DETAILS

PROJECT NUMBER		M141/10
PROJECT TITLE		Integrated control of maize ear rots
PROJECT MANAGER		BC Flett
CO-WORKERS	Internal	WG Khali, B Janse van Rensburg, TM Ramusi, A Schoeman, E Ncube
	External	Medical Research Council (MRC), University of Free State, University of Stellenbosch, Plant Research International, Wageningen, Netherlands, North West University.
PROJECT STATUS		Complete
DURATION		01/04/1999 to 31/03/2010

GENERAL INTRODUCTION

Ear rots of maize in South Africa are caused by *Stenocarpella maydis, S. macrospora*, *Fusarium verticillioides*, *F. proliferatum*, *F. subglutinans*, and *F. graminearum*. These pathogens occur world-wide, but vary in prevalence and importance from area to area (Booth, 1973; Booth & Waterston, 1964a, b; Sutton & Waterston, 1966a, b). In South Africa, ear rot epidemics are sporadic and may result in severe yield reductions and downgrading of grain. In addition to the effect on yield and grain quality, maize ear rot pathogens are known to produce mycotoxins. Grain infected with S.*maydis* can kill rats and ducklings (Rabie *et al.,* 1977) or cause diplodiosis in sheep and cattle (Marasas, 1977). Consumption of *F. verticillioides*-infected grain may cause leucoencephalomalacia in Equidae (Coetzer *et al.,* 1985) while high incidences of human oesophageal cancer in Transkei have been associated to the intake of maize meal containing *F. verticillioides* (Kriek *et al.,* 1977). Zearalenone produced by *F. graminearum* has an oestrogenic effect on pigs (Bryden *et al.,* 1987).

Symptoms caused by ear rot pathogens vary markedly. Symptoms of S. *maydis* ear rot are initially a yellowing and drying of infected leafbracts on the green maize plant. Growth of the white fungal mycelium generally starts at the ear base and may infect the entire ear (Fig. 1). On breaking an infected ear pycnidia are exposed at the kernel bases (Fig. 2). Symptomless infections may occur when kernel moisture is low. Discoloured kernel embryos are obvious when such ears are broken transversely. Symptoms caused by S. *macrospora* are similar to those of S. *maydis* (Fig. 3). However, on breaking an infected ear, no pycnidia are evident. Two symptom types of *F. verticillioides* occur in the field. The first is growth of white-pink cottony mould on kernels where insect or bird damage is apparent (Fig. 4). A second symptom is pink discolouration in undamaged kernels (Fig. 5). Symptomless infections are, however, most common and are not identified during grain grading (Flett *et al.,* 1996). Symptoms of *F. subglutinans* ear rot cannot be distinguished from *F. verticillioides* ear rot and infected material has to be plated on medium to determine the casual organism. *F. graminearum* ear rot symptoms are obvious when infection begins at either the base or the tip, covering the kernels with a dark red mould (Fig. 6). Early infections result in total rotting of ears with husks adhering tightly to the ear (Flett *et al.,* 1996).

Effective and economical control of maize ear rot can only be achieved using an integrated approach based on resistant hybrids. However, resistance breeding is a long-term programme, and reactions of local hybrids to maize ear rots have not yet been adequately quantified. Chemical control of ear rots would not be economically viable in commercial fields due to high production costs. Chemical control has been used with limited success in seed production (D.C. Nowell, Pannar Seed Co. - personal communication). Reduced tillage systems have been shown to increase levels of S. *maydis* ear rot but not those caused by *F. verticillioides* and *F. subglutinans* (Flett & Wehner, 1991). However, the advantages of reduced tillage, which include soil moisture retention (Berry *et a!.,*1985), constant soil temperatures (Onderdonk & Ketcheson, 1973),maintenance of pH (Doran, 1980), improved soil structure (Duley, 1957; McCalla, 1958) and lower production costs (Mannering & Fenster, 1983) make this control measure undesirable and alternative sanitation (reduction of initial inoculum) measures require study.

The present study was undertaken to investigate hybrid resistance and various production practices that could contribute to an integrated ear rot control programme. Due to the amount of work and subjects covered only the abstracts have been included in this final report. Reprints/theses are available on request.

INCIDENCE OF EAR ROT PATHOGENS UNDER ALTERNATING CORN TILLAGE PRACTICES (1998)

The efficacy of periodic plowing in reduced-tillage fields in reducing corn ear rot caused by *Stenocarpella maydis, Fusarium verticillioides, F subglutinans,* and *F graminearum* was determined over three seasons at Bloekomspruit, South Africa. A positive linear relationship was recorded for Stenocarpella ear rot incidence and surface stubble mass. Moldboard plow plots consistently had lower stubble mass and Stenocarpella ear rot incidence than did reduced tillage practices. A cross-moldboard plow applied after one, two and three seasons of reduced tillage reduced stubble mass and Stenocarpella ear rot incidence in the respective season only. Stenocarpella ear rot incidence increased during the subsequent season in which the original tillage practices were again applied. Alternating tillage practices would therefore not reduce Stenocarpella ear rot in the long term. Reduced disease incidence can only be achieved by moldboard plowing during each season. Alternating tillage practices had no effect on ear rots caused by *Fusarium* spp. during all seasons.

INCIDENCE OF *STENOCARPELLA MAYDIS* EAR ROT OF CORN UNDER CROP ROTATION SYSTEMS (2001)

The efficacy of crop rotation in reducing corn ear rot caused by *Stenocarpella maydis* in reduced and conventional tillage systems was determined over five and four seasons, respectively, at two sites in South Africa. Stenocarpella ear rot and S. *maydis* were isolated from kernels more frequently in monoculture maize and crop rotation where maize was planted for two consecutive seasons than where monoculture maize was interrupted by a rotation crop. Surface stubble mass, and consequently inoculum pressure, were affected similarly by crop rotation. Positive linear relationships were recorded between Stenocarpella ear rot incidence, surface stubble mass, and pycnidial counts. Wheat, soybean, and peanut were the most effective, and sunflower the least effective, rotation crops for reducing S. *maydis* ear rot.

