SS 3

Table 2. Analysis of variance on the impact of various cropping systems on disease severity at 21, 70 and 100 days after planting (DAP), over two localities (Ventersdorp and Viljoenskroon) as evaluated over two seasons (2012/13 and 2013/14).

	21DAP	70 DAP	100 DAP
Loc	<.0001	0.3991	0.0661
Year	<.0001	<.0001	0.3520
Year*Loc	<.0001	0.6048	0.1424
Tmt	0.5038	0.0103	0.2537
Loc*Tmt	0.8959	0.2639	0.2540
Year*Tmt	0.9609	0.0390	0.0560
Year*Loc*Tmt	0.9966	0.1857	0.0802
PlantPart	0.1656	<.0001	<.0001
Loc*PlantPart	0.5606	0.1092	0.0408
Year*PlantPart	0.0010	<.0001	0.0001
Year*Loc*PlantPart	0.0008	0.1582	0.0040
Tmt*PlantPart	0.3548	0.495	0.2260
Loc*Tmt*PlantPart	0.2641	0.0062	0.2888
Year*Tmt*PlantPart	0.9585	0.3724	0.6406
Year*Loc*Tmt*PlantPart	0.5480	0.0416	0.6205

Table 4. Analysis of variance on the effect of different cropping systems on the frequency (%) of fifteen soilborne pathogens as measured over two localities (Viljoenskroon and Ventersdorp) and two years (2012/13 and 2013/14) in both the root and crowns of maize plants 70 days after planting.

	Alternaria spp.	*Aspergillus spp.*	*Fusarium clamydosporum*	*Fusarium culmorum*	*Fusarium graminearum*	*Fusarium oxysporum*	*Fusarium scirpi*	*Fusarium semitectum*	*Fusarium solani*	*Fusarium subglutinans*	*Fusarium verticillioides*	*Rhizoctonia spp.*	*Trichoderma spp.*	*Macrophomina phaseolina*	*Stenocarpella maydis*
Loc	0.2181	0.0034	0.0244	0.0618	0.8121	0.0009	0.0136	0.0745	0.1023	0.003	0.6179	0.0324	0.1242	0.0147	0.3370
Year	0.0207	0.0796	0.0090	0.0169	0.2742	0.7845	0.0076	0.0141	0.9574	0.0124	0.0065	0.4006	0.0993	0.3351	0.3370
Year*Loc	0.3667	0.0359	0.1401	0.1605	0.1516	0.3224	0.0410	0.1833	0.3746	0.0076	0.0134	0.1578	0.8373	0.6869	0.3370
Tmt	0.1406	0.5947	0.3302	0.3132	0.7776	0.6804	0.4304	0.1439	0.3649	0.5057	0.0182	0.0047	0.1261	0.8350	0.4040
Loc*Tmt	0.1416	0.2097	0.2899	0.4485	0.7746	0.1182	0.2942	0.5141	0.3113	0.2571	0.0862	0.0047	0.2835	0.8567	0.4040
Year*Tmt	0.0217	0.8930	0.1678	0.5514	0.6205	0.7151	0.5894	0.3422	0.3919	0.4315	0.3914	0.0001	0.2093	0.7050	0.4040
Year*Loc*Tmt	0.0013	0.9941	0.2192	0.2429	0.2410	0.8827	0.3145	0.1940	0.1339	0.1922	0.4847	0.0004	0.5875	0.8868	0.4040
PlantPart	0.7676	0.0007	0.3864	0.0052	0.3572	0.0048	0.7964	0.9178	0.0090	0.0380	<.0001	0.0188	<.0001	0.0028	0.3223
Loc*PlantPart	0.9921	0.0051	0.2853	0.2150	0.0455	0.0106	0.2466	0.0081	0.1841	0.0536	0.9835	0.0019	0.0087	0.0035	0.3223
Year*PlantPart	0.2088	0.0988	0.5273	0.0056	0.4985	0.4106	0.7332	0.0287	0.4359	0.0900	0.0287	0.2300	0.0001	0.2610	0.3223
Year*Loc*PlantPart	0.0659	0.0957	0.7867	0.3730	0.0570	0.2532	0.5709	0.2271	0.2733	0.0972	0.0369	0.7143	0.3892	0.1790	0.3223
Tmt*PlantPart	0.4097	0.5325	0.2650	0.6332	0.8778	0.3424	0.1156	0.5737	0.4919	0.2109	0.0178	0.0039	0.5805	0.7618	0.4010
Loc*Tmt*PlantPart	0.3746	0.2492	0.2913	0.2297	0.6452	0.7004	0.1141	0.3648	0.6357	0.2824	0.0880	0.0170	0.6945	0.8586	0.4010
Year*Tmt*PlantPart	0.2476	0.9845	0.9073	0.5315	0.3318	0.0873	0.0870	0.4418	0.7658	0.3195	0.6027	0.0007	0.2937	0.6986	0.4010
Year*Loc*Tmt*PlantP	0.8679	0.9956	0.9614	0.4718	0.9109	0.2684	0.1318	0.4528	0.0848	0.3736	0.1398	<.0001	0.4447	0.2821	0.4010

