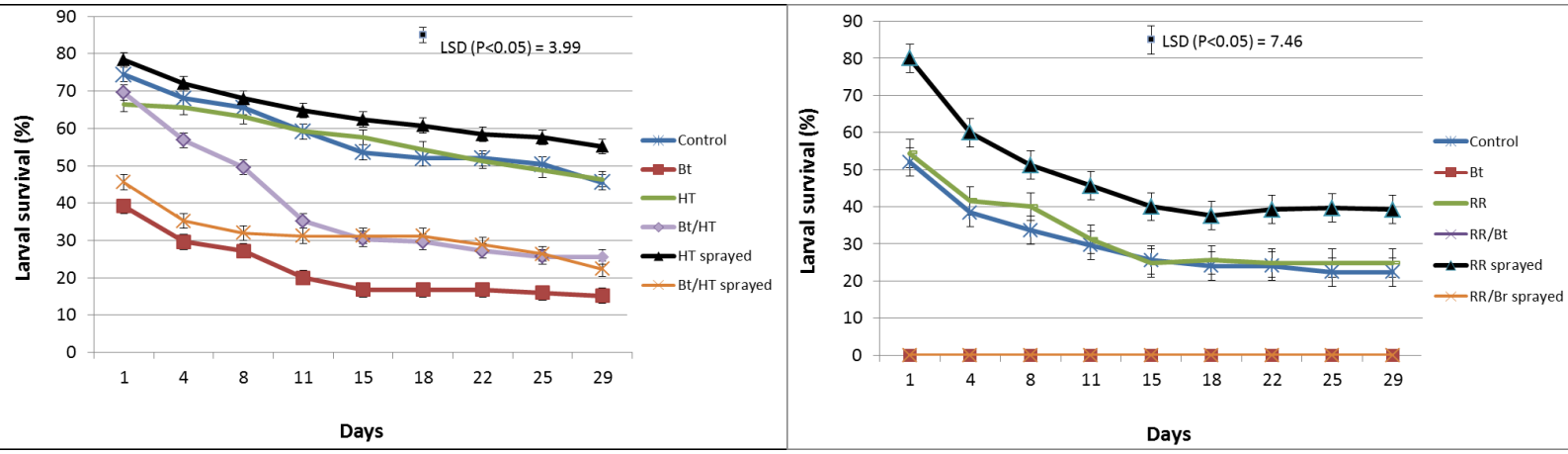

Figure 3. (Left) *Busseola fusca* and (right) *Chilo partellus* larval survival over time on Bt/Ht stacked maize.