VARIATION IN THE IDENTIFICATION OF *FUSARIUM* SPP. IN MAIZE SAMPLES DUE TO ENUMERATOR AND GROWTH MEDIUM (2008)

Fusarium spp. have variable phenotypic and morphological features when cultured on different media. Inaccurate identifications could result in variation in the quantification of *Fusarium* spp. associated with ear rot of maize when two or more researchers identify the same *Fusarium* isolates based on morphological characteristics. Two hundred and fifty kernels from maize samples collected from each of five localities were surface-sterilized and plated on selective rose Bengal-glycerine-urea (RbGU) medium from which isolates were subsequently transferred to split plates containing Carnation Leaf Agar (CLA) and Potato

Dextrose Agar (PDA) for further identification. Three different enumerators independently identified and enumerated *Fusarium* spp. on RbGU and CLA/PDA media to determine the relative frequency of *Fusarium* spp. within maize samples, to compare the accuracy of species identification on different media and to detect and quantify bias among enumerators (inter-enumerator reliability). It was concluded that enumerators were consistent in the identification and quantification of *F. verticillioides* and *F. subglutinans* on both RbGU and CLA/PDA media. *F. proliferatum* enumerations yielded an interaction between locality and enumerator with no significant differences for media. The significant locality x enumerator interaction for *F. proliferatum* suggests that enumerators had difficulty to distinguish between *F. proliferatum* and *F. verticillioides.* The use of RbGU and/or CLA/PDA media were eliminated as a possible source of variation in the identification of *Fusarium* spp. The inter-enumerator reliability study indicated that *Fusarium* spp. enumerations by three different enumerators were reliable and accurate and were not a source of variation in the quantification of *Fusarium* spp. associated with maize ear rot.

QUANTITATIVE DETECTION OF FUMONISIN-PRODUCING *FUSARIUM* SPP. AND ITS CORRELATION WITH FUMONISIN CONTENT IN MAIZE FROM SOUTH AFRICAN SUBSISTENCE FARMERS (2008)

A quantitative detection tool was developed to enable the monitoring of fumonisin-producing fungi in food and feed commodities. To this end, a quantitative PCR (TaqMan) was developed that targets a conserved region in the polyketide synthase gene *fum1*, which is involved in the biosynthesis of fumonisin. Hence, this method specifically detected isolates from the fumonisin-producing species *Fusarium verticillioides*, *F. proliferatum*, *F. nygamai* and *F. globosum* whereas isolates of the fumonisin non-producing species *F. equiseti*, *F. graminearum*, *F. oxysporum*, *F. semitectum* and *F. subglutinans* that commonly occur on maize were not detected. Moreover, a few fumonisin non-producing *F. verticillioides* isolates did not generate any fluorescent signals and were therefore not detected. The correlation between quantitative PCR and mycotoxin content was determined using field samples collected at homestead farms in South Africa. Among 40 samples from the Eastern Cape collected in 2005 a good correlation (R^2=0.8303) was found between pg fungal DNA and fumonisin content. A similar correlation (R^2=0.8658) was found among 126 samples collected from four provinces in South Africa in 2007. These observations indicate that samples containing ≥ 40 pg fungal DNA/mg sample are suspected of also exceeding the 1 mg/kg total fumonisin level and therefore do not comply with the European Commission limit for fumonisins B_1+B_2 for maize intended for direct human consumption that applies from 1 October 2007. Combined with the very high maize intake, our results indicate that fumonisin levels in maize from South African homesteads regularly exceed the tolerable daily intake for fumonisins.

SAMPLING VARIATION IN THE QUANTIFICATION OF FUMONISINS IN MAIZE SAMPLES (2011)

Fumonisins produced by *F. verticillioides* and *F. proliferatum* cause mycotoxicoses in horses, swine and rats and have been associated with oesophageal cancer in humans. Accurate measurement of mycotoxins is essential for determining the safety of grain and their products for consumption. Four sources of variation were studied, namely sub-sample size, variation within a single maize sub-sample, number of replicates and toxin detection techniques used by independent laboratories. Variation in detected fumonisin levels within a single maize sample was high using the 25-g sub-samples proposed in the Neogen Veratox protocols. A 250-g sub-sample significantly reduced variation in fumonisin levels of samples. An incremental increase in sample size also improved the number of positive samples recorded. Increasing the number of replicates using the recommended sub-sample size (25 g) did not reduce variation except when the sample had high fumonisin levels. Improved accuracy was recorded when a 250-g sub-sample was used in conjunction with increased replicates. Data from laboratory analyses indicated that ELISA reactions (Agricultural Research Council – Grain Crops Institute) correlated significantly with HPLC results of the Medical Research Council (MRC), but neither of these correlated with results from an independent laboratory. Concentrations determined using ELISA were consistently higher than those from the HPLC (MRC) technique. Quantification technique, sample size, replicate number and laboratory where analyses are conducted, appear to be important sources of variation for quantification of fumonisins.

FUSARIUM SPP. AND LEVELS OF FUMONISINS IN MAIZE PRODUCED BY SUBSISTENCE FARMERS IN SOUTH AFRICA (2011)

Fusarium spp. produce fumonisins - mycotoxins that are of importance to maize production in South Africa. Fumonisins have been associated with human oesophageal cancer and cause various diseases in animals that are of concern to the animal feed industry. Maize samples, collected from subsistence farm fields in the Eastern Cape, KwaZulu-Natal, Limpopo and Mpumalanga provinces of South Africa during the 2006 and 2007 growing seasons, were analysed for *Fusarium* spp. and contamination with fumonisins. *Fusarium verticillioides* was the most common *Fusarium* species in maize followed by *F. subglutinans* and *F. proliferatum*. Levels of contamination with fumonisins ranged from 0 µg/g to 21.8 µg/g, depending on the region where samples were collected. Levels of fumonisins were highest in northern KwaZulu-Natal (Zululand) where 52 % and 17 % of samples collected in 2006 and

2007, respectively, exceeded 2 μg/g. Regression analyses showed a positive correlation between fumonisin-producing *Fusarium* spp. determined by real-time polymerase chain reaction and concentration of fumonisins (r = 0.93). Many samples from Zululand, and some from Mokopane (Limpopo) and Lusikisiki (Eastern Cape), contained fumonisins at levels well above the maximum levels of 2 μg/g set by the Food and Drug Administration (USA) and therefore also the limit of 1 μg/g set by the European Union for food intended for direct human consumption. Regulations governing contamination of grain with fumonisins are not yet implemented in South Africa. The high incidence of fumonisins in subsistence farming systems indicates the need for awareness programmes and further research.

