# MAIZE TRUST BIANNUAL REPORT 2020/21 (1 April 2020 to 30 September 2020)

# PATHOLOGY

**DETAILS**

| | | |
|---|---|---|
| PROJECT NUMBER | | P05000005 (101597) |
| PROJECT TITLE | | Development of a charcoal rot screening protocol for maize and the establishment of resistance levels within commercially available hybrids |
| PROJECT MANAGER | | B Janse van Rensburg |
| CO-WORKER(S) | Internal | M Craven, DP Nkoko, DB Biya, NY Maila, TJ Baas, MLP Motlhatlhego |
| | External | None |
| PROJECT STATUS | | Continue |
| DURATION | | 01/04/2016 to 31/03/2020 |

**ACTIONS TAKEN TO DATE**

**Commercial cultivar screening (field trials)**

It is well known from literature that inoculation methods with mechanical damage (such as the toothpick method) can bypass the plant's natural defence system resulting in hybrids being labelled as susceptible, whilst under natural conditions they might have been resistant. Therefore, a field trial consisting of 30 cultivars and replications (randomised) were planted mid-November of 2019/2020. Three of the replications were randomly chosen and used to screen cultivar responses under natural infection (no plant wounding) and inoculated (with charcoal rot toothpicks) conditions. Two rows of each cultivar were planted, the first row were inoculated at flowering stage and the second row were left to infect naturally. Destructive sampling took place at soft dough stage and results of the natural infection vs. inoculation (with mechanical damage) will be discussed in this report. It is important to make this comparison in order to provide producers and breeders with accurate information. The information generated should thus be an outcome that the farmer / breeder can expect to observe in a farming / breeding system. The remaining three replications were treated in the same way as the first three replications, but were left in the field until physical maturity. The thirty cultivars selected for this project, is planted in cultivar evaluation trials and the aim will be to compare yield of the inoculated and naturally infected maize plants in a field with a high charcoal rot inoculum load to the yield of the same cultivars planted in soil that was not infected by charcoal rot. Therefore, a second field trial was planted on the same date in a different field, but at the same farm in order to obtain yield results that can be compared with yield of the trial that was planted first (uninoculated plants). Yield loss information in South Africa due to charcoal rot is currently limited and the proposed study will greatly contribute to understand and determine yield loss. It is mentioned by Kendig *et al*. (2000) that early infections, at seedling stage, can result in greater colonization up into the stem of the host and in turn greater yield loss compared to when infection only starts at flowering stage. Trials were successfully completed and data captured and results will be conveyed in this report.

**Quick screening method (seedling stage)**

With the project initiation, one of the goals was to develop a viable evaluation method that would allow for the fast and efficient screening of maize hybrids and lines for resistance to charcoal rot. Various quick screening methods (seedlings) were evaluated with a glasshouse cone seedling trial giving good results, although this method is labour intensive and results were not always consistent. A method is optimized where germinating seed can be screened after 11 days of applying inoculum (no mechanical damage) and incubation at 37ºC. This method is cheaper, less labour intensive and can be performed under controlled conditions which can allow for a greater number of cultivars/lines that can be screened.

Optimization of this method is now completed. Two inoculum concentrations were used, different aged cultures (14 days, 34 and 60 days) and time of incubation (7 days, 11 days and 14 days). Optimum conditions for this method was identified: using the 60 day old culture, lowest inoculum concentration and incubation at 11 days at a temperature of 37ºC. Materials were prepared to test the 15 common cultivars under optimized conditions. These were repeated three times and infection results correlated to data from common cultivars obtained from field trials. Laboratory trials were successfully completed and data captured and results will be conveyed in this report. Irrespective of correlation results (seedling screening vs field plant screening), it is also important to note that charcoal rot can reduce mean seed weight, germination, and seedling vigour index, emphasising the importance of screening cultivar seedling reactions to charcoal rot infection.

**PROGRESS MADE**

**Commercial cultivar screening**

All objectives/activities were completed as set out in the extension motivation. The 2020/2021 season will be the last season that field trials and screening will be conducted. This will also provide a second year of data to determine yield losses. Results from the 2019/2020 season will be discussed in this report.

**Quick screening method (seedling stage)**

A method is optimized and repeated in order to correlate data of cultivar reactions to that of results from cultivar reactions in the field. Results from the 2019/2020 season will be discussed in this report.