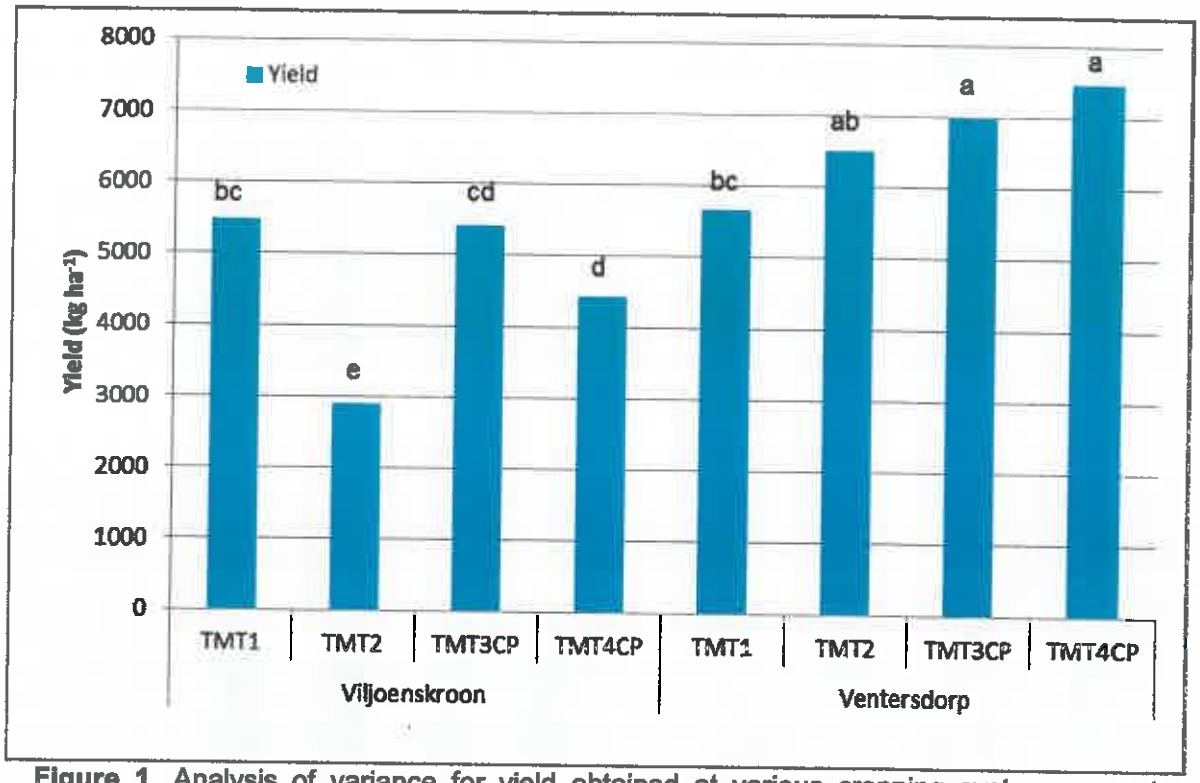

Figure 1. Analysis of variance for yield obtained at various cropping systems over two localities (Viljoenskroon and Ventersdorp) during two seasons (2012/13 and 2013/14) combined (P=0.05)

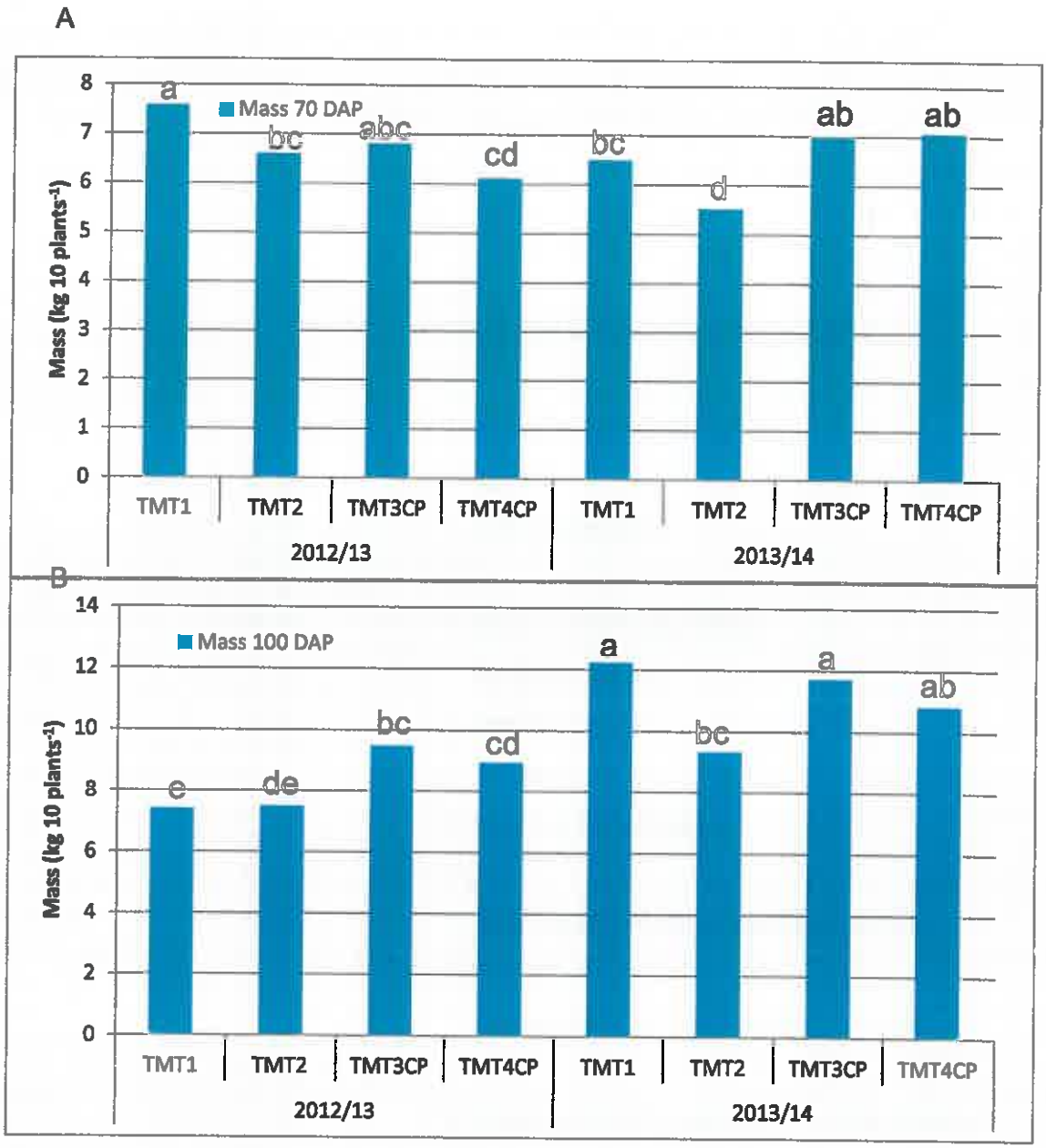

Figure 3. Analysis of variance for the effect of various cropping systems on the plant mass of 10 randomly selected plants at 21, 70 and 100 DAP as measured over seasons (2012/13 and 2013/14) (localities combined).