RESISTANCE IN MAIZE INBRED LINES TO *FUSARIUM VERTICILLIOIDES* AND FUMONISIN ACCUMULATION *(SUBMITTED)*

Fusarium ear rot of maize, caused by *Fusarium verticillioides*, is an important disease affecting maize production world-wide. Apart from reducing yield and grain quality, *F. verticillioides* produces toxic secondary metabolites, called fumonisins, which have been associated with mycotoxicoses of animals and humans. Currently, no maize breeding lines are known with resistance to *F. verticillioides* in South Africa. The objective of this study, therefore, was to evaluate 24 genetically diverse maize inbred lines as potential sources of resistance to Fusarium ear rot and fumonisin accumulation in grain. The inbred lines were evaluated in field trials under natural infection conditions at a Potchefstroom location in 2006/07, and through artificial silk channel inoculation with *F. verticillioides* isolate MRC 826 at Potchefstroom and Vaalharts during 2007/08. An artificially inoculated greenhouse trial consisting of seven potentially resistant and five highly susceptible lines chosen according to field results from 2008 was carried out in 2009. Visual rating of Fusarium ear rot symptoms was performed and fumonisins B_1, B_2 and B_3 in grain kernels quantified with high performance liquid chromatography tandem mass spectrometry for all trials. Insufficient infection took place under natural infection conditions in Potchefstroom during 2007 with mean visual symptom ratings ranging from 0.1 ± 0.1 % to 4.2 ± 8.3 %, which prevented the differentiation of resistant from susceptible inbred lines. Artificial inoculation with *F. verticillioides* in 2008 increased the level and consistency of infection with a range of mean visual symptoms for inbred lines from 0.2 ± 0.1 % to 26.5 ± 7.8 % at the Potchefstroom location, and 0.2 ± 0.2 % to 24.0 ± 9.2 % at Vaalharts. Toxin contamination levels varied between locations with mean fumonisin content of inbred lines planted at Potchefstroom ranging from 0.5 ± 0.1 mg kg^{-1} to 7.5 ± 5.2 mg kg^{-1}, while the fumonisin content of inbred lines planted at Vaalharts ranged from 2.6 ± 0.8 mg kg^{-1} to 61.1 ± 33.5 mg kg^{-1} (B_1, B_2 and B_3). This resulted in sufficient differentiation between susceptible and potentially resistant inbred lines based on visual rating of Fusarium ear rot symptoms and fumonisin content. The greenhouse trials produced highly conducive conditions for infection and led to significant differentiation between susceptible and resistant lines that correlated with field results. Maize inbred lines with resistance to *F. verticillioides* would provide the southern African maize industry with major opportunities to develop maize cultivars with resistance to the pathogen and the fumonisins produced by it.

We are still working on a chapter from Ian Smalls thesis which may result in another paper on induced resistance of maize on Fusarium ear rots and fumonisins.

GENERAL DISCUSSION

This study has clarified a number of issues regarding the integrated management of maize ear rot. Various aspects of sanitation to reduce the nutrient base on which pathogens survive, and hence the initial inoculum levels, were studied for inclusion into an integrated disease management system with resistance. The incidence and spread of ear rots and mycotoxins in both commercial and subsistence farming systems was determined and high risk areas identified.

Resistance of local maize genotypes to ear rot was successfully quantified for the first time. Previous quantifications of ear rot resistance resulted in inconsistent results over seasons and localities (Du Toit & Nordier, 1989, 1990, 1991; Klapproth & Hawk, 1991) which made hybrid comparisons under different climatic conditions and inoculum levels unreliable. Inconsistent reactions were explained by the nonlinear response of hybrids to ear rot over various disease potentials due to G x E interactions. The latter was defined as the mean ear rot severity in a hybrid evaluation trial within a season. All maize genotypes evaluated were to a greater or lesser extent susceptible to ear rot, depending on disease potential. Hybrids differed in the rate of resistance breakdown with increasing disease potential. The use of standard hybrids for measurement of disease potential may add consistency to the quantification of disease potential and requires further study. The different reactions of hybrids to S. *maydis* ear rot resistance but not so for *F. verticillioides* ear rot, implies that local hybrids can be used in an integrated control programme for certain ear rots but not others.

Resistance in USA and local sweetcorn maize hybrids to *Fusarium verticillioides* and *Fusarium subglutinans* kernel infections was identified (Headrick & Pataky, 1989; Holley *et al.,* 1989; Rheeder *et al.,* 1990). Locality effects have been reported to confound genetic effects (Holley *et al.,* 1989) and repeatability of hybrid reactions (King & Scott, 1981). However, in the current study the relationship between the incidence of infected ears, quantification of fumonisin producing fungal tissue (real-time PCR) and fumonisins varied over localities. This implies that genotype screening for resistance to *F. verticillioides* and *F. subglutinans must be conducted* using a number of localities and seasons. Genotypic resistance to *F. verticillioides* ear rots differ significantly. The efficacy of local hybrids in the control of *F. verticillioides* is presently limited and of restricted value in an integrated control programme. Sources of resistance to ear rot caused by *Fusarium* spp. identified in this study and by others (King & Scott, 1981; Holley *et al.,* 1989) may be used in future breeding programmes, may improve levels of resistance in local hybrids.