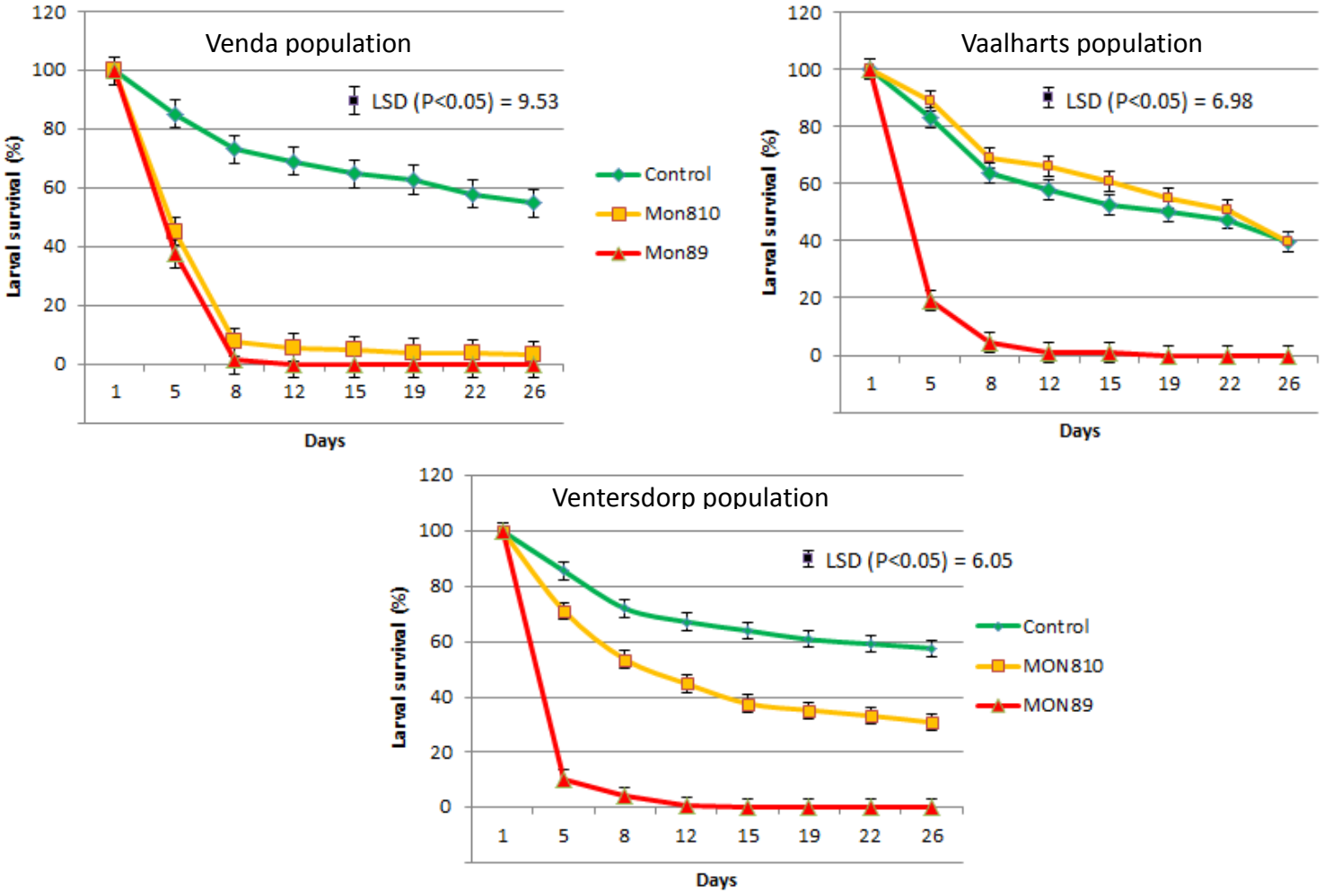

Figure 4. *Busseola fusca* larval survival over time for Venda, Vaalharts and Ventersdorp populations. MON810 = Cry1Ab event and MON89 = Pyramid Bt event.

PATHOLOGY

DETAILS

PROJECT NUMBER		M141/20
PROJECT TITLE		Characterisation of *Exserohilum turcicum* isolates within South African maize production areas.
PROJECT MANAGER		M Craven
CO-WORKER(S)	Internal	F Mashinini, JGC Kroukamp (vacant), MM Mahlobo, MO Motheketlela, KA Tantasie, TJ Baas, VJ Gobiyeza, RE Terblanche, LA Madubanya
	External	B Crampton (UP), I Barnes (UP), M Haasbroek (UP)
PROJECT STATUS		Continue
DURATION		01/04/2011 to 31/03/2016

ACTIONS TAKEN TO DATE

Commercial greenhouse trials

Twelve F1 lines were planted in a commercial greenhouse in order to create a F2 segregation population that will be used to test generated *Ht*-resistance gene markers. The material was selfed during June to December 2013.

In vitro fungicide study

The fungicide sensitivity of five different *Exserohilum turcicum* isolates was evaluated *in vitro.*

Molecular marker development: (University of Pretoria)

Established the mating types of isolates obtained from KwaZulu-Natal and obtained complete SSR marker genotypes of 292 *E. turcicum* isolates collected from various South African provinces.

Molecular development-ARC-GCI

SSRs markers routinely used for fingerprinting purposes were evaluated for their possible use as polymorphic SSR markers for the mapping of the four *Ht* resistance genes.

PROGRESS MADE

Commercial greenhouse trials

F2 generated lines have been harvested.

In vitro fungicide study

Five *E. turcicum* isolates obtained from Potchefstroom, Jacobsdal, Orania (all three belong to Race 23N), GWK isolates #101 and #110 (both belonging to Race 13N) respectively were selected for the study. Two fungicides were included in the study i.e. azoxystrobin/difenocolazole and carbendazim/flusilazole. The isolates were tested at seven different concentrations (0.5µg/ml, 5µg/ml, 10µg/ml, 50µg/ml, 75µg/ml, 100µg/ml and a control that did not receive any active ingredients).

Molecular marker development (UP)

Thirteen polymorphic SSR markers were screened on 292 *E. turcicum* isolates from maize and two isolates from sorghum collected from six South African provinces during 2005-2013. Of these, 251 isolates were collected during 2013 from three locations in the KwaZulu-Natal province. Additionally, mating types of samples collected from three locations in KwaZulu-Natal (Cedara, Baynesfield and Greytown) have been established. Population genetic analyses have been conducted

Molecular development-ARC-GCI

SSR markers targeted for *Ht1* included umc1256, phi101049, phi96100 (panel 2), umc1126 (panel 6), phi083 (panel7), phi109642, umc1555 (panel 10), bnlg2277 (panel 11) and umc1230 (panel 12). For *Ht2* and *HtN*; phi233376 (panel 1), umc1304 (panel 4), umc1161 (panel 6), phi080k15 (panel 8), umc1777 (panel 9) and phi100175 (panel 10) For Ht3; phi114 (panel 3), umc1295 (panel 4), phi112 (panel 5), phi034 (panel 6), umc1545 (panel 7), bnlg1792k8, phi328175 (panel 8), umc1407, phi116 (panel 9) and umc1932 (panel 11).