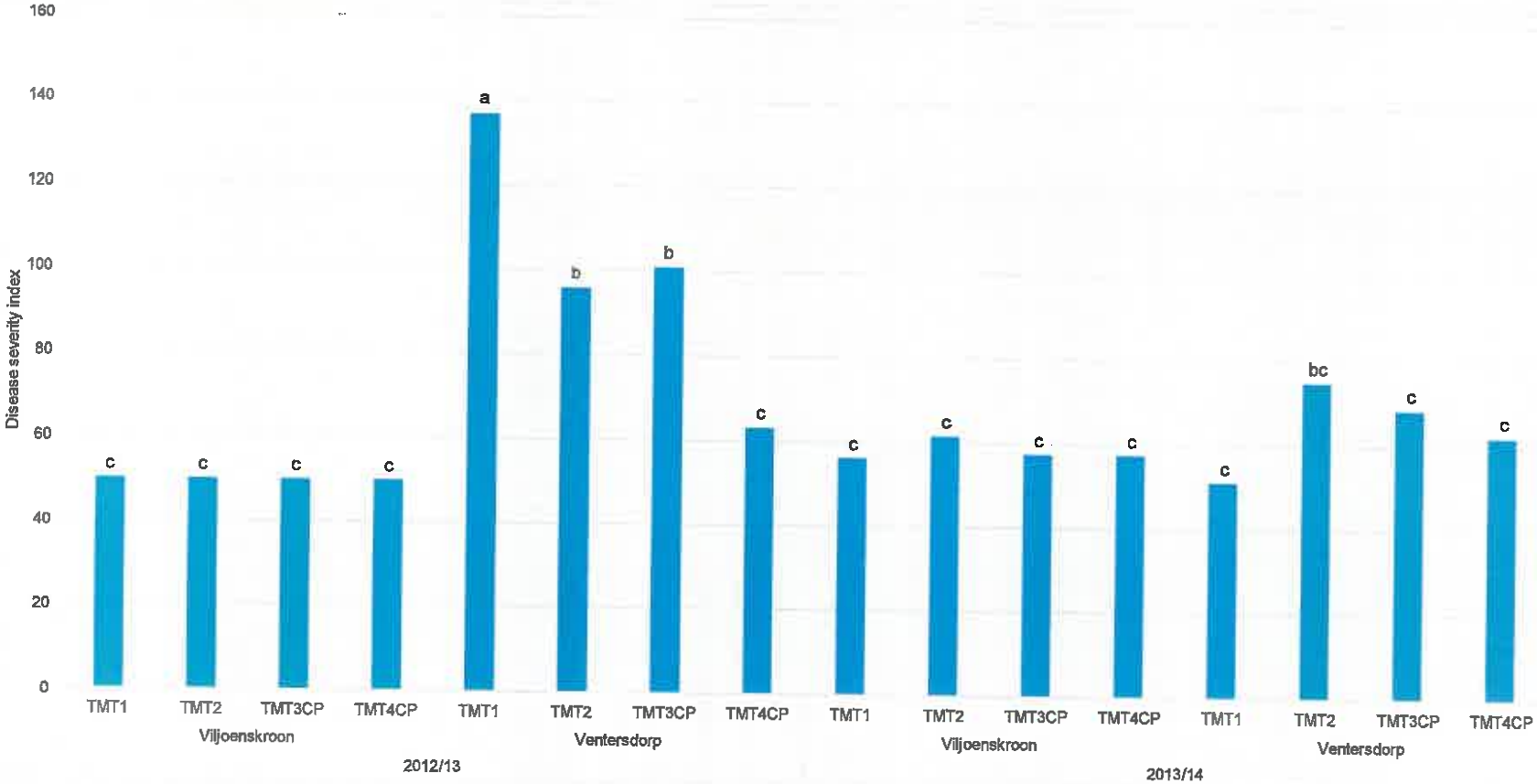

Figure 5. Analysis of variance for disease severity at 100 DAP obtained at various cropping systems over two localities (Viljoenskroon and Ventersdorp) during two seasons (2012/13 and 2013/14) combined (P=0.1)

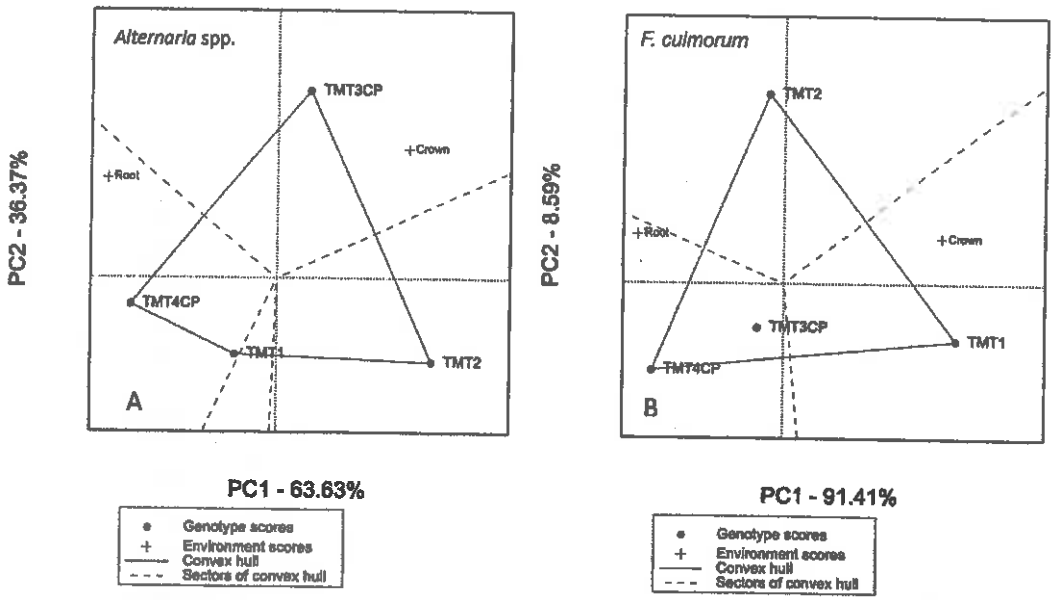

Figure 7. Biplot analysis on the impact that treatment had on the frequencies of *Alternaria spp.* and *F. culmorum* with regard to the plant part (root or crown) from which it was isolated from at 21 days after planting.