**RESULTS ACHIEVED TO DATE**

**Results from the 2019/2020 commercial cultivar screenings: Inoculated vs natural infection**

Cultivar disease scores in this study ranged from 1 to 4 where a score of 1 indicate a healthy stalk or slight discolouration at the site of inoculation. A score of 2, 3 and 4, means that up to 50%, 51-75% and 76-100% of the inoculated internode is discoloured, respectively. A score of 5 means that 100% of the inoculated internode is discoloured with less than 50% discoloration of the adjacent internode, no scores was captured in this category. A new score had to be developed for natural infections which occurred mainly in the roots and crown, growing systemically upwards (Figure 1).

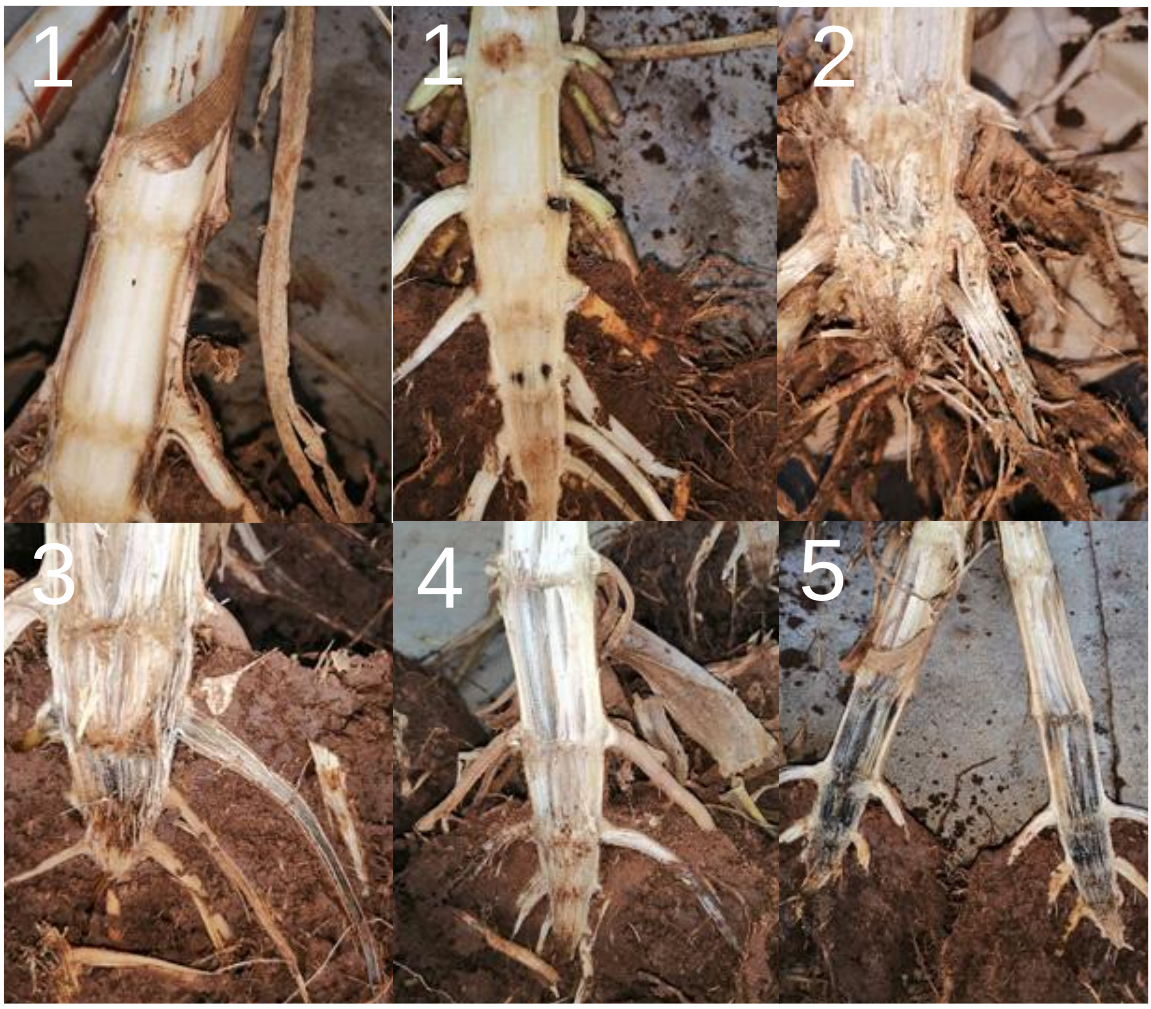



**Figure 1: Rating scale for naturally charcoal rot infected plants, mainly at the taproots, crowns and lower internodes.**