RESULTS ACHIEVED TO DATE

In vitro fungicide study

Preliminary results indicated slight differences in the sensitivity of the various isolates to the activate ingredients tested. The Jacobsdal isolate e.g. was the most sensitive to both fungicides applied, whilst the 0.5µg/ml azoxystrobin/difenocolazole application provided significantly lower control with the GWK isolate

#110 compared to the other isolates and concentrations tested. The study will be repeated and the concentrations refined in order to clarify NCLB isolate sensitivity to active ingredients.

Molecular marker development - UP
Microsatellite markers and mating type PCR primers were developed and applied to study the population genetic structure and reproduction mode of *E. turcicum* in South Africa. Results indicated that no population subdivision occurred within samples collected from maize during 2012-2013, and isolates from different locations clustered together (viz. no geographical boundaries). Furthermore, shared genotypes were observed between isolates from different provinces, indicating that gene flow is occurring between locations. Cross-species amplification with *E. rostratum* was obtained for one marker (SSR27). Interestingly, the sorghum isolates had unique haplotypes and 13 private alleles were observed. Mating type distributions revealed evidence for sexual recombination in samples collected from Cedara in KwaZulu-Natal. However, the high genotypic diversity observed in isolates collected from all three locations in KwaZulu-Natal is indicative of sexual reproduction. The MAT markers were specific to *E. turcicum* and could be used to differentiate between isolates of *E. turcicum* and *E. rostratum.* The markers developed in this study revealed evidence for migration and sexual reproduction in *E. turcicum* populations from South Africa, and will be useful in future global population genetic studies.

Molecular development-ARC-GCI
None of the SSR markers currently used with maize fingerprinting yielded any usable results in order to map any of the four *Ht* resistance genes. With the help of the maize genome database website, http://www.maizegdb.org/, we accordingly managed to verify the chromosomal sections (bins) where each of the genes were previously mapped. The *Ht1* gene maps to bin 2.07, *Ht2* to bin 8.05, *HtN* to bin 8.06 while *Ht3* was not previously mapped nor was there any information online. We thus downloaded the sequences of the three above mentioned chromosomal sections. Gene *Ht1* is within an area of 551 155 base pairs on bin 2.07 using the IBM2 2008 neighbours map. There is a total of 33 microsatellite regions discovered to date as listed in table 1. Gene *Ht2* is within an area of 87 722 base pairs and a total of 45 microsatellite regions were discovered to date. Gene *HtN* is within an area of 761 275 base pairs and a total of 25 microsatellite regions were discovered. We will screen the following markers for polymorphisms in the following crosses; B68 x V26*Ht1*, B68 x A632*Ht1*, V26 x B68*Ht1*, A632 x V26*Ht2*, B68 x V26*Ht2*, V26 x B68*HtN*, B68 x *A632HtN.* Polymorphic markers from the crosses will be further screened in F2 populations with the use of the bulked segregant analysis. We will only ascertain their efficiency once genotype-phenotype associations are performed. Tightly linked SSR markers will thus be used in marker assisted breeding for northern corn leaf blight.

PROBLEMS ENCOUNTERED
Growth chambers are not functioning effectively.
We need to screen more race typed *E. turcicum* isolates in order to confidently associate an SSR marker or haplotype with a particular race.

DETAILS

PROJECT NUMBER		M141/22
PROJECT TITLE		Survey, screening maize hybrids for resistance to and determination of inoculum source of bacterial leaf streak of maize
PROJECT MANAGER		BC Flett
CO-WORKER (S)	Internal	F Mashinini, JGC Kroukamp (vacant)
	External	Northwest University Potchefstroom, Pannar Seed Co, Pioneer Hybrid International.
PROJECT STATUS		Continue
DURATION		01/04/2011 to 31/03/2014

ACTIONS TAKEN TO DATE

Diseased plant material showing symptoms of bacterial leaf streak (BLS) were collected from localities throughout South Africa during the 2011, 2012 and 2013 maize production seasons. Results from pathogenicity tests confirmed that only isolates identified as *Xanthomonas* species caused characteristic BLS symptoms. A genus specific molecular marker (XGum-D) was then used to identify and to confirm all the isolates belonging to the genus *Xanthomonas.* The isolation method was altered by grinding the plant material with a sterile mortar and pestle and creating a dilution range. The objectives were (I) to determine the incidence, severity of the disease and distribution range of the pathogen through locality surveys, (II) to identify and rapidly detect the causal agent, (III) to establish the resolution power of ribosomal genes and a protein encoding gene (gyraseB) to differentiate amongst the isolates via the construction of phylogenies, (IV) to illuminate possible variation amongst the population (intrapathovar diversity), (VI) to determine whether or not these molecular methods are capable of differentiating between *X. campestris* pv. *zeae* and the closely related *X. axonopodis* pv. *vasculorum*, which is also capable of infecting maize and (V) to use biochemical and phenotypic methods to determine the specific characteristics of the causal agent of BLS.