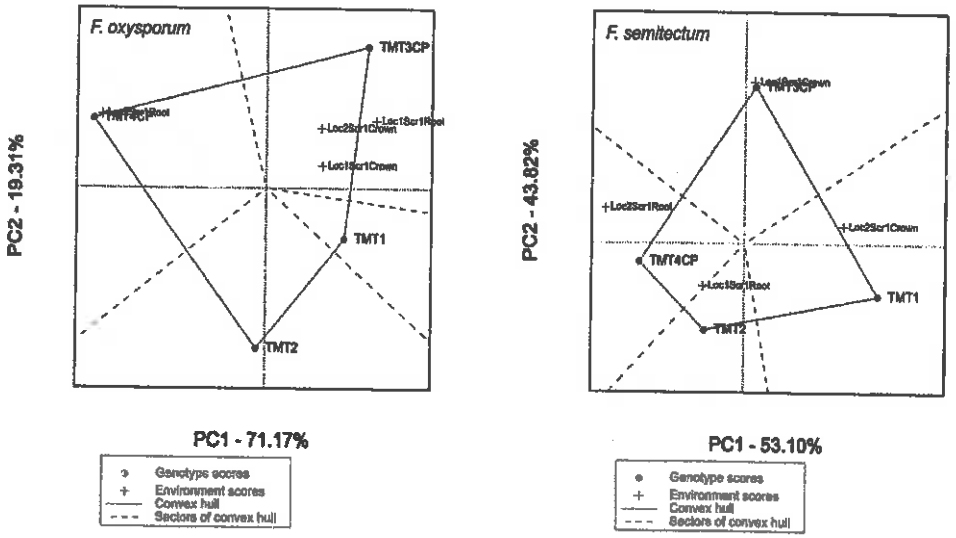

Figure 8. Biplot analysis on the impact that treatment had on the frequencies of *F. oxysporum* and *F. semitectum* with regard to the locality and plant part (root or crown) from which it was isolated from over two localities at 21 days after planting. (Y5 - 2012/13; Y6 - 2013/14; Scr1 - Screening at 21 days after planting).

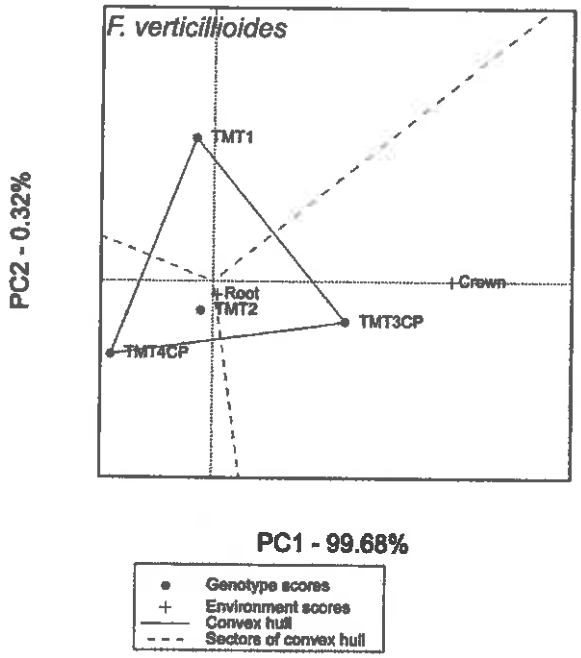

Figure 11. Biplot analysis on the impact that treatment had on the frequencies *of F. verticillioides* with regard to the plant part (root vs. crown) from which it was isolated from 70 days after planting.

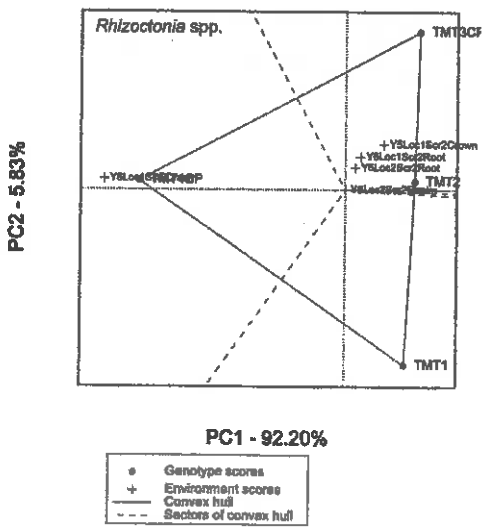

Figure 12. Biplot analysis on the impact that treatment had on the frequencies of *Rhizoctonia* spp. with regard to the season, locality and plant part (root or crown) from which it was isolated format 70 days after planting. (Y5 - 2012/13; Y6 - 2013/14; Loc1 - Viljoenskroon; Loc2 - Ventersdorp; Scr2 - screening at 70 days after planting)

