Table 6. Analysis of variance on the effect of different cropping systems on the frequency (%) of fifteen soilborne pathogens as measured over four selected treatments at the Viljoenskroon trial over three years (2011/12, 2012/13 and 2013/14) in both the root and crowns of maize plants 100 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*
Year	0.0190	0.0133	0.0215	0.0091	0.0173	0.0086	0.6736	0.0021	0.6378	0.0167	<.0001	0.1083	0.0854	*	0.6215
Tmt	0.1238	0.1842	0.7691	0.6210	0.1254	0.4287	0.0017	0.2856	0.4888	0.4954	0.3426	0.6208	0.3500	*	0.1382
Year*Tmt	0.1099	0.1559	0.2769	0.8389	0.6215	0.4595	0.8638	0.1815	0.0983	0.5053	0.0187	0.8548	0.5307	*	0.8015
PlantPart	0.0018	0.0005	0.4528	0.0003	0.0395	<.0001	0.4665	0.192	0.0046	0.3214	0.1768	0.8201	<.0001	*	0.1662
Year*Plantpart	0.0063	0.0024	0.8257	0.0184	0.0874	0.0187	0.2297	0.0288	0.8702	0.0472	0.4716	0.7286	0.3411	*	0.6097
Tmt*Plantpart	0.0060	0.3984	0.2682	0.5634	0.0633	0.7187	0.6569	0.1908	0.6272	0.0773	0.1234	0.5273	0.7789	*	0.1318
Year*Tmt*plantpart	0.0011	0.6385	0.5046	0.7904	0.4416	0.0589	0.1955	0.1683	0.9119	0.0298	0.7347	0.0776	0.8988	*	0.8029

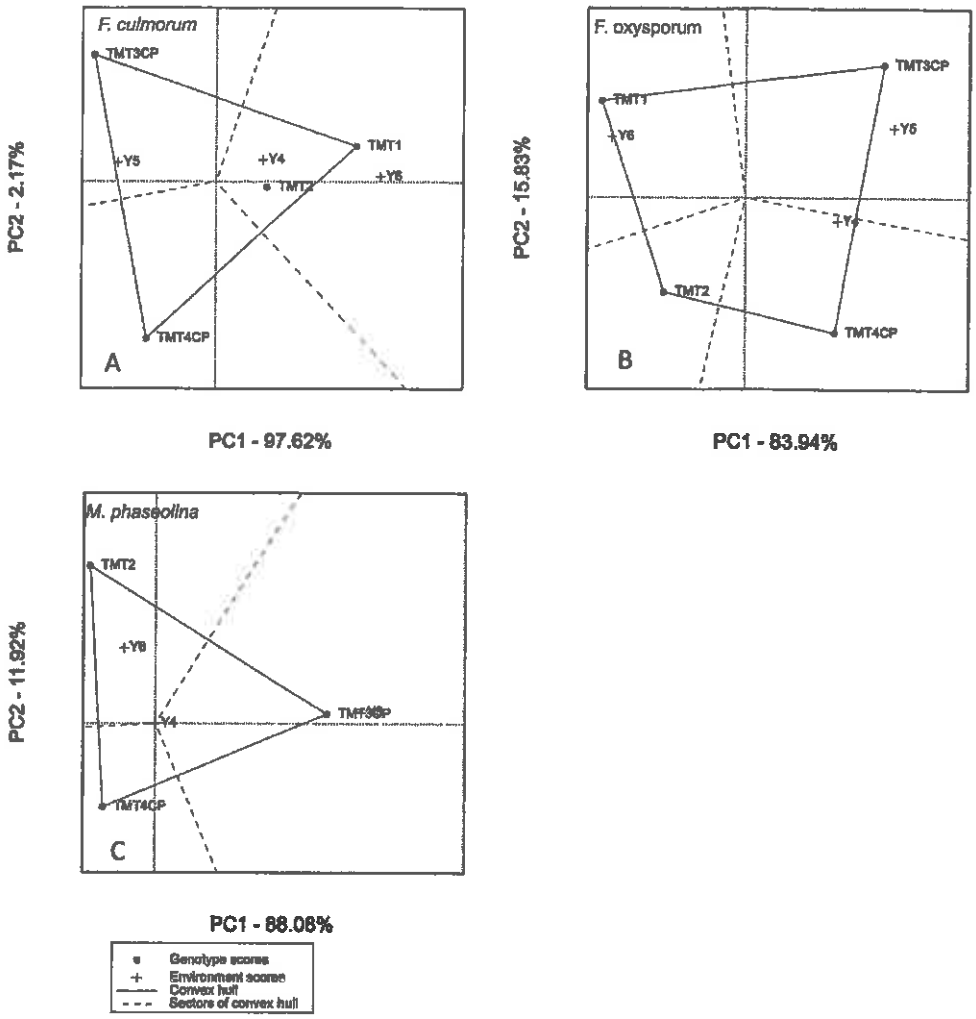

Figure 2. Biplot analysis on the impact that treatment had on the frequencies of *F. culmorum, F. oxysporum* and *M. phaseolina* over seasons 21 days after planting. (Y4 – 2011/12, Y5 – 2012/13 and Y6 – 2013/14).

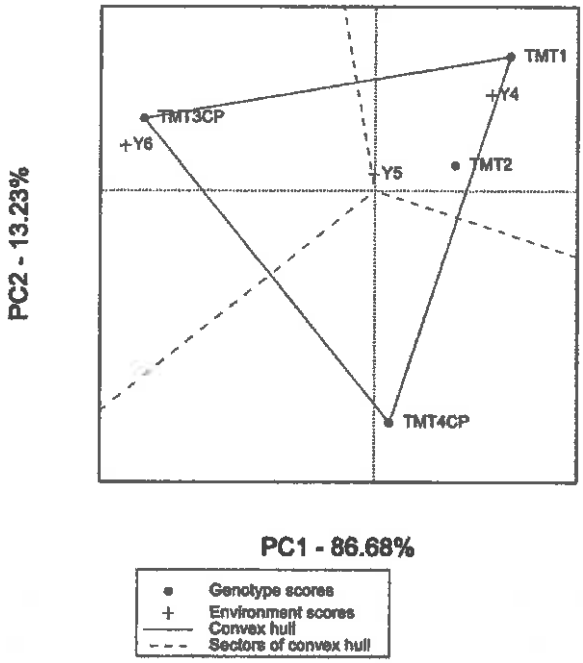

Figure 5. Biplot analysis on the impact that treatment had on the frequencies of *F. verticillioides* over three seasons at 100 days after planting. (Y4 - 2011/12, Y5 - 2012/13 and Y6 - 2013/14)