The role of sanitation in the control of S. *maydis* ear rot was studied. The reduction in S. *maydis* ear rot associated with stubble removal has previously been reported (Kerr, 1965; Flett & Wehner, 1991). The reluctance of producers to revert to ploughing to reduce stubble due to the advantages of reduced tillage resulted in further studies on practices which could reduce S. *maydis* inoculum. Alternating of maize tillage systems reduced stubble, inoculum and S. *maydis* incidence in the season that ploughing was practiced. Reversion to the former reduced tillage system resulted in a rapid increase in stubble, inoculum and S. *maydis* incidence. Thus, the use of alternating tillage systems to control S. *maydis* ear rot was unsuccessful and persistent mouldboard ploughing is the only tillage system which significantly reduces S. *maydis* ear rot.

The efficacy of crop rotation in reducing S. *maydis* ear rot was studied and reported for the first time. The success of crop rotation in the control of a pathogen depends on the pathogen's ability to infect alternative hosts and/or the time needed for inoculum reduction (Curl, 1963). The inability of S. *maydis* to infect other major commercial crops in local maize production areas fulfils the first requirement stated by Curl (1963) for successful control. Similarly, alternating non-host crops with maize reduced S. *maydis* ear rot incidence in comparison with monoculture maize and crop rotation systems where two seasons of maize were followed by the rotation crop. The linear relationships between ear rot incidence and surface stubble mass for both the alternating tillage and crop rotation systems were previously reported (Flett & Wehner, 1991) and emphasised the role of sanitary measures for control of S. *maydis* ear rot.

Sanitation was unsuccessful in the control of ear rot caused by *Fusarium verticillioides* and *F. subglutinans.* Fusarium ear rots were previously reported not to be affected by tillage practice (Flett & Wehner, 1991). The effect of alternating tillage systems on incidence of fusarium ear rot was non-significant. *Fusarium* spp. overwinter on host residues (Nyvall & Kommedahl, 1970; Bolkan *et al.,* 1979; Manzo & Claflin, 1984; Skoglund & Brown, 1988) both below and above the ground and thus the burying of stubble will not necessarily reduce inoculum.

Results from the study have shown that use of sanitation practices and resistant hybrids could play a major role in controlling maize ear rots and mycotoxins. Effective breeding programmes can improve the levels of resistance and recent studies indicated a definite shift towards improved resistance in new releases (personal observation). Sanitation measures must be applied each season to control inoculum levels as S. *maydis* inoculum build-up is rapid. Due to the wide host range and ubiquitous nature of *F. verticillioides* and *F.*

subglutinans (Booth & Waterston, 1964a, b) crop sanitation is not a viable control option for controlling these pathogens. However, as available sources of resistance, identified in this study, to fusarium ear rot (King & Scott, 1981; Holley *et al.,* 1989) become included in breeding programmes, resistance may play an important role in disease control. The timeous control of *B. fusca* stalkborer damage should also maintain low fusarium ear rot levels. The integration of various control measures to control maize diseases is important but decisions should be made bearing specific localities and climatic conditions in mind.

REFERENCES

ANONYMOUS, 1996. Maize Board Annual Report. Maize Board, Pretoria, South Africa.

ANONYMOUS, 1997. Maize Board Annual Report. Maize Board, Pretoria, South Africa.

BERRY, W.A.J., MALLET, J.B. & JOHNSTON, M.A., 1985. Soil-water conservation as affected by primary tillage practices. South African Journal of Plant and Soil 2: 21 - 26.

BOLKAN, H.A., DIANESE, J.C. & CUPERTINO, F.P., 1979. Survival and colonization potential of *Fusarium Moniliforme* var. *subglutinans* in soil. Phytopathology 69: 1 298 - 1 301.

BOOTH, C., 1973. *Gibberella zeae* (Conidial State: *Fusarium graminearum).* C.M.I. Descriptions of pathogenic fungi and bacteria. Number 384, Commonwealth Agricultural Bureaux, Kew, Surrey, England.

BOOTH, C. & WATERSTON, J.M., 1964a. *Gibberella fujikuroi* var. *subglutinans. C*.M.I. Descriptions of pathogenic fungi and bacteria. Number 22, Commonwealth Agricultural Bureaux, Kew, Surrey, England.

BOOTH, C. & WATERSTON, J.M., 1964b. *Gibberella fujikuroi:* C.M.I. Descriptions of pathogenic fungi and bacteria. Number 23, Commonwealth Agricultural Bureaux, Kew, Surrey, England.

BRYDEN, W.L., LOVE, R.J. & BURGESS, L.W., 1987. Feeding grain contaminated with *Fusarium graminearum* and *Fusarium moniliforme* to pigs and chickens. Australasian Veterinary Journal 64: 225 - 226.

COETZER, J.A.W., KELLERMAN, T.S. & NAUDE, T.W., 1985. Neurotoxins of livestock caused by plants and fungi in southern Africa. Technical Communication No 199, Department of Agriculture, RSA. 138 pp.

CURL, E.A., 1963. Control of plant diseases by crop rotation. The Botanical Review 29: 413 - 479.

DAVIS, R.M., KEGEL, F.R., SILLS, W.M. & FARRAR, J.J., 1989. Fusarium ear rot of corn. Californian Agriculture 43: 4 - 5.

DORAN, J.W., 1980. Soil microbial and biochemical changes associated with reduced tillage. Soil Science Society of America Journal 44: 765 - 771.

DULEY, F.L., 1957. Stubble mulching in the great plains. Journal of Soil and Water Conservation 12: 7 - 11.

DU TOIT, W., 1997. Handleiding vir die verbouing van mielies in die Somerreënvalgebied. Landbounavorsingsraad - Instituut vir Graangewasse. 74 pp.

DU TOIT, W. & NORDIER, G.A., 1989. National cultivar trials with commercial maize cultivars. Department of Agricultural Development. Republic of South Africa.

DU TOIT, W. & NORDIER, G.A., 1990. National cultivar trials with commercial maize cultivars. Department of Agricultural Development. Republic of South Africa.

DU TOIT, W. & NORDIER, G.A., 1991. National cultivar trials with commercial maize cultivars. Department of Agricultural Development. Republic of South Africa.

FLETT, B.C., BENSCH, MJ., SMIT, E. & FOURIE, H., 1996. A field guide for identification of maize diseases in South Africa. Agricultural Research Council - Grain Crops Institute. 68pp.