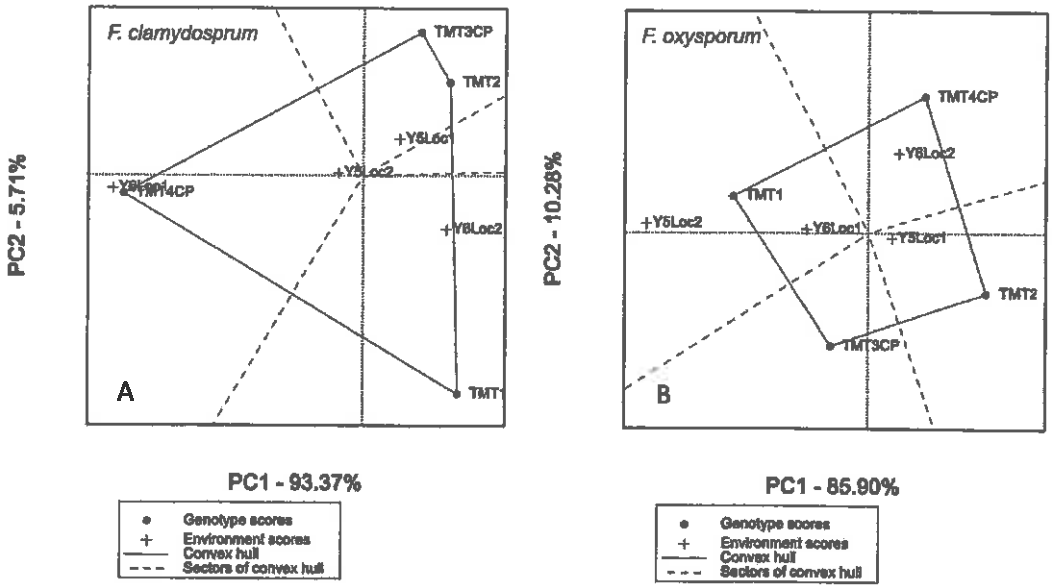

Figure 15. Biplot analysis on the impact that treatment had on the frequencies of *F. clamydosporum* and *F. oxysporum* with regard to the season and locality from which it was isolated from 100 days after planting. (Y5 - 2012/13; Y6 - 2013/14; Loc1 - Viljoenskroon; Loc2 - Ventersdorp)

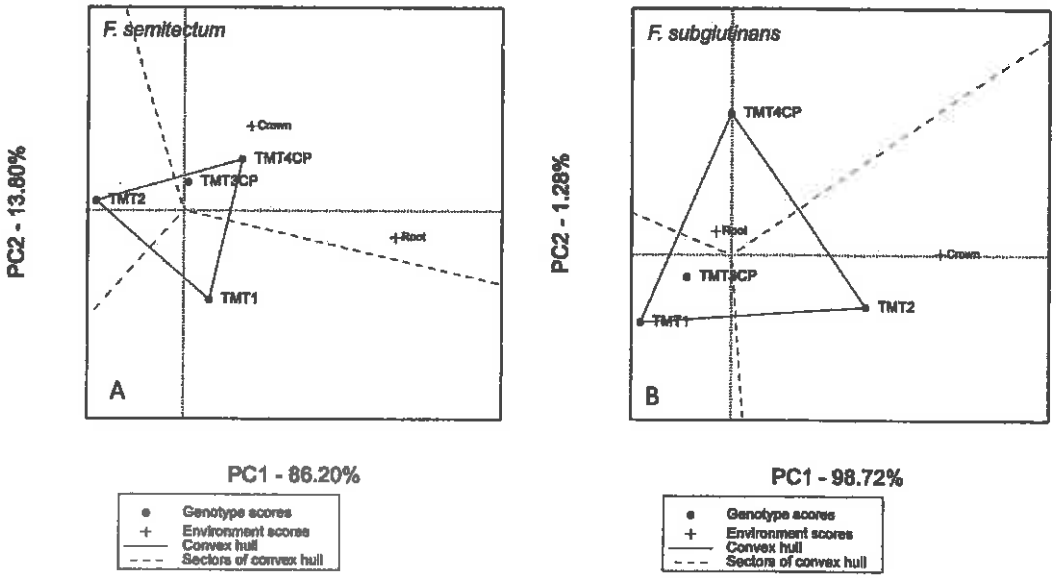

Figure 16. Biplot analysis on the impact that treatment had on the frequencies of *F. semitectum* and *F. subglutinans* with regard to plant part (root vs. crown) from which it was isolated from 100 days after planting.