PROGRESS MADE

Bacterial leaf streak (BLS) of maize was first reported in South Africa in 1949 and has not been reported elsewhere. Very little is known about the pathogen(s) involved and therefore it is deemed necessary to compile a characteristics profile for the pathogen(s). Most of the initial objectives have been achieved: These were:

- To isolate the pathogen responsible for BLS
- To confirm pathogenicity by applying Koch's postulate
- To create metabolic fingerprints of the pathogen using Biolog GN2 plates
- To create a protein profile for the pathogen
- To establish the incidence, spread and severity of the disease through locality surveys (geographical mapping of isolates)
- To further characterise the collected isolates (PCR of: ribosomal genes and repetitive elements, sequencing)
- To construct a phylogenetic tree(s) based upon the ribosomal gene(s) and a protein encoding/housekeeping gene of the bacteria
- To highlight possible differences/intra-pathovar diversity between the isolates

Primary objectives not met in this project include:

- The development of a new PCR protocol or to alter an existing protocol for its (genus) specific and rapid detection was not successfully achieved in this project.
- To determine whether the bacterial leaf streak causal organism is seed transmitted or not using a PCR technique specific to *X. campestris* pv. *zeae.*

The results of this study have been written up as two MSc theses by Mr Nicky Niemann and Ms Jesse Nienaber. The promotors and co-promotors are presently working with the two students to complete the theses for examination and then the results to date will be published.

RESULTS ACHIEVED TO DATE

Sequencing revealed that all 47 isolates belonged indeed to the genus *Xanthomonas*. Due to the delay caused by the inadequate initial isolation method and consequent re-isolation, all the objectives for the study were not reached. The intention is to submit a new funding application to address these outstanding objectives. Some of the isolates have been characterised through rep-based genomic profiling and Biolog

GN2 plates. Protein profiling of the isolates are in an advanced stage. Molecular and protein profiling of *Xanthomonas* isolates that are completed has shown characteristic patterns. The *Xanthomonas* isolates used to create carbon utilization patterns with the Biolog GN2 system, showed a clear metabolic fingerprint that is distinguishable from other plant bacteria. It can be concluded that an altered isolation method is needed to extract *Xanthomonas* species from plant material. It is proposed that *X. campestris* pv. *zeae* is the causal agent of bacterial leaf streak of maize. A distribution map has been compiled to show the occurrence of bacterial leaf streak in the South African maize production area. Details of the results have been included in two MSc theses which are being finalised for examination.

PROBLEMS ENCOUNTERED

Major problems were encountered with the isolation of the causal organism. This only became evident when Koch's postulates were done by inoculating maize plants grown in the greenhouse with a spectrum of species isolates from maize leaves. Only *X. campestris* pv *zeae* produced symptoms indicating the other isolated species to be epiphytic and not pathogens. This problem with isolating the causal organism hampered progress and achievement of two of our original goals. These include the development of a PCR protocol specific to *X. campestris* pv. *zeae* and to determine whether the causal bacterium is seedborne or not. The development of a suitable inoculation technique will also enable us to screen maize cultivars for resistance to bacterial leaf streak.

DETAILS

PROJECT NUMBER		M141/23
PROJECT TITLE		Screening maize hybrids for resistance to cob and tassel smut
PROJECT MANAGER		BC Flett
CO-WORKER (S)	Internal	TM Ramusi, F Mashinini
	External	Pannar Seed Co
PROJECT STATUS		Continue
DURATION		01/04/2011 to 31/03/2016

ACTIONS TAKEN TO DATE

It must be noted that this study includes the routine screening of maize hybrids to cob and tassel smut of maize. This means the actions taken annually will not vary except the hybrids included in the screening study. Medium- to long-season and short-season hybrids were planted in two separate trials at two localities (Greytown and Standerton) for rating of resistance to cob and tassel smut. At Greytown two rows of each hybrid were planted of which one was inoculated and the other was an uninoculated control row. Trials were inoculated at planting using a 50/50 spore/sand mixture. At Standerton a decision was made together with the farmer where the trial is planted to only use natural inoculum and infections to screen the hybrids. A random block design with three replicates was used for each trial. Ratings of ears and tassels were carried out at physiological maturity and were reported as a percentage of the total plants in the row.

PROGRESS MADE

Both trials were successfully planted and inoculated. The Standerton trial had no cob and tassel smut infection and will not be rated. The Greytown trial has adequate infection levels and will rated for cob and tassel smut prior to harvest. We presently have four seasons data which together with the 2013/14 data can be analysed to study cultivar x locality x season interactions for cob and tassel smut using both ANOVA and AMMI analyses. This data will be published in a scientific journal.

RESULTS ACHIEVED TO DATE

Significant variations in hybrid resistance to cob and tassel smut were observed over the previous three seasons we have screened. Unfortunately the publication of such data has not prevented the spread of the disease into new production areas. Additional studies on the potential control of cob and tassel smut using new seed treatments appear promising. This work falls outside the aims of this funded project but has been done on contract for various agrochemical companies.