FLETT, B.C. & WEHNER, F.C., 1991. Incidence of Stenocarpella and Fusarium cob rots in monoculture maize under different tillage systems. Journal of Phytopathology 133: 327 - 333.

HEADRICK, IM. & PATAKY, J.K., 1989. Resistance to kernel infection by *Fusarium moniliforme* in inbred lines of sweet corn and the effect of infection on emergence. Plant Disease 73: 887 - 892.

HOLLEY, R.N., HAMILTON, P.B. & GOODMAN, M.M., 1989. Evaluation of tropical maize germplasm for resistance to kernel colonization by *Fusarium moniliforme.* Plant Disease 73: 578 - 580.

KERR, W.E., 1965. Ear and cob rot diseases of maize. Rhodesian Agricultural Journal 62:1 - 23.

KING, S.B. & SCOTT, G.E., 1981. Genotype differences in maize to kernel infection by *Fusarium moniliforme.* Phytopathology 71: 1 245 - 1 247.

KLAPPROTH, J.C. & HAWK, J.A., 1991. Evaluation of four inoculation techniques for infecting corn ears with *Stenocarpella maydis.* Plant Disease 75: 1 057 - 1 060.

KOEHLER, B., 1942. Natural mode of entrance of fungi into corn ears and some symptoms that indicate infection. Journal of Agricultural Research 64: 421 - 442.

KRIEK, N.P.J., MARASAS, W.F.O., STEYN, P.S., VAN RENSBURG, S.J. & STEYN, M., 1977. Toxicity of a moniliformin-producing strain of *Fusarium moniliforme* var. *subglutinans* isolated from maize. Food Cosmetic and Toxicology Journal 15: 579 - 587.

LE ROUX, P.M., 1988. *Diplodia* cob rot. Proceedings of the 8th South African Maize Breeding Symposium. Department of Agriculture and Water Supply Technical Communication 222.

MANNERING, J.V. & FENSTER, C.R., 1983. What is conservation tillage? Journal of Soil and Water Conservation 38: 141 - 143.

MANZO, S.K. & CLAFLIN, L.E., 1984. Survival of *Fusarium moniliforme* hyphae and conidia in grain sorghum stalks. Plant Disease 68: 866 - 867.

MARASAS, W.F.O., 1977. The genus Diplodia pp. 119 - 128 *in:* T.D. Wyllie & L.G. Morehouse (eds). Mycotoxic fungi, mycotoxins, mycotoxicoses. An encyclopedic handbook. Marcel Dekker, New York.

McCALLA, T.M., 1958. Microbial and related studies of stubble mulching. Journal of Soil and Water Conservation 13: 255 - 258.

NELSON, S.W., 1988. *Diplodia.* Proceedings of the 8th South African Maize Breeding Symposium. Department of Agriculture and Water Supply Technical Communication 222.

NYVALL, R.F. & KOMMEDAHL, T., 1970. Saprophytism and survival of *Fusarium moniliforme* in corn stalks. Phytopathology 60: 1 233 - 1 235.

ONDERDONK, J.J. & KETCHESON, J.W., 1973. Effect of stover mulch on soil temperature, corn root weight and phosphorus fertilizer uptake. Proceedings of the Soil Science Society of America 37: 904 - 906.

RABIE, C.J., VAN RENSBURG, S.J., KRIEK, N.PJ. & LüBBEN, A., 1977. Toxicity of *Diplodia maydis* to laboratory animals. Applied Environmental Microbiology 34: 111 - 114.

RHEEDER, J.P., MARASAS, W.F.O., VAN WYK, P.S., DU TOIT, W., PRETORIUS, AJ. & VAN SCHALKWYK, D.J., 1990. Incidence of *Fusarium* and *Diplodia* species and other fungi in naturally infected grain of South African maize cultivars. Phytophylactica 22: 97 - 102.

SKOGLUND, J.G. & BROWN, W.M., 1988. Effects of tillage regimes and herbicides on *Fusarium* species associated with corn stalk rot. Canadian Journal of Plant Pathology 10: 332 - 338.

SMELZER, D.G., 1959. Relationships between Fusarium ear rot and corn earworm infestation. Agronomy Journal 51: 53 - 64.

SUTTON, B.C. & WATERSTON, J.M., 1966a. *Diplodia macrospora*. C.M.I. Description of Pathogenic Fungi and Bacteria Number 83, Commonwealth Agricultural Bureaux, Kew, Surrey, England.

SUTTON, B.C. & WATERSTON, J.M., 1966b *Diplodia maydis.* C.M.I. Description of Pathogenic Fungi and Bacteria Number 84, Commonwealth Agricultural Bureaux, Kew, Surrey, England.

ACHIEVEMENTS OF PROJECT M141/10

EXTENSION MANUALS

FLETT, B.C. 1999. Kopvrotweerstand. MIG '99. Compiled by ARC-Grain Crops Institute, Potchefstroom.

FLETT, B.C. 2000. Control of major maize diseases. MIG 2000. Compiled by ARC-Grain Crops Institute, Potchefstroom.

FLETT, B.C. 2007. Maize disease situation during the recent season. MIG 2007. Compiled by ARC-Grain Crops Institute, Potchefstroom.

FLETT, B.C., JANSE VAN RENSBURG, B., & NCUBE, E. 2008. Maize disease and mycotoxin situations during recent seasons. MIG 2008. Compiled by ARC-Grain Crops Institute, Potchefstroom.

FLETT, B.C. 2009. Maize diseases during the 2008/09 season. MIG 2009. Compiled by ARC-Grain Crops Institute, Potchefstroom.

FLETT, B.C. 2010. Maize diseases during the 2009/10 season.MIG 2010. Compiled by ARC-Grain Crops Institute, Potchefstroom.

POPULAR ARTICLES

FLETT, B.C. Kopvrotsiektes benadeel graanopbrengs. *Landbouweekblad*. Augustus 1999.

FLETT, B.C. Diplodia kopvrot moeilik om te bestry. *Landbouweekblad bylaag*. 25 Augustus 2000.

FLETT, B.C. Fusarium kopvrot 'n sluimerende gevaar. *Landbouweekblad bylaag*. 25 Augustus 2000.