Natural infection disease score range from 1 to 5 in this study (scores will be added when infection symptoms increase). A score of 1 indicate roots/crowns or slight discolouration at the infection site. A score of 2 is given when up to 50% of the first internode/crown is infected, a 3 is given when the 1st internode/crown in infected and the infection is spread to the second internode or taproots, a 4 is given when there is infection of the

taproots and upwards infection (partial) into the third internode, a 5 is given if this infection colonizes the whole area up to the third internode.

Data were analysed by Mrs. N. Cochrane (ARC - Biometry Services), using a split-plot ANOVA with cultivars as main treatments and treatments (inoculated and non-inoculated) as sub-plots. From the Anova analyses, both cultivar and treatment had a highly significant effect on charcoal rot infection (Figure 2). Charcoal rot infected toothpick-inoculated plants showed a mean disease score of 2.25 (lower than the previous year with a score of 3.41) while the naturally infected plants had a mean disease score of 1.68 which is similar to the previous year (1.63) although the methods differ (controls in the previous year were inoculated with clean sterilized toothpicks). The cultivar with the highest mean average disease score (Figure 2) is PAN5R-791BR (3.00) and the lowest mean average disease score is recorded for BG5785BR (1.46). As a main effect, cultivars BG5785BR (1.33), PAN5R-785BR (1.37), VP8405BR (1.50) and DKC78-79BR (1.50) had significantly lower infection scores (P=0.01) compared to the other cultivars. An interaction between the treatment (inoculated and naturally infected plants) and cultivar had a significant effect on infection scores (P=0.01). Although cultivars SC506 and PAN4A-156 had low infection scores (Figure 2) with the toothpick method (1.53 and 1.60, respectively) at the adult plant stage, they had significantly higher scores under natural field infections (2.40 and 3.40 respectively) indicating they were more susceptible during the seedling stage. These results suggest that resistance screening should include both plant stages. A second year of data (from the last planting in mid-November 2020) will give more clarity on the responses of cultivars to natural infections vs that of toothpick infected plant responses.