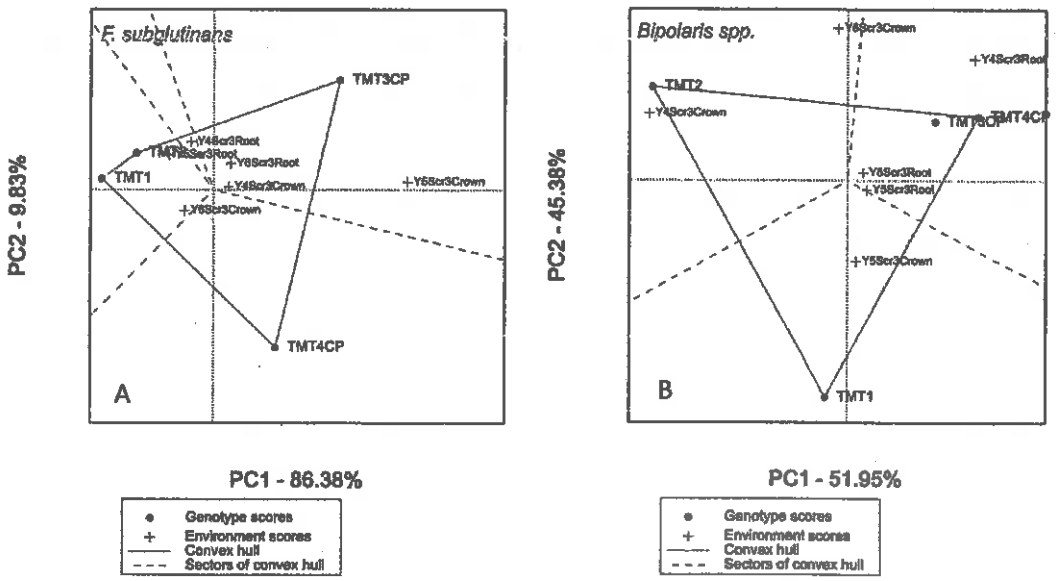

Figure 6. Biplot analysis on the impact that treatment had on the frequencies of *F. subglutinans* and *Alternaria* spp. with regard to the season and plant part (root or crown) from which it was isolated from at 100 days after planting. (Y4 - 2011/12, Y5 - 2012/13 and Y6 - 2013/14; Scr3 - sampling at 100 days after planting)

Over a period of six years of evaluation, only one season resulted in significant disease development (2012/13), which emphasised the importance that climate plays in whether root and crown rot develop or not. Based on the findings of especially the 2012/13 season, is it clear that Conservation agriculture can play a very important role in the management of charcoal rot especially in the dryland production areas of the North West province which often suffers from the disease due to the generally hotter and dryer conditions experienced.

3.1. Aim

The aim of the current study is to investigate the impact that six selected CA cropping systems had on the plant mass and root and crown rot disease severity of maize over a three year period (2011/12 to 2013/14) on a sandy loam soil in the Ventersdorp area. A second aspect investigated is the impact of such systems on the fungal frequencies of known root and crown rot pathogens.

3.2. Material and methods

3.2.1. Field trial

The trial was planted at Buffelsvallei (Ventersdorp district, South Africa: sandy loam soil) over a five year period (2008/09 to 2012/13) under dryland conditions as indicated under "GENERAL MATERIAL AND METHODS".

3.2.2. Parameters evaluated

Parameters evaluated included yield, plant mass at 21, 70 and 100 DAP, disease severity for roots and crowns at 21, 70 and 100 DAP as well as fungal frequencies of fifteen known root and crown rot pathogens as listed under "GENERAL MATERIALS AND METHODS".

3.2.3. Statistical analysis.

Yield, mass and disease severity

Data generated during 2011/12, 2012/13 and 2013/14 were used in statistical analysis. Analysis of variance was conducted on the yield as well as plant mass at 21, 70 and 100 DAP over the three years. A Split-split plot analysis was conducted on root and crown rot disease severity obtained for the three years combined (year = main plot, treatment = sub-plot, plant part from which isolated from (i.e. roots or crowns = sub-sub plot). Additional analysis was conducted which focussed on the results of the 2012/13 season in order to better explain the findings of the first set of analysis. Correlation analysis was in addition conducted to determine which of the fungi isolated correlated the highest with crown rot observed as well as the yield obtained.

screened (Figure 5), but due to the low levels of severity not much note is taken at this stage of the differences.

70 Days after planting

At 70 DAP the impact of the various treatments on disease severity was significantly affected by the season and plant part which was analysed (Table 2). Analysis of variance (Figure 6) indicated that crown rot was in general not present in any of the seasons at this particular sampling date. During 2011/12, root rot was significantly lower in TMT3CP, TMT3SF as well as TMT4SF compared to TMT1, whilst root rot severity during 2012/13 was similar for all treatments. During 2013/14 root rot severity for TMT1 followed by TMT2 were significantly higher than that of the remaining crop rotation based treatments.

100 Days after planting

Analysis of variance indicated that the year-by-plant part interaction was significant for disease severity (Table 2). The majority of disease severity was in general limited to the roots, with root rot severity being higher than that of the crowns. Root rot severity did, however, not differ significantly between seasons (Figure 7). In contradiction to the roots, 2012/13 had higher levels of crown rot (Photo 1), which was not present during the 2011/12 or 2013/14. The year-by-treatment interaction was significant at P=0.1 (Table 2), which indicated that during 2012/13 disease severity measured for TMT1 was significantly higher compared to the remaining five treatments of which all were CA based treatments which contained a cover layer.

during 2013/14 when the fungus was most affected by both the cowpea rotation treatments. *F. semitectum*'s frequencies (Figure 10E) were the most affected by TMT4CP during both the 2011/12 and 2012/13 seasons. During the following season (2013/14), the fungus was most affected by the sunflower crop rotation treatments (TMT3SF as well as TMT4SF).