FLETT, B.C. Diplodia resistance in maize and selection of hybrids. *SA Graan/SA Grain*, Oktober 2000.

FLETT, B.C. Gee mieliekopvrot nekslag voordat dit stoom opbou. *Landbouweekblad bylaag*. 24 Augustus 2001.

FLETT, B.C. Diplodia ear rot of maize and its control. *The Farmer (Zimbabwe)*. 4 - 10 September 2001.

FLETT, B.C. Maize our staple food: Should toxins concern us? http://www.scienceinafrica.co.za/2001/november/maize.htm. 12 October 2001.

FLETT, B.C. Mieliepap: Is daar enige gevare? *SA Graan/SA Grain*, Februarie 2002.

FLETT, B.C. Downgrading of maize caused by ear rot fungi. *SA Graan/SA Grain*, September 2006.

FLETT, B.C. Behoud van stoppels het risiko's. *Landbouweekblad*. 2 April 2010. pp. 36 - 38.

FLETT, B.C. Bogrondse mieliesiektes onder bewaringsboerdery. *SA Graan/SA Grain*, Februarie 2011. pp. 28 - 31.

FLETT, B.C. & JANSE VAN RENSBURG, B. Beginsels vir die geintegreerde beheer van gewassiektes. *Ons Eie*. Februarie 2008.

FLETT, B.C. & JANSE VAN RENSBURG, B. Belangrikste mieliesiektes in Noordwes en Vrystaat. *Ons Eie*. April 2008.

FLETT, B.C. & SCHULTZ, A. Diplodia steek weer kop uit. *Landbouweekblad*. 2 Julie 2010.

FLETT, B.C. & SMALL, I. Reducing maize mycotoxins in the production chain. *SA Graan/SA Grain*, August 2009.

FLETT, BC. & SNYMAN, A.J. Combating Cobrot. *Farmer's Weekly*. 8 July 1988.

JANSE VAN RENSBURG, B. & FLETT, B.C. Vat mikotoksiene vas en verseker veilige voedsel. *Landbouweekblad*. 11 November 2005.

JANSE VAN RENSBURG, B. & FLETT, B.C. Bestuurspraktyke vir die vermindering van toksiene in mielieproduksie. *SA Graan/SA Grain*, September 2006.

JANSE VAN RENSBURG, B. & FLETT, B.C. Zearalenone - possibly the most significant mycotoxin threat. *SA Graan/SA Grain*, January 2010.

JANSE VAN RENSBURG, B. & FLETT, B.C. Natural occurrence of Fusarium verticillioides and fumonisins: surveys help to detect possible threats. *SA Graan/SA Grain*, September 2010. pp 88 - 89.

JANSE VAN RENSBURG, B. & FLETT, B.C. Mieliekopvrot- en fumonisienopnames verbeter voedsel veiligheid. *SA Koop*, Vol 28 no. 4. April/Mei 2011. pp. 10-12.

NCUBE, E. & FLETT, B.C. Mycotoxins: a potential threat to food safety in Africa. *Farmers Weekly*. 9 February 2007.

NCUBE, E. & FLETT, B.C. Bt technology and its implications for mycotoxin contamination of grain. *SA Graan/SA Grain*, May 2011. pp 58.

SCIENTIFIC ARTICLES

BOUTIGNY, A., WARD, T.J., VAN COLLER, G.J., FLETT, B.C., LAMPRECHT, S.C., O'DONNELL, K. & VILJOEN, A. 2011. Analysis of the Fusarium graminearum species complex from wheat, barley and maize in South Africa provides evidence of species-specific differences in host preference. *Fungal Genetics and Biology*. (In press).

FLETT, B.C., N.W. McLAREN & F.C. WEHNER, 2001. Incidence of *Stenocarpella maydis* ear rot of corn under crop rotation systems. *Plant Disease*. 85: 92 - 94.

JANSE VAN RENSBURG, B., B.C. FLETT, N.W. McLAREN & A.H. McDONALD. 2008. Variation in the identification of *Fusarium* spp. in maize samples due to enumerator and growth medium. *South African Journal of Plant and Soil*. 25: 105 - 110.

NCUBE E., FLETT B.C., WAALWIJK C. & VILJOEN A. 2011. Fusarium spp. and levels of fumonisins in maize produced by subsistence farmers in South Africa. *SA Journal of Science*. 107:1 - 7. ISI=0.42

VAN RENSBURG, J.B.J. & FLETT, B.C. 2010. A review of research achievements on maize stem borer, *Busseola fusca* (Fuller) and Diplodia ear rot caused by *Stenocarpella maydis* (Berk. Sutton). *South African Journal of Plant and Soil*. 27: 74 - 80.

WAALWIJK, C., KOCH, S.H., NCUBE, E., ALLWOOD, J., FLETT, B.C., DE VRIES, I. & KEMA, G.H.J. 2008. Quantitative detection of Fusarium spp. and its correlation with fumonisin content in maize from South African subsistence farmers. *World Mycotoxin Journal*. 1: 39 - 47.

CONGRESSES and SYMPOSIA

McLAREN, N.W. & FLETT, B.C. (1992). The role of non-linearity of the disease x environment relationship in resistance screening. Proceedings of the 10th South African Maize Breeding Symposium, Summer Grain Centre, Potchefstroom.

FLETT, B.C. McLAREN, N.W. & WEHNER, F.C. (1999). Incidence of *Stenocarpella maydis* ear rot under alternating maize tillage systems. Proceedings of the 37th South African Society of Plant Pathology Congress, Pietermaritzburg.

FLETT, B.C., RHEERDER, J.P. LEGOTT, N.L. & STOCKENSTRöM, S. (2002). Damage to maize ears and concomitant incidence of *Fusarium verticillioides* and fumonisin. Proceedings of the 40th Southern Africa Society of Plant Pathology Congress. Dikholholho, Brits.

SMIT, E. & FLETT, B.C. (2002). Incidence of *Fusarium* spp., section Liseola, and associated mycotoxins from maize grain silos. Proceedings of the 40th South African Society of Plant Pathology Congress, Dikholholho, Brits.