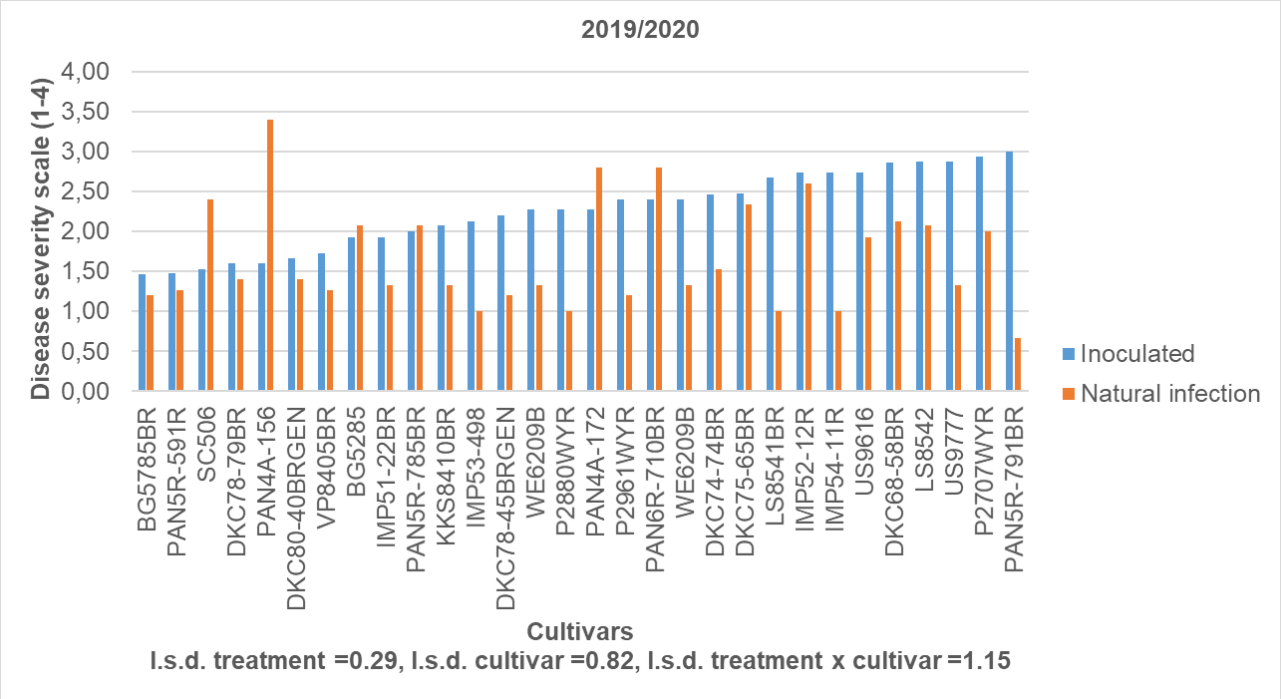



**Figure 2: Cultivar reactions to *M. phaseolina* infection under dry land conditions (inoculated and natural infection with no plant wounding).**

The average of disease scores from common cultivars over a three year period (2018-2020) were calculated, except for cultivar SC419 (two year period, 2018-2019) that was replaced with cultivar WE6209B in the 2019/20 season (results of the latter cultivar shown in Figure 2). Cultivar PAN5R-785BR had overall the best resistance (disease severity score of 1.69) against charcoal rot infections (using the toothpick method). Cultivars US9777 and US9616 are the most susceptible to charcoal rot infections with disease severity scores of 3.71 and 3.75 respectively (Figure 3).