PROBLEMS ENCOUNTERED

Even though trials are inoculated the development of disease is dependent on favourable climatic conditions. This results in failure of some trials to produce results. Since the decision was made in conjunction with the farmer at Standerton due to his concerns of increasing inoculum levels on his farm the cultivar trials have not had adequate disease levels to screen for differences between hybrids. It might be worthwhile moving the Standerton trial to Potchefstroom and inoculate the same as at Greytown to get more reliable results.

DETAILS

PROJECT NUMBER		M141/24
PROJECT TITLE		Screening maize hybrids for resistance to Diplodia ear rot
PROJECT MANAGER		BC Flett
CO-WORKER (S)	Internal	MT Ramusi, F Mashinini, B Janse van Rensburg
	External	Pannar Seed Co, Monsanto SA, UFS
PROJECT STATUS		Continue
DURATION		01/04/2011 to 31/03/2016

ACTIONS TAKEN TO DATE

It must be noted that this study includes the routine screening of maize hybrids to cob and tassel smut of maize. This means the actions taken annually will not vary except the hybrids included in the screening study. Medium- to long-season and short-season Phase 2 hybrids were planted at four localities (Potchefstroom, Greytown, Petit and Vaalharts) and three localities (Potchefstroom, Greytown, and Vaalharts), respectively, for evaluation of hybrids for resistance to Diplodia ear rot. These localities are included to ensure an adequate range of potentials to analyse data using a technique developed by Flett & McLaren (1994). Potchefstroom and Vaalharts are low disease potential areas, Petit a moderate disease potential area and Greytown a high potential area. The aim of this study is to identify maize hybrids resistant and/or susceptible to Diplodia ear rot over various potentials. Trials were inoculated using a technique where *S. maydis* inoculated maize is ground to a meal and put in the plant whorl (5 ml) at the 14 - 16 leaf stage. Trials will be harvested in May 2014 and ear rot counts were carried out and expressed as a percent infected ears. In addition to this screening study inoculum is made for private seed companies to screen potential maize hybrids prior to release. This ensures that high risk hybrids are identified before release and are either withdrawn or marketed with a warning that these high risk hybrids be used under certain low risk production systems only.

PROGRESS MADE

This study is a routine screening for resistance to Diplodia ear rot in commercial maize hybrids in South Africa. Data for previous seasons has been captured and will be analysed using the regression analysis technique developed by Flett and McLaren (1994) and using an AMMI analysis. All trials have been successfully planted and inoculated and will be harvested and rated in May 2014.

RESULTS ACHIEVED TO DATE

This project is merely a screening project to ensure susceptible hybrids are kept off the market or used in low Diplodia ear rot risk production systems. We have partially achieved our objective by getting the major seed companies to initially screen hybrids for ear rot resistance prior to releasing them for commercial plantings. This data will be published in popular journals and in a scientific journal at the end of the 2014/15 season.

PROBLEMS ENCOUNTERED

The only problems encountered are with contamination of inoculum during preparation. This is due to high contamination levels of saprophytic fungi and mites in the growth room. We are consistently fumigating and buying new consol jars and lids to reduce contamination levels.

DETAILS

PROJECT NUMBER		M141/26
PROJECT TITLE		A rapid, accurate detection method for identification of specific maize root rot and seedling blight fungal pathogens to enable farmers to combat these diseases successfully
PROJECT MANAGER		A Schoeman
CO-WORKER(S)	Internal	Prof BC Flett, Dr M Craven, F Mashinini, NY Maila, TJ Baas, KA Tantasi, MB Kwele.
	External	Stellenbosch University
PROJECT STATUS		Continue
DURATION		01/04/2013 to 31/03/2016

ACTIONS TAKEN TO DATE

Primers were searched for in literature for eleven fungi that are most often associated with root and crown rots and seedling blight: *Pythium* spp. (seedling blight/death), *Rhizoctonia solani* (damping off/failure to geminate/seedling blight), *Curvularia eragostidis*, *Macrophomina phaseolina* (charcoal rot), *Exserohilum pedicellatum*, *Phoma* spp. (root and crown rot/red root rot), *Trichoderma* spp., *Fusarium chlamydosporum*, *F. oxysporum*, *F. equiseti* and *F. verticillioides* (organisms causing Fusarium root, crown and stalk rots) and *F. graminearum* (Gibberella root, crown and stalk rots). In literature to date, primers were found for *Pythium* spp., *Rhizoctonia solani*, *Macrophomina phaseolina*, *Phoma* spp., *Trichoderma* spp., *Fusarium oxysporum*, *F. equiseti*, *F. graminearum* and *F. verticillioides*. qPCR SYBR green protocols were optimised using these primers for each of these organisms.