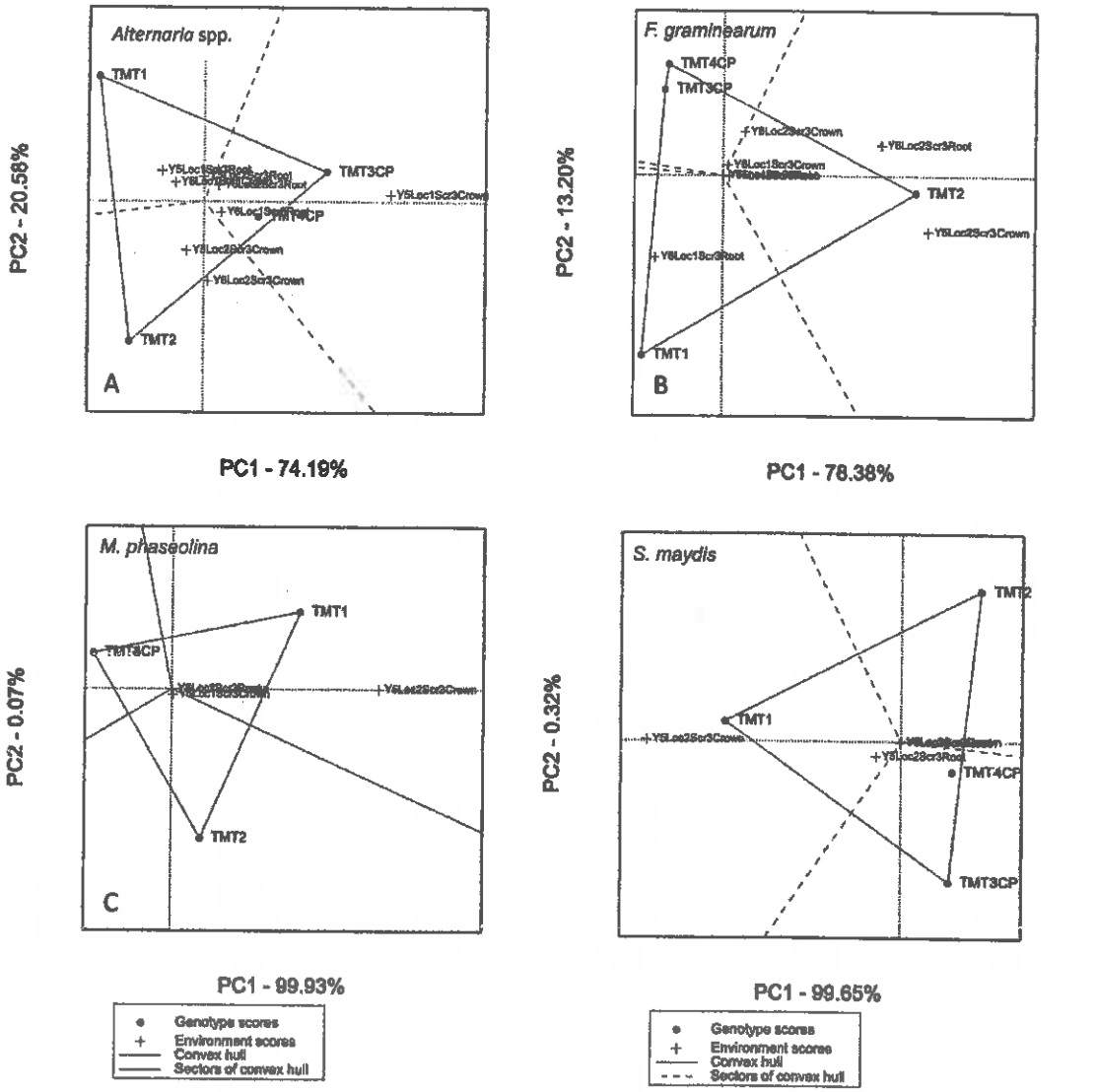

Figure 18. Biplot analysis on the impact that treatment had on the frequencies of *Alternaria spp. F. graminearum, M. phaseolina* and *S. maydis* with regard to the season, locality and plant part (roots vs. crowns) from which it was isolated from 100 days after planting. (Y5 - 2012/13; Y6 - 2013/14; Loc1 - Viljoenskroon; Loc2 - Ventersdorp; Scr3 - sampling at 100 days after planting).

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remains a problem in every field, every year, with the only variable being severity (White, 1999). It is well known that soilborne fungi that cause root and stalk rots are responsible for yield decreases in monoculture maize worldwide (Lipps, 1988, Sumner and Bell, 1982; Sumner *et al.*, 1990, Williams and Schmitthenner, 1963). Root rot is accordingly also very common in the major maize producing areas of South Africa (Du Toit, 1968) since many producers are practicing monoculture maize without using an alternative crop (Lamprecht *et al.*, 2006). Although crop rotation is often associated with healthier root systems (Cook, 1993), some crop rotation systems might result in more severe root rot on maize, as observed by Nel and Lamprecht (2011) in canola-maize rotation systems.

Root and crown rot is the result of infection by a complex of fungi (Nel and Lamprecht, 2011, White, 1999). The incidence and severity of this complex is affected by soil type, tillage and crop rotation systems, as influenced by locality as well as seasonal effects (Lamprecht *et al.*, 2006, Smith and Van Rensburgh, 1997). White (1999) listed *Fusarium* spp. *Pyrenochaeta terrestris, Pythium* spp. and *Rhizoctonia* spp. to be the most important pathogens associated with soilborne diseases. Studies conducted under South African conditions which focused on fungi isolated under various cropping systems listed some of the more prominent fungi isolated from root rots to be *Exserohilum pedicellatum, F. equiseti, F. oxysporum, F. solani, Macrophomina phaseolina, Phoma* spp.and *Pyrenochaeta terrestris,Trichoderma* spp. (Nel and Lampbrecht, 2011, Smit and Van Rensburg, 1997).

Quantification of the true impact associated with root and crown rot on yield is problematic, due to the difficulty of obtaining a healthy control (White, 1999). Nel and Lamprecht (2011) were, however, able to demonstrate a yield decline of 1,81 t ha^{-1} for each unit increase in root rot severity, whilst Lamprecht *et al.* (2006) demonstrated that maize yield could potentially increase with nearly 2 t ha^{-1} in the absence of soilborne diseases (under no-till, wheat-maize rotational systems in the Winterton area of KwaZulu-Natal).

Due to the complexity and unpredictability of root and crown rot, the ultimate goal for the management of the disease would be the development of cultivation practices that would result in, or contribute to, healthier root systems. Anhydrous ammonia (AA) is considered to be a good source of fertilizer N as it has a very high nitrogen content of approximately 82% (Jackson and Chang, 1947, Tisdale and Nelson, 1967). A direct effect of AA application to soil is an observed increase in soil pH. AA has amongst some other effects also a biocidal effect killing fungi as well as some soil fauna (Andrews, 1956, Huber, 1991, Tenuta and Lazarovits, 2002). Such prospects accordingly created the possibility that soilborne diseases could be managed to some extent with the use of AA (Eno and Blue, 1954). Anhydrous ammonium application would, however, also

During 2010, both treatments were administered on 23/11/2010. The two trials were planted on 30/11/2009 and 29/11/2010, respectively.

Photo 1. Anhydrous Ammonia application at Buffelsvallei (Ventersdorp, North West province).

Photo 2. Methyl bromide application at Buffelsvallei (Ventersdorp, North West province).

conducted with regard to the fungal frequencies obtained. The results indicated that fungal frequencies differed between season, SD and plant part from which it was isolated from. It was accordingly decided to report on the split-plot analysis conducted on the different SD over the two seasons separately, with treatment being the main effect and the plant part (root or crown) from which the isolation was made the sub-plot effect. Student's t-LSD (Least Significant Difference) was calculated at a 5% significance level to compare means. Correlation between fungal species/genera identified and mass, disease severity and yield was calculated for seasons x SD x plant part individually.

Canonical variate analysis (CVA) was conducted to establish possible grouping in the various treatments as well as to determine which of the fungi that were identified over the course of the study were responsible for the observed grouping. The analysis was conducted separately for fungi isolated (i.e. fungal frequencies) from each plant part (roots or crowns) at a specific SD, as observed over the two seasons combined. It was important to compare fungal frequencies over the two seasons in order to determine if the observed effect on mass might be attributed to one or more fungi. Seventeen fungal genera were selected based on their frequency of occurrence within the trials as well as known association with disease development (indicated in table 2 with an asterisk - *). All data analyses were done using the GenStat statistical program (Version 14, Payne *et al.*,2011).

3. Results

3.1. Yield

Statistical analysis conducted over the separate seasons indicated that none of the treatments significantly affected maize yield in either of the two seasons at the 5% level (Table 1).