The impact that the various treatment had on *Alternaria spp., F. clamydosporum, F. oxysporum, F. semitectum* and *Trichoderma* spp. at 21 DAP were all dependant on the plant part from which it was isolated from. *Alternaria* spp. (Figure 11A) was more prominent in the roots of plant in TMT4CP and the crowns of TMT3SF. *F. clamydosporum* (Figure 11B) was most prominent in the roots of TMT4CP, and in the crowns of TMT4SF. *F. oxysporum* (Figure 11C) was most prominent in the roots of TMT4CP and in the crowns of TMT4SF. *F. semitectum* (Figure 11D) were also most prominent in the roots of TMT4CP and in the crowns of TMT4SF. *Trichoderma* spp. was lastly more prominent in the roots of TMT1 and in the crown of TMT2.

Fusarium graminearum as well as *F. scirpi* were the only fungi that were significantly affected by the 3rd order interaction at 21 DAP. During 2011/12, *F. graminearum* (Figure 12A) was most prominent in roots of TMT3CP and with TMT4CP together with TMT1 in the crowns. The cowpea rotation systems accordingly played a large role early in the season on the occurrence of the fungus. During 2012/13, all treatments impacted equally on the frequency of the fungus whilst in 2013/14 the two sunflower rotations treatment (TMT3SF and TMT4SF) resulted in the highest frequencies of this fungus in the roots, whilst TMT3CP had the largest impact on the fungus's frequencies in the crowns. With regard to *F. scirpi*, (Figure 12B) the majority of the data points fell into the sector dominated by TMT3SF, possibly indicating a strong association with this crop rotation system.

70 Days after planting

At 70 DAP *F. oxysporum, F. verticillioides* as well as *Rhizoctonia* spp. were the only fungi that indicated significant differences with regard to their frequencies at various treatments. *F. oxysporum* occurred at higher frequencies in TMT1 followed by TMT2 whilst both *F. verticillioides* and *Rhizoctonia* spp. were more prominent in TMT3CP (Table 6).

100 Days after planting

Similar to the 70DAP both *F. oxysporum* and *Rhizoctonia* spp. again was significantly influenced by the various treatments applied. *F. oxysporum* occurred at significantly lower

rot severity) was experienced in TMT1 (P=0.1) 2012/13. The lack of a cover layer resulted in the plants being more water stressed, which in the end led to a higher degree of infection by pathogens. Correlation analysis indicted that *M. phaseolina*as well as *S. maydis* to be highly correlated with the crown rot, plant mass as well as the eventual yield measured during 2012/13. *Macrophomina phaseolina* (the causal organism of charcoal rot) is in general associated with warm dry conditions. During the 2012/13 season, the late drought that occurred towards the latter part of the season, resulted in plant stress due to lack of insufficient water. The fact that *M. phaseolina* occurred at higher frequencies in TMT1, is an indication that the absence of a cover layer resulted in the stress plants becoming more susceptible to infection. In the remaining treatments, the respective cover layers of the various CA associated treatments resulted in unfavourable conditions for the pathogen to infect or added to the plants being less stress and accordingly less susceptible infection by the pathogen. With regard to the infection by *S. maydis*, the importance of crop rotation was emphases by the 2012/13 results. *S. maydis* the causal organism of diseases such as Diplodia stalk and cob rot, can only infect and reproduce on maize, it was accordingly not much of a surprise when it occurred at significantly higher levels in TMT1 and TMT2, as these treatments represent maize cultivated in monoculture.

The Ventersdorp trial represents a more established CA trial as it has been under CA since 2008/9. This longer than usual monitoring of a single trial allowed to demonstrate a very important aspect of root and crown rot based research. As already stated, various soilborne pathogens are capable to infect and cause root and crown rot. Each of them, however, differ with regard to the climatic conditions or hosts they prefer. Various season can according pass by without any indication of root or crown rot development, because either the host (crop planted) were not suitable for the pathogen, or the climatic conditions were not favourable for the pathogen to infect. Over a period of six years of evaluation, only one season resulted in significant disease development (2012/13), which emphasised the importance that climate plays in whether root and crown rot develop or not. By screening the site over a longer than usual period, a season was finally identified which were able to highlight the differences that was brought about by the various treatments with regard to the disease development that occurred. Based on the findings of especially the 2012/13 season, is it clear that Conservation agriculture can play a very important role in the management of charcoal rot especially in the dryland production areas of the North West province which often suffers from the disease due to the generally hotter and dryer conditions experienced. In general *M. phaseolina* is very difficult to control due to its large host range which include cowpea and sunflower, which doesn't make crop rotation a very effective way to control the disease. With the 2012/13 season results, it appeared as though the cover layer provided by the various CA treatments

Table 3. Analysis of variance on the effect of different cropping systems on the frequency (%) of fifteen soilborne pathogens as measured over six selected treatments at the Ventersdorp trial over three years (2011/12, 2012/13 and 2013/14) in both the root and crowns of maize plants 21 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*
Year	0.1210	<.0001	0.3834	0.0829	0.0365	0.0006	0.0394	0.1747	0.1134	0.0084	0.0012	0.6651	0.2774	0.0001	0.4053
Tmt	0.0137	0.0114	0.0990	0.1996	0.3850	0.0030	0.0138	0.0413	0.1518	0.0099	0.1221	0.6047	0.0704	0.4047	0.4287
Year*Tmt	0.0082	0.0152	0.0278	0.1030	0.4716	0.1073	0.1783	0.0202	0.0334	0.2236	0.2502	0.5995	0.2108	0.4671	0.4579
PlantPart	0.4576	0.0005	0.6093	0.2281	0.8293	0.0048	0.0394	0.1008	0.2969	0.6357	0.9023	0.1550	0.4946	0.0002	0.3218
Year*Plantpart	0.8258	0.0002	0.0367	0.0343	0.8636	0.0119	0.0394	0.9170	0.0185	0.7599	0.9556	0.4971	0.8820	<.0001	0.3746
Tmt*Plantpart	0.0007	0.1397	0.0060	0.4655	0.2835	0.0022	0.0394	0.0020	0.1939	0.2100	0.4571	0.2252	0.0106	0.9306	0.4267
Year*Tmt*plantpart	0.5745	0.0543	0.1568	0.5547	0.0318	0.2791	0.0394	0.7259	0.3732	0.7308	0.5018	0.6923	0.7446	0.4866	0.4553