Optimisation of a qPCR reaction is performed to ensure that only the target DNA (in this case the specific fungal species) is amplified and no other unnecessary amplification of non-target DNA occurs (other fungal species). This process is time consuming as various technique scenarios must be tested and many qPCR runs performed to optimise the protocol to accurately quantify the specific target organism. Firstly, the template or target DNA of each organism studied must be of suitably high quality and purity. After DNA extraction an agarose gel must be run to ensure there is no protein contamination. This is ensured by measuring the DNA concentration where a A260/280 of ~1.8 was accepted as "pure" for DNA. DNA of poor quality or contaminated DNA can cause the qPCR to fail and no amplification to occur. In a reaction the DNA concentration must be adjusted and optimised to improve amplification. The DNA in the 0.6 to 0.8 ng/µl range is usually regarded as optimal. The concentration of specific organism primers and dNTP's (consists of adenine, thymine, guanine and cytosine) were also adjusted and optimised for specific amplification. A too high DNA concentration will lead to all the primers and dNTP's in the qPCR mixture to bind to the DNA and thus, no amplification will occur. A too low DNA concentration and a too high primers and dNTP concentration will lead the primers to bind to one another and unspecific amplification will occur and result in primers dimers. Primers dimers will be quantified by the qPCR reaction. $MgCl_2$ is used to improve the accuracy of amplification and adding 0.6 µl to a 10 µl reaction usually worked well for the organisms being targeted. Amplification was also performed at different melting temperatures in order to obtain specific and accurate amplification for each organism being quantified. Usually temperatures above 60°C will improve specific amplification.

Fusarium verticillioides and *F. graminearum* primers obtained from literature successfully amplified the target species DNA. Specificity of these primers was tested and it was found that no other root rot fungal isolates showed amplification. Optimisation was performed and it was found that at a higher temperature (60°C or at 65°C) and the addition of 0.6µl of $MgCl_2$ to the PCR mixture, specificity was obtained.

Successful amplification was achieved with *F. oxysporum* primers obtained from literature, but qPCR requires the PCR product to be not longer than 300 bp. The product size of these primers was between 300 and 400 bp and thus new primers were required and designed.

Primers cited in literature for *F. equiseti*, *Trichoderma* spp., *Pythium* spp. and *Rhizoctonia* solani did not amplify the target DNA extracted from maize roots and thus, necessitated the design of new primers. Primers were also designed for *Curvularia eragostidis*, *Macrophomina phaseolina*, *Exserohilum pedicellatum* and *F. chlamydosporum*. Primer design also involves specific requirements. Firstly, the primers must not form hairpin loops as this will inactivate amplification. Also, the GC (guanine:cytosine) content of the primers must not be more than 60%, as this will increase the melting temperature to unacceptably high values which will render primers useless. Also, more than 3 base pair stretches of G's and C's must be limited and primers should not have a G on the end. The four main nucleotides in a DNA sequence is guanine, cytosine, adenine and thymine. In order to form a typical DNA helix structure guanine binds to cytosine using three hydrogen

bonds. Adenine and thymine binds together with two hydrogen bonds. A high GC content makes the DNA structure more thermostable and higher temperatures will be required in order to break these bonds. Thus, the more GC basepairs in the primers the more difficult it will be to achieve a perfect match to the target DNA and achieve qPCR amplification. Care was taken that primer dimers did not form, thus the primers did not have complementarity in the sequences especially at the 3' end. Primers dimers could interfere in the qPCR process as these dimers will be amplified along with the target DNA, resulting in unspecific (unwanted) amplification.

Primer designs involve the use of submitted DNA sequences of specific genes of the target fungal species on the National Centre for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/) website. The gene sequences of the fungal species were downloaded and sequences of no longer than 150bp were chosen and the programs Primer 3 (http://bioinfo.ut.ee/primers3-0.4.0/) and Beacon Designer software (http://www.premierbiosoft.com/molecular_beacons/) were used to design primers for that chosen specific region. The primers were then tested for specificity on the NCBI website, as well as specificity of the chosen region using the Basic Local Alignment Search Tool (BLAST). When using the BLAST tool the only fungal species that must be recognised must be the target species, otherwise non-specific amplification will occur, other species will be amplified which defeats the object of the study which is to quantify specific organisms in the maize root rot complex. Fifteen new primer pairs for the remaining fungal species were designed.

Although amplification with the newly designed primer pairs occurred, there was a problem regarding species specificity and protocols had to be re-optimised. Finally qPCR protocols were established for *Rhizoctonia solani*, *F. oxysporum*, *F. equiseti* and *Curvularia eragostidis*. Most of these protocols required higher annealing temperatures (above 60°C) and $MgCl_2$ (0.6µl) in order to improve species-specificity.

The following species again required new primers to be redesigned: *F. chlamydosporum*, *Trichoderma* spp., *Phoma* spp.¸ *Pythium* spp. and *Macrophomina phaseolina.*

After obtaining primers and the necessary protocols for *Rhizoctonia solani*, *F. oxysporum*, *F. equiseti* and *Curvularia eragostidis* other tests must be performed to ensure that this is a viable protocol. Firstly, melt curve analyses must be carried out as this can also assist to distinguish different species and this is typically shown by only one peak being observed in the melt curve. Different species can also render different melting values. For example: The melt curve value for *F. oxysporum* is 77°C, while for *Rhozoctonia solani* it is 74°C. Thus, in the same run if the primers were design for *F. oxysporum* and a product is amplified but the melting value is not that of *F. oxysporum* then the wrong species was amplified and the results are totally incorrect. The PCR efficiency of the qPCR reaction must be determined by setting up a standard curve on which the slope will indicate the curves efficiency. The efficiency will be 100% if the product doubles with every cycle. A serial dilution of the DNA template is made and the qPCR protocol is run in order to test efficiency. A good qPCR protocol must give reliable quantification and must have good efficiency, reproducibility, high sensitivity and only render one product in the melt curve analysis.