PURCHASE, J.L., FLETT, B.C., CILLIERS, A.J. & VERMAAK, N.J. (2003). Producers perspective on mycotoxins. 17th SAAFOST International Congress and Exhibition, CSIR, Pretoria.

FLETT, B.C. (2004). Preparation and inoculation of maize plants with *Stenocarpella maydis* to screen for resistance to Diplodia ear rots. Proceedings of the fifth Plant Breeding Symposium, San Lameer.

VISMER, H.F., RHEEDER, J.P., VD WESTHUIZEN L., IMRIE, G.C., SHEPHARD, G.S., MARASAS, W.F. & FLETT, B.C. (2005). Effect of Bt corn hybrids on insect damage, incidence of fumonisin-producing Fusarium species and fumonisin levels in South Africa. APS 2005 Annual Meeting, Austin, Texas. 30 July - 03 August 2005.

JANSE VAN RENSBURG, B., FLETT, B.C. & McDONALD, A.H. (2006). Sources of variation in the quantification of fumonisin concentrations in maize samples. Proceedings of the 44th South African Society of Plant Pathology Congress, Magalies Park.

JANSE VAN RENSBURG, B., FLETT, B.C., McLAREN, N.W. & McDONALD, A.H. (2006). Sources of variation in the detection of fumonisins in maize samples. Myco-Globe International Conference on "Advances on genomics, biodiversity and rapid systems for detection of toxigenic fungi and mycotoxins". Monopoli, Bari, Italy. 26 - 29 September 2006. (poster)

JANSE VAN RENSBURG, B., FLETT, B.C., McLAREN, N.W. & McDONALD, A.H. (2008). Distribution and quantification of *Fusarium verticillioides* in South African maize and its effect on grain quality and toxicity. Proceedings of the Combined Congress, Grahamstown.

JANSE VAN RENSBURG, B., FLETT, B.C., McLAREN, N. W. & McDONALD, A.H. (2008). Variance in fumonisin quantification in maize samples in South Africa using the ELISA Veratox protocol. 10th International Fusarium and Fusarium Genomics Workshop. Alghero, Italy. 30 August - 2 September 2008. (poster)

NCUBE, E., FLETT, B.C. & VILJOEN, A. (2008). Mycotoxin levels in subsistence farming systems in South Africa. Proceedings of the Combined Congress, Grahamstown.

NCUBE, E., FLETT, B.C., WAALWIJK, C. & VILJOEN, A. (2008). Geographic distribution of *Fusarium* spp. and production of fumonisins in maize of subsistence farmers in South Africa. 10th International Fusarium and Fusarium Genomics Workshop. Alghero, Italy. 30 August - 2 September 2008. (presentation)

SMALL, I., FLETT, B.C., MARASAS, W.F.O., MCLEOD, A. & VILJOEN, A. (2009). Potential resistance in South African maize inbred lines to *Fusarium verticillioides* and fumonisin accumulation. Proceedings of the 46th South African Society of Plant Pathology Congress. Villa Via Hotel, Gordons Bay.

SMALL, I., FLETT, B.C., McLEOD, A., MARASAS, W.F.O. & VILJOEN, A. (2009). Resistance in South African maize inbred lines to *Fusarium verticillioides* and fumonisin accumulation. International Society for Mycotoxicology Conference, Tulln, Austria, 9 - 11 September

BELGROVE, A., FLETT, B.C. & VILJOEN, A. (2010). Comparing methods for measuring the pathogenicity and toxicity of *Fusarium verticillioides* in maize. Proceedings of the Combined Congress, Bloemfontein.

JANSE VAN RENSBURG, B., FLETT, B.C., VILJOEN, A. & McLAREN, N.W. (2010). Genotype x environment interactions associated with fumonisin contamination of commercial maize. 8th SAPBA Symposium 15 - 17 March 2010, Spier Estate, Stellenbosch.

NCUBE, E., FLETT, B.C., WAALWIJK, C. & VILJOEN, A. (2010). Fumonisin and aflatoxin contamination of maize and groundnut in South African subsistence farming systems. 8th SAPBA Symposium 15 - 17 March 2010, Spier Estate, Stellenbosch.

ROSE, L.J., SLABBERT, J.P., VAN DER VYVER, C. FLETT, B.C. & VILJOEN, A. (2010). Radiation breeding: A potentially valuable application in a plant breeders toolbox. 8th SAPBA Symposium 15 - 17 March 2010, Spier Estate, Stellenbosch.

SMALL, I., FLETT, B.C., MARASAS, W.F.O., McLEOD & VILJOEN, A. (2010). Resistance in maize inbred lines to Fusarium verticillioides and fumonisin accumulation. 8th SAPBA Symposium 15 - 17 March 2010, Spier Estate, Stellenbosch.

BELGROVE, A., FLETT B.C. & VILJOEN A. (2011). Pathogenicity of Fusarium Ear Rot (Fusarium verticillioides) isolates over two seasons on different maize cultivars using two inoculation techniques. Proceedings of the 47th Southern African Society for plant Pathology Congress. Berg ‘n Dal, Kruger National Park. 23 - 26 January 2011.

BELGROVE, A., FLETT B.C. & VILJOEN A. (2011). Genetic diversity and fumonisin production in a South African population of *Fusarium verticillioides.* Mycored Africa. Cape Town, South Africa. 4 - 6 April 2011. (poster).

JANSE VAN RENSBURG B., FLETT B.C., VILJOEN A. & MC LAREN N.W. (2011). The occurrence of fumonisin spp, and their fumonisins from commercial maize production areas in Southern Africa. Proceedings of the 47th Southern African Society for Plant Pathology Congress . Berg ‘n Dal, Kruger National Park. 23 - 26 January 2011.