Table 5. Analysis of variance on the effect of different cropping systems on the frequency (%) of fifteen soilborne pathogens as measured over six selected treatments at the Ventersdorp trial over three years (2011/12, 2012/13 and 2013/14) in both the root and crowns of maize plants 100 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*
Year	0.4971	0.3096	0.0035	0.0874	0.1151	0.0663	0.3329	<.0001	0.9209	0.5958	0.0030	0.0427	0.0384	0.0152	0.0213
Tmt	0.5896	0.8228	0.3159	0.9455	<.0001	0.0458	0.7014	0.0059	0.3636	0.1029	0.3868	0.0413	0.4869	0.0239	0.0001
Year*Tmt	0.4956	0.2296	0.1209	0.1803	0.0040	0.0907	0.6438	0.0136	0.3127	0.1879	0.4557	0.3528	0.4662	0.0130	0.0034
PlantPart	0.4265	0.2329	0.2083	0.6804	0.5271	<.0001	0.3945	0.0001	0.0068	0.1540	0.0019	0.0277	0.0041	0.0032	0.0229
Year*Plantpart	0.1626	0.0026	0.3519	0.0047	0.0002	<.0001	0.1889	<.0001	0.0034	0.6310	<.0001	0.0405	0.0334	0.0003	0.0001
Tmt*Plantpart	0.0747	0.5000	0.0140	0.3803	0.7733	0.2152	0.2407	0.0575	0.4361	0.5306	0.9441	0.5673	0.7048	0.0086	0.0749
Year*Tmt*plantpart	0.9772	0.5362	0.0071	0.4271	0.4794	0.0883	0.7338	0.2911	0.2249	0.0475	0.6462	0.3195	0.8323	0.0027	0.0028

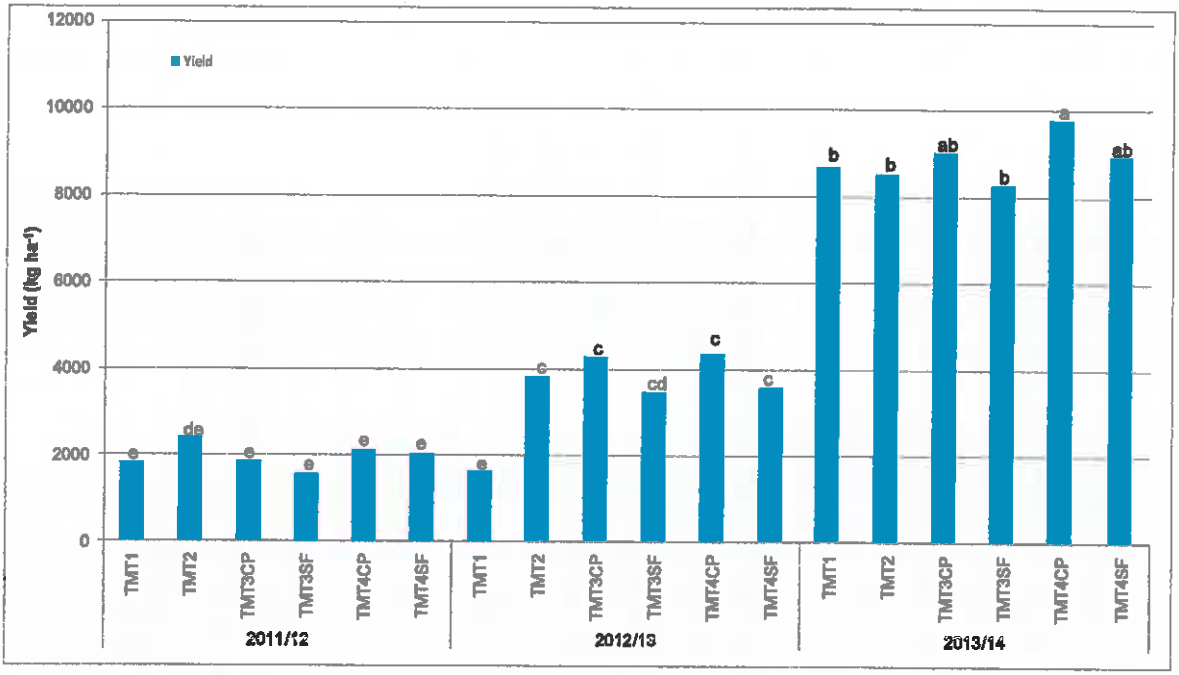

Figure 1. Analysis of variance for yield obtained with six various cropping systems at the Ventersdorp trial over three seasons (2011/12, 2012/13 and 2013/14) (P=0.05). (TMT 1 = mono-cropped maize under conventional tillage treatment, TMT2 = mono-cropped maize under minimum soil disturbance (CA), TMT3CP = two two-year maize rotation system treatments with cowpea under CA, TMT3SF = two-year maize rotation system treatments with sunflower under CA, TMT4CP = three year rotation system with millet and cowpea under CA, TMT4SF = three year rotation system with millet and sunflower under CA)