The optimised protocols *F. verticillioides*, *F. graminearum, Rhizoctonia solani*, *F. oxysporum*, *F. equiseti* and *Curvularia eragostidis* are being tested for reliable quantification, efficiency, sensitivity and reproducibility. Melt curve analyses have already been performed.

PROGRESS MADE

- SYBR green qPCR protocols that is species-specific for six fungal species *F. verticillioides*, *F. graminearum, Rhizoctonia solani*, *F. oxysporum*, *F. equiseti* and *Curvularia eragostidis*.
- Melt curve analysis have been performed for *F. verticillioides*, *F. graminearum, Rhizoctonia solani*, *F. oxysporum*, *F. equiseti* and *Curvularia eragostidis*.
- qPCR protocols for *F. verticillioides*, *F. graminearum, Rhizoctonia solani*, *F. oxysporum*, *F. equiseti* and *Curvularia eragostidis* are being tested for reliable quantification, efficiency, sensitivity and reproducibility.
- New primer pairs have been designed for *F. chlamydosporum*, *Trichoderma* spp., *Phoma* spp.¸ *Pythium* spp. and *Macrophomina phaseolina* and must still be tested for qPCR amplification and species specificity.

RESULTS ACHIEVED TO DATE

qPCR protocols were optimised for *F. graminearum* and *F. verticillioides* (literature primers), *Rhizoctonia solani*, *F. oxysporum*, *F. equiseti* and *Curvularia eragostidis* (designed primers). The protocols for the primers found in literature for the *F. graminearum* and *F. verticillioides* were optimised by using different

annealing temperatures and adding $MgCl_2$. The primers that were designed using the NCBI website were species specific for *Rhizoctonia solani*, *F. oxysporum*, *F. equiseti* and *Curvularia eragostidis* and thus amplification with other fungal species was not found. However, the primer pair for *F. oxysporum* did show amplification with other species, but since amplification of other fungal species only occurred after more than 10 cycles the amplification was not considered as *F.* oxysporum. Also, the melt curve values of the amplified product were also different from the *F. oxysporum* values. New primer pairs have been designed for a second time for *F. chlamydosporum*, *Trichoderma* spp., *Phoma* spp., *Pythium* spp. and *Macrophomina phaseolina* after species specific amplification was not obtained with primers found in literature or with the first primer pairs that were designed.

PROBLEMS ENCOUNTERED

National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) website was not found to be a reliable source for evaluating species specificity and extensive testing of primers were required to ensure species specificity and delivering an optimised qPCR protocol.

NEMATOLOGY

DETAILS

PROJECT NUMBER		M151/12
PROJECT TITLE		Yield loss and host plant resistance as a management tool for the management of root-knot nematodes on maize
PROJECT MANAGER		Dr S Steenkamp
CO-WORKER(S)	Internal	L Bronkhorst, SS Kwena, R Jantjies
	External	Prof AH Mc Donald, Prof H Fourie (North-West University)
PROJECT STATUS		Continue
DURATION		01/04/2013 to 31/03/2016

ACTIONS TAKEN TO DATE

Glasshouse trials

Four glasshouse trials have been planted to date to screen maize, sunflower, oats and wheat cultivars obtained from various companies to root-knot nematodes. Screenings included 38 maize cultivars and 22 sunflower cultivars inoculated with *Meloidogyne incognita* and 19 oat cultivars and 30 wheat cultivars inoculated with *M. javanica*.

Small field trial

A small-field crop rotation trial was established by planting a susceptible maize cultivar (DKC8010) and inoculated with a mixed *M. incognita* and *M. javanica* nematode population.

Microplot trials

Two microplot trials were planted to quantify yield losses and nutritional value of a susceptible maize cultivar (DKC8010) and inoculated with eight inoculation levels of *M. incognita* and of *M. javanica*, respectively.

Radio talks

- Steenkamp, S. 2013. Monsterneming vir aalwurmbesmetting. *RSG Landbou* 15 May 2013.
- Steenkamp, S. 2014**.** Simptome van aalwurmbesmetting. *RSG Landbou* 5 February 2014.