JANSE VAN RENSBURG B., FLETT B.C., VILJOEN A. & MC LAREN N.W. (2011). Development of an epidemiological model of fumonisin-producing Fusarium spp. on commercial maize in South Africa. Mycored Africa. Cape Town, South Africa. 4 - 6 April 2011. (poster)

NCUBE E., FLETT B.C., VAN RENSBURG J.B.J**.,** VAN DEN BERG J. & VILJOEN A. (2011). The interactive effect of *Busseola fusca* and *Fusarium verticillioides* on fusarium ear rot and

production of fumonisins in maize. Proceedings of the 47th Southern African Society for Plant Pathology Congress . Berg 'n Dal, Kruger National Park. 23 - 26 January 2011.

ROSE, L.J., , J.P., VAN DER VYVER, C. FLETT, B.C. & VILJOEN, A. (2011). Optimizing gamma irradiation dosages to induce resistance in maize for control of Fusarium verticilliodes and its fumonisins. Proceedings of the 47th South African Society of Plant Pathology Congress, Berg 'n Dal, Kruger National Park. 23 - 26 January 2011.

SMALL, I., FLETT, B.C., MARASAS, W.F.O., MCLEOD, A. & VILJOEN, A. (2011). Use of resistance elicitors to reduce Fusarium ear rot and fumonisin accumulation in maize. Proceedings of the 47th South African Society of Plant Pathology Congress, Berg 'n Dal, Kruger National Park. 23 - 26 January 2011.

INVITED PAPERS AND SEMINARS

FLETT, B.C. (1999). Disease triangle and maize ear rots. Crop Protection Course. Potchefstroom.

FLETT, B.C. (1999). Maize ear rots. PANNAR Information day. Kroonstad.

FLETT, B.C. (2000). Beheer van Diplodia kopvrot. Mooiveld Boerevereeniging. Wesselsbron.

FLETT, B.C. (2000). Beheer van Diplodia kopvrot. Loskuil Boerevereeniging. Bothaville.

FLETT, B.C. (2000). Maize ear rots, storage diseases and mycotoxins. Western Transvaal pigfarmers study group, Klerksdorp.

FLETT, B.C. (2001). Important diseases of maize in South Africa. Pioneer Information Day, Pretoria.

FLETT, B.C. (2001). Control of maize ear rots. Banket farmers day, Banket, Zimbabwe.

FLETT, B.C. (2001). Control of maize ear rots. SeedCo Seed Growers Farmers' Day, Harare, Zimbabwe.

FLETT, B.C. (2001). Control of maize ear rots. No-till club Farmers' Day, Winterton.

FLETT, B.C. (2002). Most Important Maize Diseases. PANNAR Information Course, Potchefstroom.

FLETT, B.C. (2003). No tillage and maize diseases. No till club meeting, Bethlehem.

FLETT, B.C. (2003). Maize diseases. Monsanto Course, Potchefstroom.

FLETT, B.C. (2003). Maize diseases in the Northern Free State. Hertzogville Farmers' Day, Hertzogville.

FLETT, B.C. (2004). Maize diseases. Pioneer Course, Potchefstroom.

FLETT, B.C. (2004). Maize diseases. SA Grain Developing Farmers Course, Nampo, Bothaville.

FLETT, B.C. (2004). Maize diseases. SA Grain Developing Farmers Course, Nampo, Bothaville.

FLETT, B.C. (2005). Resistance to important maize diseases. Monsanto seminar, Petit.

FLETT, B.C. (2007). International tendencies in Fusarium and mycotoxin research. Maize Forum, Pretoria.

FLETT, B.C. (2007). Report on international Fusarium and mycotoxin meetings. Fusarium Discussion Group, Plant Protection Research Institute, Stellenbosch.

FLETT, B.C. (2008). Belangrikste mieliesiektes in Suid Afrika. Yara Opleiding, Villiers.

FLETT, B.C. (2009). Belangrikste mieliesiektes. Bayagro Opleiding (BAYER), Protea Hotel, Christiana.

FLETT, B.C. (2009). International tendencies regarding maize mycotoxins. Maize Forum, Pretoria.

FLETT, B.C. (2010). Analysis of non-linear G X E interactions in disease evaluation, Scientific Workshop SASPP Free State Branch, Stonehenge, Parys.

FLETT, B.C. (2010). Belangrikste mieliesiektes. Soetdoring Boere-Unie, Coligny.

FLETT, B.C. (2010). Belangrikste mieliesiektes. Monsanto - Sardiensvlei boeredag.

FLETT, B.C. (2010). Diplodia ear rot of maize. Grain SA - cooperative information day - Standerton.

FLETT, B.C. (2010). Mycotoxin research at ARC-GCI in collaboration with various Institutions. (Representatives from GSA) - Potchefstroom.

FLETT, B.C. (2010). Diplodia ear rot of maize. Pioneer information day - Bergville.

FLETT, B.C. (2010). Ear rot of maize - emphasis on Diplodia ear rot. Namib Milling Agronomy Information Day. Otavi, Namibia.

FLETT, B.C. (2011). Belangrikste mieliesiektes. Monsanto - Delmas boeredag.

FLETT, B.C. (2011). Belangrikste mieliesiektes. Summerfield, Namibia - boeredag.

FLETT, B.C. (2011). Belangrikste mieliesiektes. Grootfontein, Namibia - boeredag.

RADIO TALKS

FLETT, B.C. March 4, 2009. Maize disease situation this season - RSG (Hennie Maas).

FLETT, B.C. May 27, 2009. Mycotoxins on maize in South Africa - RSG (Hennie Maas).

FLETT, B.C. July 28, 2010. Effect of ear rots on maize grain quality - RSG (Gerrit Bezuidenhout).

FLETT, B.C. March 9, 2011. 2011 Maize disease situation - RSG (Lisa Roberts).

TV BROADCASTS

April 2000 - Diplodia kopvrot beheer.

POSTGRADUATE SUPERVISION

Belinda J. van Rensburg (2007). Distribution and quantification of *Fusarium verticillioides* in South African maize and its effect on grain quality and toxicity. M.Sc. Thesis, North West University.

Edson Ncube (2008). Mycotoxin levels in subsistence farming systems in South Africa. M.Sc. thesis, Stellenbosch University.

Ian Small (2010). Resistance in maize to Fusarium verticillioides and fumonisin. M.Sc. Thesis, Stellenbosch University.