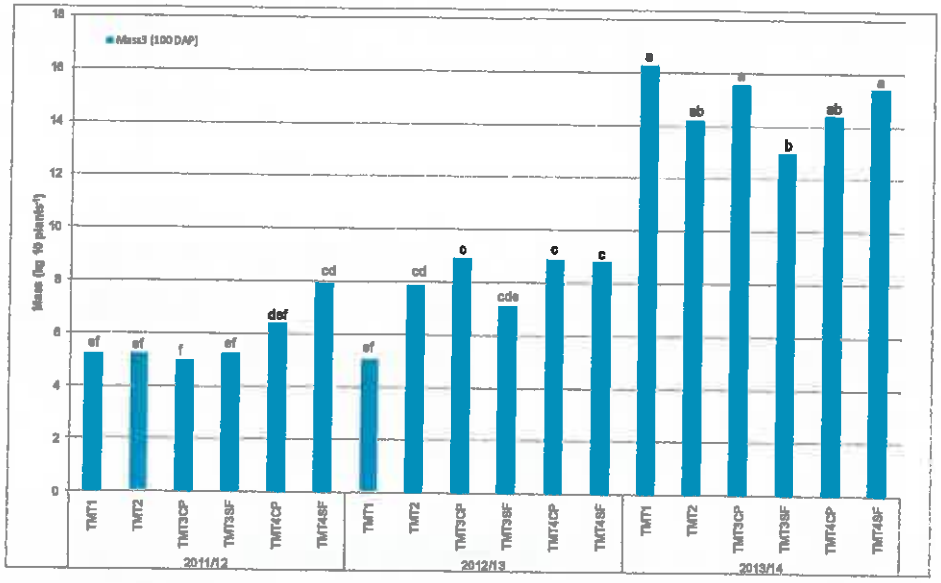

Figure 4. Analysis of variance for plant mass 100 days after planting obtained with six various cropping systems at the Ventersdorp trial over three seasons (2011/12, 2012/13 and 2013/14). (TMT 1 = mono-cropped maize under conventional tillage treatment, TMT2 = mono-cropped maize under minimum soil disturbance (CA), TMT3CP = two two-year maize rotation system treatments with cowpea under CA, TMT3SF = two-year maize rotation system treatments with sunflower under CA, TMT4CP = three year rotation system with millet and cowpea under CA, TMT4SF = three year rotation system with millet and sunflower under CA).

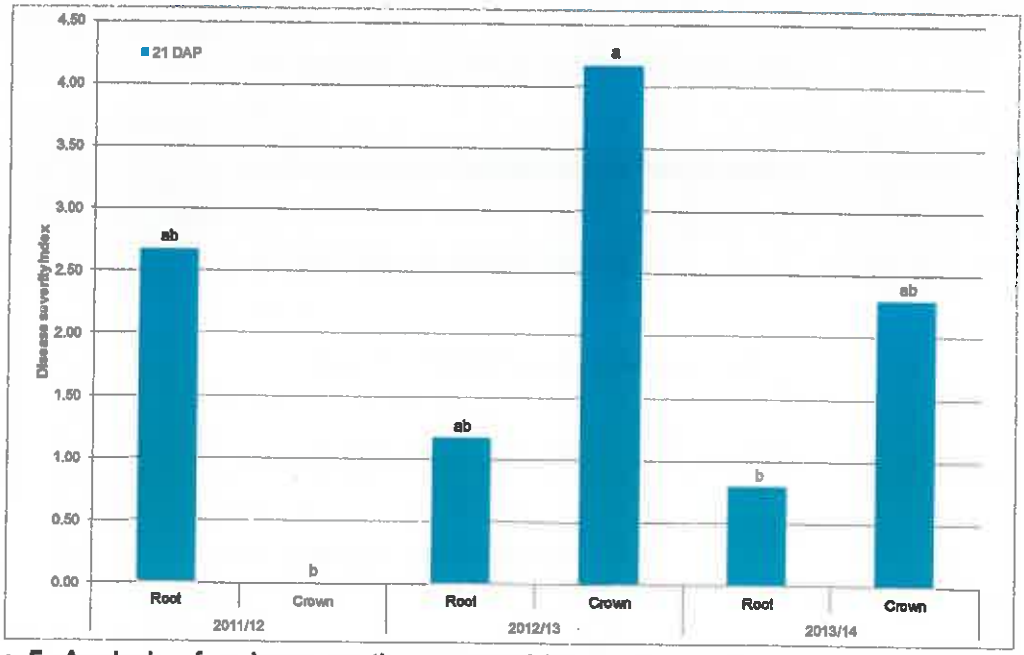

Figure 5. Analysis of variance on the seasonal impact of on disease severity measured in roots and crowns at 21 days after planting (DAP), at the Ventersdorp trial as evaluated over three seasons (2011/12 to 2013/14).

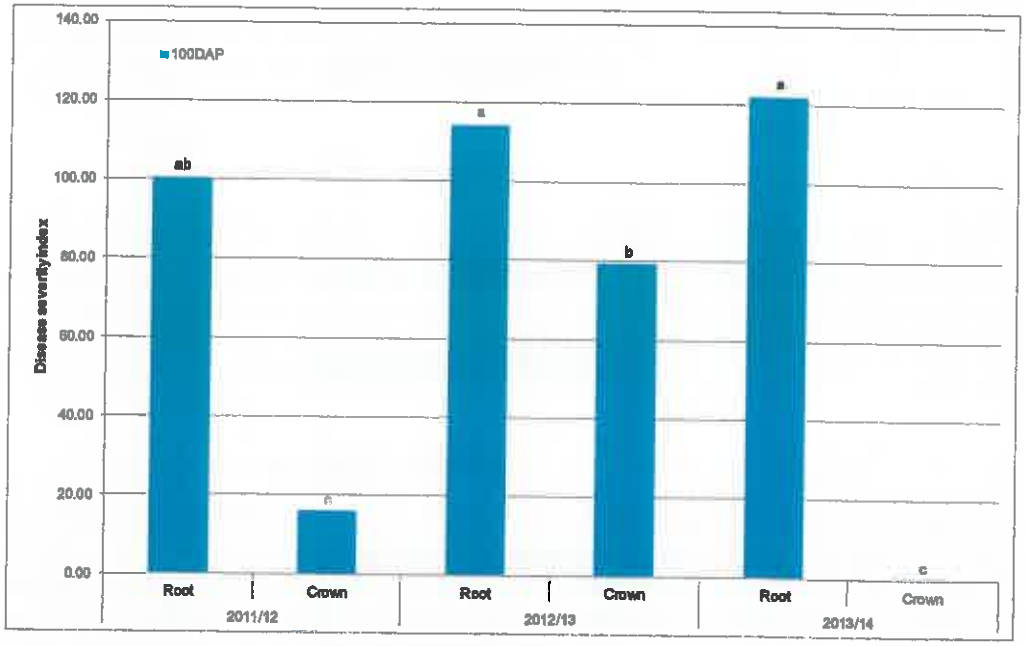

Figure 7. Analysis of variance on the seasonal impact on disease severity measured in roots and crowns at 100 days after planting (DAP) at the Ventersdorp trial as evaluated over three seasons (2011/12 to 2013/14).