3.2. Mass

Plant mass was affected by treatment in both seasons. During 2009/10, the mass of the MB treatment was respectively 91 and 62% higher than that of the AA and control at the first sampling and 21% higher than both treatments at the second SD. Mass were similar among treatments at the third sampling. During the following season (2010/11), plant mass was significantly lower with AA treatment compared to MB and the control at all three SD. The average reduction in mass varied from 255% with the 1st SD, to 46 and 32% with the 2nd and 3rd SD. No signs of toxicity or wilting were

the first order interaction. Of these, *Aspergillus* spp. is probably the most important. The levels of *Aspergillus* spp. were significantly lower on the roots for all three treatments. The highest frequency was obtained for the MB treatments as determined within the crowns. During the 2010/11 season only *F. oxysporum* were significantly affected with the first SD, yielding the lowest levels on the roots of the MB treatment and the highest with the roots of the AA treatment. Fungi that were noteworthy within the 2nd sampling analysis (2009/10) were *F. oxysporum* and *M. phaseolina.* Theses fungi recorded the lowest frequencies on the roots of the MB treatment, which differed significantly from those obtained by the control and the AA treatment. During the 2010/11 season similar results were obtained for *F. oxysporum.*At the 3rd SD (2009/10), *F. oxysporum* levels were the highest on roots of control as well as AA treatments, whilst *M. phaseolina* were significantly higher on roots of the control treatment. During the 2010/11 season *Trichoderma* spp. were the lowest in crowns of the MB treatment, which did not differ from the frequencies observed on the roots of the AA treatment.

3.5.2. Correlation analysis

Fungal frequencies that correlated significantly with mass, disease severity or yield are listed in Table 4. As very limited correlations could be obtained between mass, disease severity and yield, results are not presented. Neither root rot severity nor mass at any of the SDs correlated significantly with maize yield. A negative correlation was obtained between mass and disease severity on the roots at the 1st SD during 2010/11 (data not shown: Roots: $r= -0.726$ $P=<0.001$).

The correlation of fungal frequencies to mass, disease severity and yield over the various seasons, SD and plant part (i.e. root vs. crown) was limited and no distinct patterns could be observed. *F. oxysporum* obtained a negative correlation with mass at the 2nd and 3rd SD of the 2009/10 season, as well as with the 1st SD of the 2010/11 season. Frequency of *F. oxysporum* on roots with the 1st SD over both seasons also correlated positively with disease severity observed on the roots.

3.6. Canonical variate analysis (CVA)

Fig. 1 provides the six generated CVA's (labelled A to F). For ease of explanation the same acronyms in the figure will be used in section 3.6.1 to 3.6.6. (i.e. Y1 = 2009/10, Y2 = 2010/11; T1 = Methyl bromide; T2 = Anhydrous Ammonia; T3 = control). A positive correlation of a fungus with either of the axis indicates that the fungal frequency increase from left to right (x-axis) or from the bottom to the top (y-axis). The opposite

important root pathogens. All three treatments of 2009/10 grouped above the x-axis, indicating higher than trial mean frequencies of *Aspergillus* spp. and *F. subglutinans* in the crowns at the 1st SD compared to the 2010/11 season.

3.6.3. Figure 1C: 2nd sampling - Roots

Three groups were created by the CVA. The 2009/10 season had lower levels of *F. semitectum* and *F. verticillioides* and higher levels of *Cheatomium* spp. and *N. vasinfecta* compared to the 2010/11 season. The MB treatment of the 2009/10 season is still grouped away from all the other treatments, indicating that the application effect is still present after 70 days, resulting in low levels of *F. oxysporum* and high levels of *Trichoderma* spp. All three treatments during the 2010/11 season grouped together and did not affect the fungi in a similar manner as the 2009/10 season.

3.6.4. Figure 1D: 2nd sampling - Crowns

A similar grouping in the crowns to that of the roots at the same SD was observed, but different fungi were responsible for the observed grouping. *F. semitectum* frequencies in the crowns were lower during the 2009/10 season when compared to the 2010/11 season, but *F. verticillioides,* was highest in the crowns during 2009/10. The effect of MB treatments on crowns at 70 days was also still present since the treatment grouped alone. The MB treatment (2009/10) accordingly resulted in higher than trial mean average levels of *F. subglutinans* (r=0.404) and *N. vasinfecta* (r=0.404). The AA application was similar to the control in both seasons.

3.6.5. Figure 1E: 3rd sampling - Roots

Trichoderma spp. (r=0.852), *F. verticillioides* (r=-0.778) and *F. semitectum* (r=-0.728) largely contributed to the different groupings obtained. The 2009/10 season had higher levels of *Trichoderma* spp. but lower levels of *F. verticillioides* and *F. semitectum*. Four groups were created which were very similar to the three created with the 2nd SD on the roots. The y-axis correlated the highest with *F. oxysporum* (r=-0.745) and *M. phaseolina* (r=-0.580). The effect of MB treatments during the first season was still apparent at 100 days after planting as observed within the *Trichoderma* spp., *F. semitectum*, *F. oxysporum* and *M. phaseolina* frequencies. The AA application did not differ from the control in both seasons.

Young and Kucharek, 1977). Rodiguez-Brljevich *et al.* (2010) in additiondemonstrated that the photosynthetic rates of maize plants tested at V2 and V4 were reduced with increased disease severity and incidence of *Fusarium* spp. The relevance of the current findings is, however, difficult to justify. Disease severity for the AA plots in the 1st SD was 35.9 (Table 1) which is low considering that disease severity is the product of disease incidence (%) x RDI (highest score = 4) with an obtainable optimum disease severity score of 400. Secondly, although the CVA for the 1st SD demonstrated a shift in the reaction of fungal populations with AA application, it was only *F. oxysporum* that was significantly affected out of 45 fungal genera/species analysed. This effect was in addition limited to the 1st SD, as the CVAs for both the 2nd and 3rd SD (Fig. 1C and E) demonstrate that all three treatments reacted similarly with regard to relevant fungal species during the 2010/11 season.

Neither root rot severity nor mass at any of the SD correlated significantly with the yield obtained (data not shown). Soonthornpoct *et al.* (2000) stated that as no correlation between the incidence of root infection on seedling material and yield could be confirmed, the colonization of maize seedling roots by soilborne fungi had not influenced the yield obtained by the study. It should, however, be stated that both seasons were not conducive for root and crown rot development. It is therefore not certain what would have been the effect on yield, had climatic conditions been favourable for development.