Popular articles

- Steenkamp, S., 2013. Plant parasitiese aalwurms op mielies. Mielie inligtingsgids (MIG). Saamgestel deur die LNR-IGG MIG. Launched at NAMPO May 2013.
- Steenkamp, S., 2014. Plant parasitiese aalwurms op mielies. Mielie inligtingsgids (MIG). Saamgestel deur die LNR-IGG MIG.
- Steenkamp, S., 2014. Monstername vir plant parasitiese aalwurms op mielies. Mielie inligtingsgids (MIG). Saamgestel deur die LNR-IGG MIG.
- Steenkamp, S., 2014. Aalwurmsimptome: Waarna moet jy oplet? *SA Graan/SA Grain*, Vol 16(2) Februarie 2014 pp. 36 - 38.

Farmers days presentations

- Steenkamp, S., 2013. Triomf, Potchefstroom. Plant-parasitiese aalwurms op mielies. 18 July 2013. 80 Triomf agents.
- Steenkamp, S., 2013. TLU, Rietfontein. Aalwurms: Wat elke boer moet weet. 29 August 2013. 60 farmers.
- Steenkamp, S., 2013. Manna Workgroup, Vierfontein. Aalwurms: Wat elke boer moet weet. 2 September 2013. 60 farmers.
- Steenkamp, S., 2013. Nampo Park, Bothaville. Aalwurms: Wat elke boer moet weet. 10 September 2013. 80 farmers.

Analytical services

To date samples were received from 51 clients and recommendations were made. Crops involved included mostly maize or crops used in rotation with maize.

PROGRESS MADE

Glasshouse trials

Data from the four glasshouse trials have been subjected to statistical analysis and interpreted.

Small field trial
A small-field crop rotation trial was established by planting a susceptible maize cultivar (DKC8010) and inoculated with a mixed *M. incognita* and *M. javanica* nematode population. This trial will be harvested in April/May.

Microplot trials
Nematode data has been collected and are in the process of being counted and subjected to statistical analysis. Harvest data will be collected during April/May.

RESULTS ACHIEVED TO DATE
Glasshouse trials

1) Maize cultivars (obtained from Pioneer, Monsanto, Sensako and Pannar)
   Most maize cultivars showed potential resistance to *M. javanica* and will be re-evaluated in the new season to confirm these results. (F-Ratio = 3.38; P-Value 0.0000).

2) Sunflower cultivars (obtained from Sensako, Agricol, Pannar and Syngenta)
   No potential *M. javanica*-resistance was identified in sunflower cultivars tested. (F-Ratio = 1.59; P-Value = 0.0778).

3) Wheat cultivars (obtained from ARC-SGI, Pannar and Sensako)
   Most wheat cultivars showed potential resistance to *M. incognita* and will be re-evaluated in the new season to confirm these results (F-Ratio = 23.96; P-Value 0.0000).

4) Oat cultivars (Obtained from Pannar and Sensako)
   Most oat cultivars showed potential resistance to *M. incognita* and will be re-evaluated in the new season to confirm these results (F-Ratio = 5.77; P-Value 0.0000).

Small field trial
First data from this project is in the process of being collected.

Microplot trials
First data from this project is in the process of being collected.

PROBLEMS ENCOUNTERED
Glasshouse problems encountered were resolved.

TECHNOLOGY TRANSFER

DETAILS

PROJECT NUMBER		M181/10
PROJECT TITLE		Maize Information Guide (MIG)
PROJECT MANAGER		SM James
CO-WORKER(S)	Internal	MA Prinsloo, SH Ma'ali, D de V Bruwer, AA Nel, AEJ Saayman-du Toit, MM van der Walt, E Hugo, B Janse van Rensburg, BC Flett, W Deale, UM Du Plessis, A Schoeman, E Ncube, M Craven, A Erasmus
	External	None
PROJECT STATUS		Continue
DURATION		01/04/2001 - 31/03/2015

ACTIONS TAKEN TO DATE

The ARC-GCI received many requests from industry to launch the MIG earlier for the farmers to take more informed decisions in the outset of the season. For this reason, the MIG 2014 information was compiled through the months of October to December and the designing thereof took place in the months preceding. The MIG 2014 was printed in the first week in March and successfully launched at the Grain SA Congress at Bothaville on 6 March 2014. The launch took place in the presence of media, stakeholders and interested public and received positive reviews in magazines such as Landbouweekblad and SA Grain. This year 4 500 copies of the booklets were printed. Nearly, 2300 copies will be posted to SA Grain members alone. The remaining copies will be sent out to various farmers and organisations on the address list as compiled by the ARC. The MIG 2014 is also made available to the public on the ARC website.

PROGRESS MADE

The Maize Information Guide has been launched earlier than usual and will be distributed to farmers and stakeholders shortly. A meeting will be held in May to discuss the MIG 2015 and to finalize what new and relevant information will appear for the 2015 guideline.

RESULTS ACHIEVED TO DATE

The booklet was successfully completed and will be distributed to all relevant stakeholders in due time. The advertisers who had advertised in the MIG 2014 will make payments for adverts placed in the new financial year.

PROBLEMS ENCOUNTERED

No significant problems were encountered during the report period. Due to the early release of the MIG the financial implication was that both the 2013 and 2014 MIG was completed in the same financial year which had budget implications.