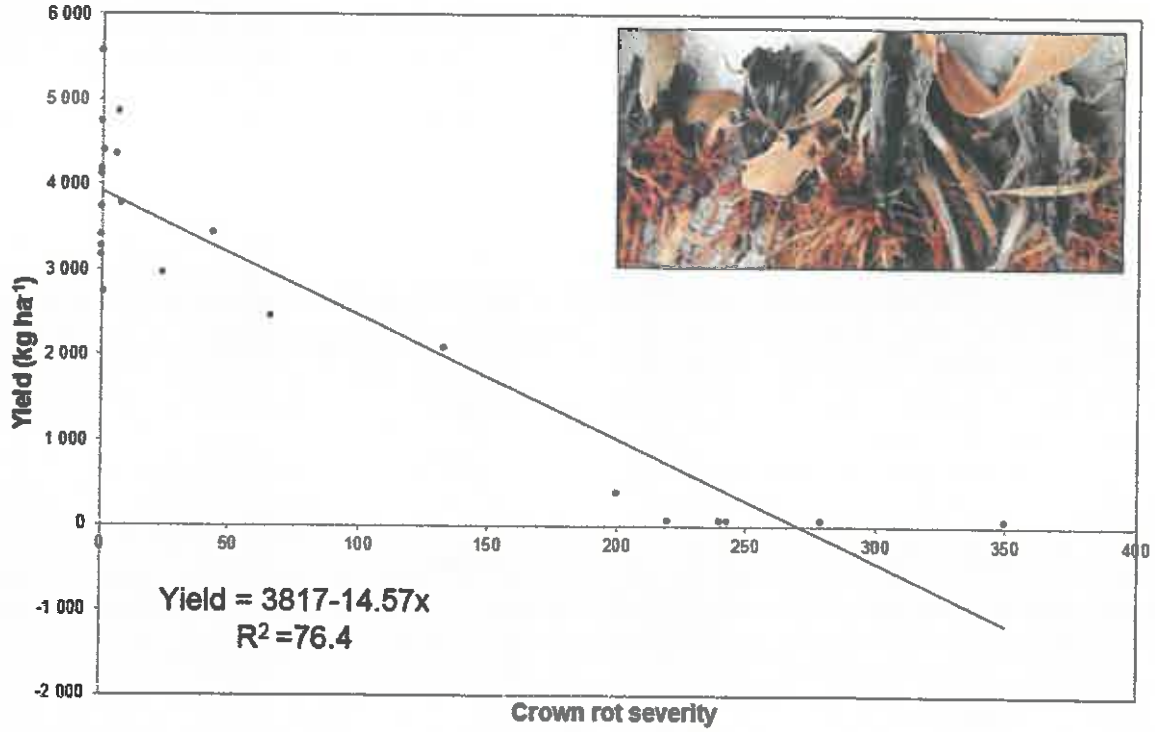

Figure 9. Regression analysis for the impact that the crown rot severity had on the yield obtained during the 2012/13 season at the Ventersdorp locality.