The seasonal effect observed with the effectiveness of the MB and AA treatments, suggests that multiple season data are required in order to formulate an accurate interpretation of the effect that such treatments would have on the plant itself as well as on the fungal populations observed in roots and crowns. Current findings also state the importance of such studies in order to understand the relevance of the risks associated with soil applications such as AA, as well as how these risks could be minimized or managed.

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Table 2. Fungi identified during the 2009/10 and 2010/11 season on maize roots and crowns.

Acremonium cf guilematii	*F. equiseti*	*N.vasinfecta**
Alternatia spp.*	*F. globosum*	*Nigrospora* spp.
Aspergillus spp.*	*F. graminearum**	*Paecilomyces* spp.
Alternaria spp.	*F. oxysporum**	*Papulaspora* spp.
Botrytis spp.	*F. polyphialidicum*	*Penicillium* spp.
Cheatomium spp.*	*F. proliferatum*	*Phoma* spp.*
Clamydosporum spp.	*F. sambucinum*	*Phytophtora* spp.
Clonostachys spp.	*F. scipri*	*Ramichloridium* spp.
Curvularia spp.	*F. semitectum**	*Rhizoctonia* spp.*
Epicoccum negrum	*F. solani*	*Talaromyces* spp.
Exserohilum spp.*	*F. subglutinans**	*Thielavia terricola*
*F. clamydosporum**	*F. verticillioides**	*Torulomyces* spp.
F. compactum	*Geotrichum* spp.	*Trichoderma* spp.*
*F. crookwellense**	*M. phaseolina**	*Sterile Hyphomycetes*
*F. culmorum**	*Morteriella* spp.	*Verticillium spp.*

Fungal species/genera included in canonical variate analysis

Table 4. Fungi that correlated significantly with mass, disease severity or yield over two seasons (2009/10 and 2010/11), three sampling dates and plant part from which they were isolated (Y = season; S= sampling date; R=root; C=crown).

	Mass	Disease severity	Yield
Aspergillus spp.	Y1S1C: r=0.429 (P=0.037)	Y1S1C: r=0.419 (P=0.042) Y2S3C: r=0.475 (P=0.019)	Y1S1R: r= 0.461 (P=0.002)
Alternaria spp.			Y2S1C: r=-0.465 (P=0.039)
Alternaria spp.			Y2S2C: r=-0.523 (P=0.018)
Cheatomium spp.	Y1S2R: r=0.515 (P=0.010)		Y1S2R: r=0.446 (P=0.029)
Clamydosporum cladosporioides			Y1S2C: r=-0.417 (P=0.043)
F. crookwellense		Y2S1R: r=0.566 (P=0.004)	Y1S2C: r=0.4676 (P=0.021)
F. culmorum	Y2S1R: r=0.504 (P=0.044)		
F. graminearum	Y2S3R: r=0.479 (P=0.024)	Y2S1C: r=0.509 (P=0.011)	
F. oxysporum	Y1S2R: r=-0.567 (P=0.004) Y1S3R: r=-0.429 (P=0.036) Y2S1R: r=-0.633 (P=0.003)	Y1S2R: r=0.529 (P=0.008) Y2S1R: r=0.658 (P=0.001)	
F. solani		Y2S2R: r=0.467 (P=0.021)	Y1S2C: r=-0.426 (P=0.038)
F. subglutinans		Y1S1R: r=0.720 (P=<0.0001)	
F. verticillioides	Y2S1R: r=-0.485 (P=0.030)	Y2S2R: r=0.526 (P=0.008)	
M. phaseolina	Y1S2R: r=-0.372 (P=0.074)		
N. vasinfecta			Y1S2C: r=0.511 (P=0.011)
Phoma spp.			Y1S2R: r=0.422 (P=0.040) Y2S2R: r=-0.578 (P=0.007)
Trichoderma spp.	Y1S2R: r=0.472 (P=0.02)		Y1S2R: r=0.542 (P=0.006)

[a] = Acronym indicating the season, sampling date and plant part which correlated with mass, disease severity or yield (Y1= 2009/10, Y2=2010/11, S1= 1st sampling date - 21 days after planting, S2= 2nd planting date - 70 days after planting and S3= 3rd sampling date - 100 days after planting; R = isolated from roots, C= isolated from crowns).

TECHNOLOGY TRANSFER

Presentations:

CRAVEN, M., 2010. Challenges in attempting to elucidate root and crown rots in different tillage systems. 2010. SASPP workshop. Parys.

CRAVEN, M., 2013. Anhydrous ammonia and soil fungi. Farmer's day. ARC-Grain Crops Institute, Potchefstroom, 2013.

CRAVEN, M., 2014. Monitoring of root and crown rot pathogens under conservation agricultural practices. ARC-GCI farmers' day, 20 February 2014. Potchefstroom.

Congress presentations:

CRAVEN, M. & MOREY, L., 2013. Observed fungal population shifts with soil applied anhydrous ammonia and the resultant effect on root rot severity, plant mass and yield of maize. 48th Congress of the Southern African Society of Plant Pathology (SASPP) at ATKV Klein Kariba, Bela-Bela, Limpopo. 20 - 24 January 2013.

CRAVEN, M., 2014. Observed fungal frequencies and their impact on plant parameters under conservation agriculture cropping systems on a sandy loam soil in the North West province during 2012/13. Combined Congress, Grahamstown, 20-23 January 2014. *Receive the Daan Retief floating trophy for best presentation by a researcher under the age of forty.*

Radio talks:

CRAVEN, M., 2011. Stamvrot van mielies. *Station: RSG Landbousake*. 19 October 2011.

CRAVEN, M. 2013. Navorsing rondom bewaringsboerdery. *Station: RSG Landbousake*. 2 October 2013.

CRAVEN, M., 2014. Grysareas van mielievrot. *Station: RSG Landbousake*. 10 September 2014.

Popular publications:

CRAVEN, M., 2010. Bestry wortel en stamvrot by mielies. *Lanbouweekblad*, 2010. p 22 - 24.