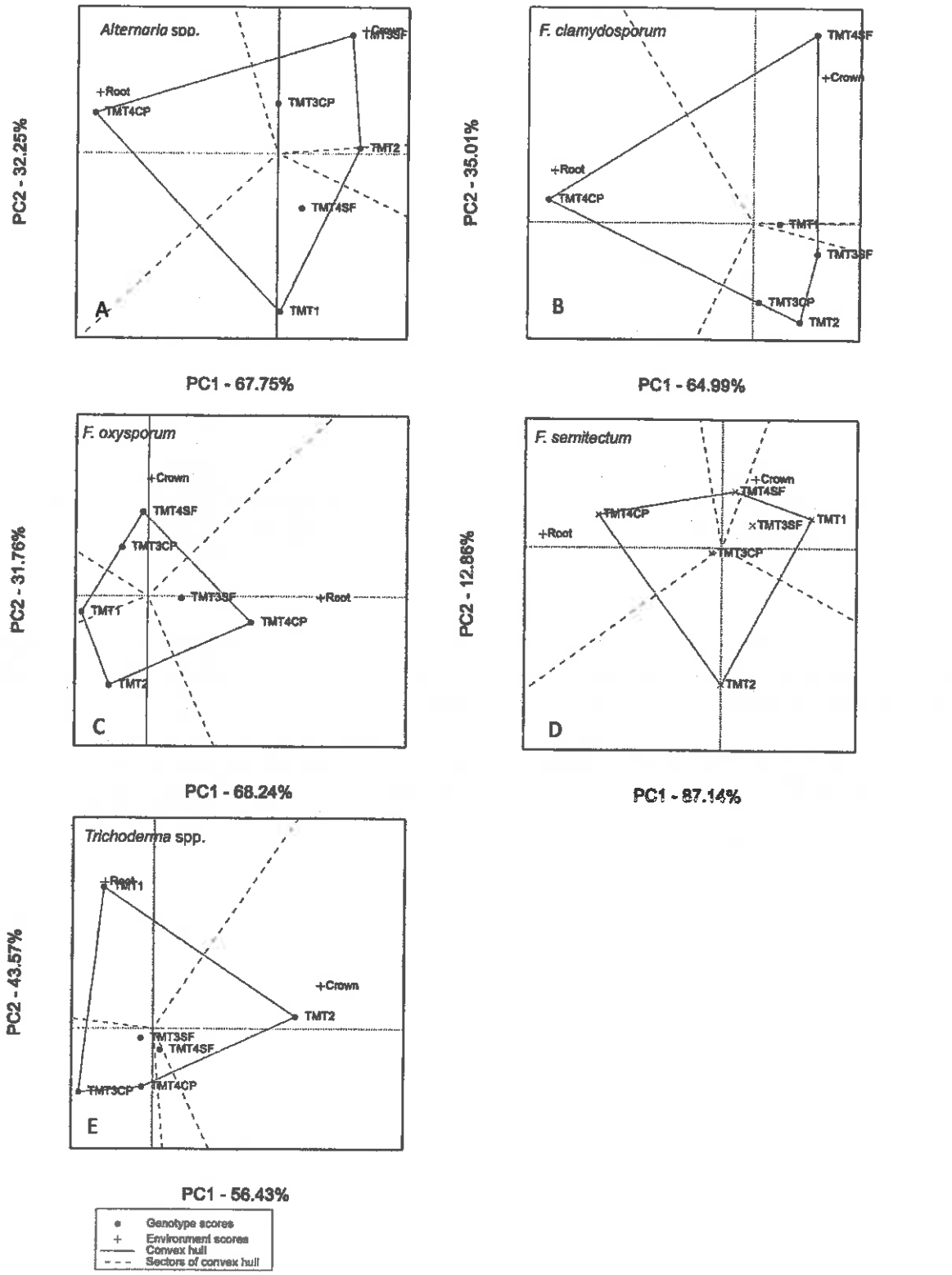

Figure 11. Biplot analysis on the impact that treatment had on the frequencies of *Alternaria spp., F. clamydosporum, F. oxysporum, F. semitectum* and *Trichoderma spp.* with regard to the plant part (root or crown) from which it was isolated from 21 days after planting.

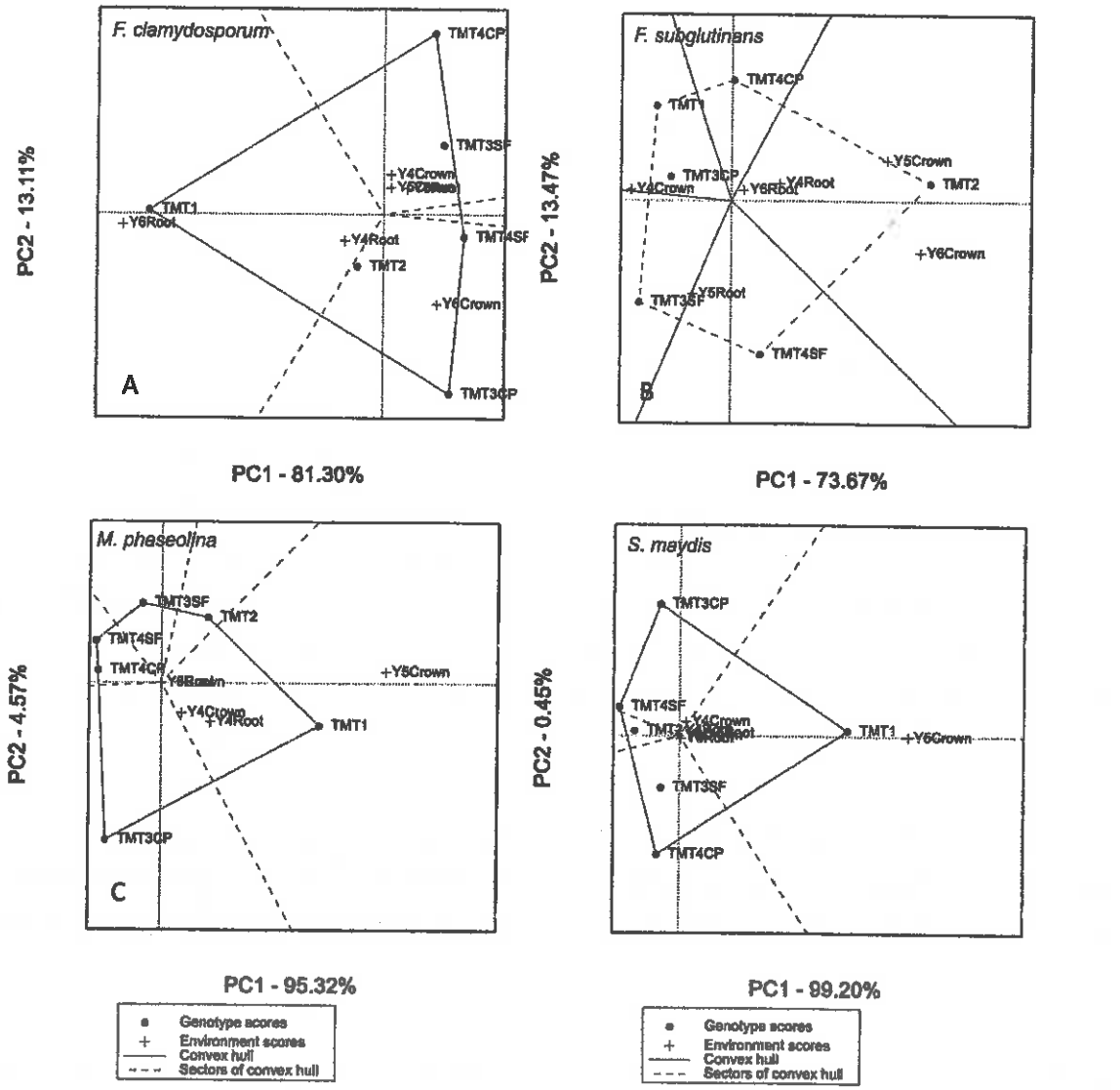

Figure 14. Biplot analysis on the impact that treatment had on the frequencies of *F. clamydosporum, F. subglutinans, M. phaseolina* and *S. maydis* with regard to the season and plant part (root or crown) from which it was isolated from at 100 days after planting. (Y4 - 2011/12; Y5 - 2012/13: Y6 - 2013/14)

4.1. Aim

The aim of this study was to compare the impact of four cropping systems associated with CA practices on parameters such as yield plant mass and root and crown rot and associated root and crown rot pathogens over two different soil types (localities) over two seasons (2012/13 and 2013/14).

4.2. Materials and methods

4.2.1. Field trial

Both the field trials planted at Viljoenskroon and Ventersdorp as indicated under "GENERAL MATERIALS AND METHODS were included in this study. Only the four corresponding treatments of the two trials were included (TMT1, TMT2, TMT3CP and TMT4CP). The last two seasons' data (2012/13 and 2013/14) were analysed, as the Viljoenskroon trial is not as established as that of the Ventersdorp trial. This was done to attempt to compare relatively established CA systems over the two localities.

4.2.2. Parameters evaluated

Parameters evaluated included yield, plant mass at 21, 70 and 100 DAP, disease severity for roots and crowns at 21, 70 and 100 DAP as well as fungal frequencies of fifteen known root and crown rot pathogens as listed under "GENERAL MATERIALS AND METHODS"

4.2.3. Statistical analysis.

Yield, mass and disease severity

Data generated during 2012/13 and 2013/14 were used in statistical analysis. Analysis of variance was conducted on the yield as well as plant mass at 21, 70 and 100 DAP over the three years. A Split-split plot analysis was conducted on root and crown rot disease severity obtained for the three years combined (year = main plot, treatment = sub-plot, plant part from which isolated from (i.e. roots or crowns) = sub-sub plot).

4.2.4. Fungal frequency

Fungal frequencies were analysed as stated under "GENERAL MATERIALS AND METHODS".

4.3. Results

4.3.1. Yield

The impact of the various treatments on yield differed according to the locality (Table 1). At the Ventersdorp trial, the two crop rotation trials (TMT3CP and TMT4CP) yielded significantly higher as opposed to the conventional monoculture trial (TMT1). At the Viljoenskroon trial,

rot was experienced compared to the previous season, with the roots of TMT2 at the Ventersdorp trial having the highest disease severity.

100 Days after planting

At 100 DAP, the year-by-treatment interaction was significant at P=0.1 (Table 2). During 2012/13 TMT1 at the Ventersdorp trial had significantly higher disease severity, followed by TMT3CP and TMT2 of the same season and locality (Figure 5).

4.3.4. Fungal frequencies

21 Days after planting

F. subglutinans frequencies was significantly affected by treatment (Table 3), with the highest frequencies measured in TMT4CP (0.471a; TMT1 - 0.104b, TMT2 -0.2456ab and TMT3 - 0.077c)

Fusarium culmorum, F. oxysporum and F. scirpi were significantly affected by the year-by-treatment interaction (Table 3). Both *F. culmorum* and *F. oxysporum* were significantly affected by TMT3CP during 2012/13, whilst TMT1 had the greatest affect during the following season (Figure 6A and B). Similarly *F. scirpi* was more prominent in TMT3 during 2012/13, but in 2013/14, TMT2 affected the frequency of this fungus the most (Figure 6C).

Treatment-by-plant part was significant for *Alternaria* spp. as well as *F. culmorum* (Table 3). Both these fungi were more frequently isolated from the roots of TMT4CP. With regards to the crowns, *Alternaria* spp. (Figure 7A) was mostly isolated from TMT3CP as opposed to *F. culmorum* which was most prominent in TMT1 (Figure 7B).

The locality-by-treatment-by-plant part interaction was significant for *F. oxysporum* and *F. semitectum* (Table 3). *F. oxysporum* was isolated most frequently form the cowpea rotation treatments (Figure 8A), with the emphasis on TMT3CP. A similar pattern could not be obtained for *F. semitectum* as the data points are scattered across the biplot (Figure 8B).

Aspergillus spp. and *Trichoderma* spp. were the only fungi that were significant for the fourth order interaction (year-by-locality-by-treatment-by-plant part; Table 3). The majority of the data points for *Aspergillus* spp. are grouped near the origin, indicating that at 21DAP *Aspergillus* spp. frequencies for especially 2012/13 were similarly affected by the various treatments (Figure 9A). A greater response is observed for the 2013/14 season, with these data points being placed furthest for the origin. Fungal frequencies measured for *Aspergillus* spp. in the

clamydosporum (Figure 15A), whilst three out of the four data points for *F. oxysporum* (Figure 15B) fell into sectors dominated by maize monoculture treatments.

F. semitectum was most frequently isolated from both root and crowns within the cowpea treatments (TMT3CP and TMT4CP; Figure 16A). *F. subglutinans* was most frequently isolated from roots in TMT4CP and from the roots of TMT2 (Figure 16B).

Three out of the four data points listed in the biplot for the locality-by-treatment-by-plant part interaction for *F. oxysporum* were placed in the sector dominated by TMT1 (Figure 17).

Four fungi indicated significant differences with regard to the 4th order interaction (Table 5). No clear grouping pattern could be obtained for *Alternaria* spp. (Figure 18A). , whilst five out of the six data points for *F. graminearum* fell into a sector dominated by TMT2 (Figure 18B). *Macrophomina phaseolina* weren't prominent during the evaluation period except for the 2012/13 sampling from Ventersdorp taken from the crowns (Figure 18C). During 2012/13 severe infection by this pathogen was observed in the crowns of TMT1 at Ventersdorp. A similar pattern is observed for *S. maydis* (Figure 18D), with the most impact on its frequencies observed at Ventersdorp during 2012/13 in both the roots and crowns of TMT1.

4.4. Discussion and conclusion

The impact of the various treatments over two localities indicated that at Viljoenskroon (sandy soil) TMT 2 produced significantly poorer yields as opposed to the other three treatments included. At Ventersdorp (sandy loam soil), TMT1 produced the lowest yield which was significantly lower than the two cowpea rotation systems. With plant mass 21 DAP, the cowpea rotation systems generally resulted in higher mass early in the season, with the effect better observed in the Viljoenskroon trial. As no significant differences were obtained for the root and crown rot severity measured at 21 DAP sampling date, the differences in plant mass cannot be attributed to the presence or absence of disease, and might rather point to an agronomical benefit that the rotation systems have. At 70 DAP this effect disappears to some extent, with TMT1 having the highest plant mass at Ventersdorp. A similar trend was observed at 100 DAP to that of yield with TMT2 at Viljoenskroon having the lowest plant mass, as opposed to TMT1 at Ventersdorp. Results obtained at 70 DAP indicate that 2012/13 suffered in general higher levels of root rot severity as opposed to the 2013/14 season. With regard to the crowns, the 2013/14 seasons suffered greater levels of crown rot in TMT2 at Ventersdorp compared to the previous season. At 100 DAP marked increase in disease seventy was observed for TMT1 at Ventersdorp, followed by TMT2 and TMT3CP during the drought stricken 2012/13 season. Fungi that demonstrated to have significantly higher levels at this specific sampling date were