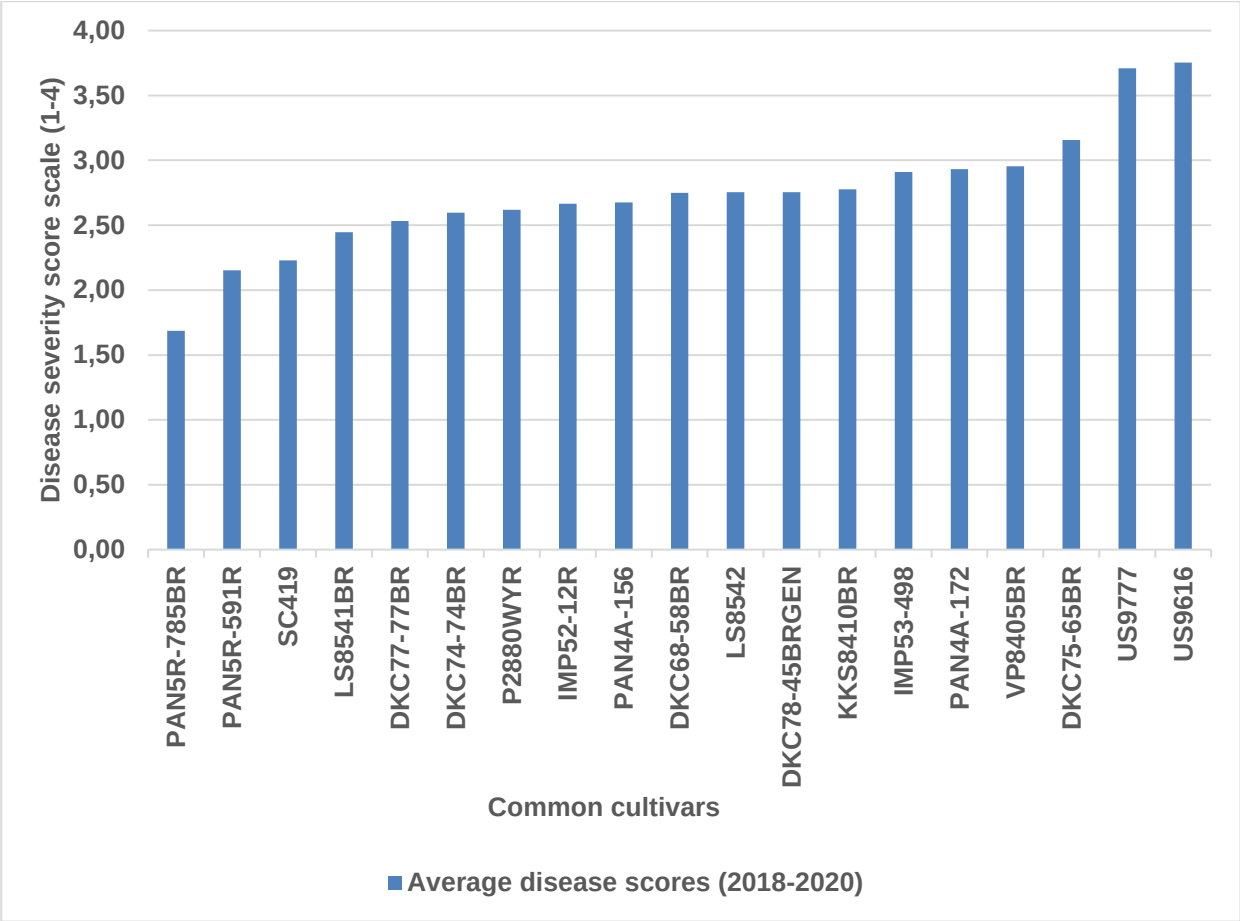



**Figure 3: The average of disease scores from common cultivars over a three year period (2018-2020) showing resistance and susceptibility to charcoal rot when infected with the toothpick method.**

**Results from the 2019/2020 commercial cultivar screenings: yield data**

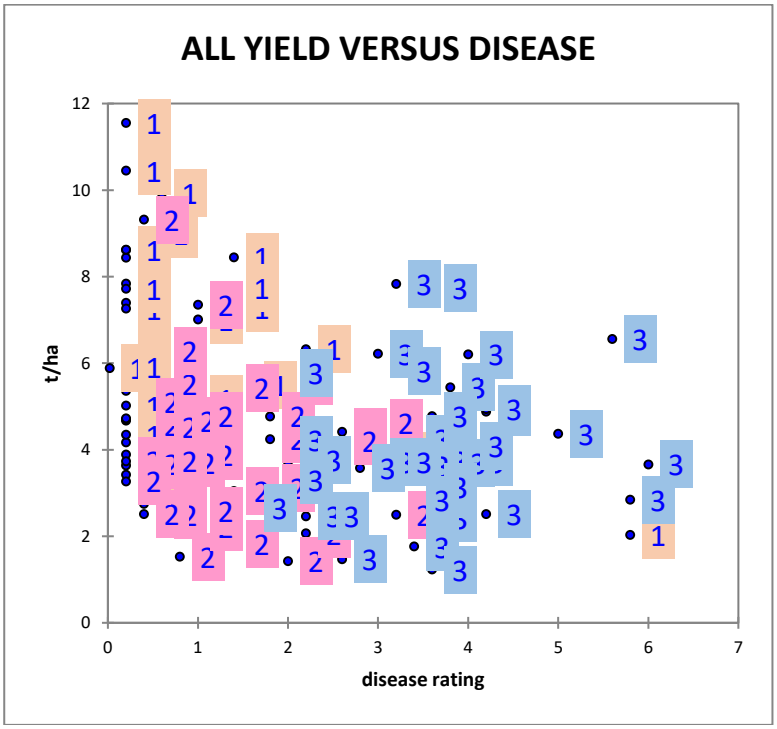
Yield data for experimental plots were calculated according to Tandzi and Mutengwa (2020) using the following formula:

Grain yield (t/ha) = [Grain weight x 10 x (100-MC)/(100-Adjusted MC)/(Plot area)]

Grain weight is in kg, moisture content (MC) in % and plot area is in $m^2$. Moisture content was adjusted to 12.5%.

Data were analysed by Mrs. N. Cochrane (ARC – Biometry Services) and the reaction of the diseases ratings to yield (t/ha) can be seen in Figure 4 where treatment 1 represent the cultivars planted in an adjacent field with low charcoal rot inoculum presence, treatment 2 represent naturally infected plants in a field with a high primary inoculum and treatment 3 represent toothpick inoculated plants, also in the same field with a high primary inoculum presence.

**Figure 4: The effect of charcoal rot infection on yield (t/ha).**




1= cultivars planted in an adjacent field with low charcoal rot inoculum presence
2= naturally infected plants in a field with high primary inoculum
3= toothpick infected plants (at flowering stage) in a field with high primary inoculum

Anova results show that treatment 1 had a highly significant (P <.0001) lowest mean disease rating of 0.75 and significant higher yield with a mean of 7 t/ha. Treatment 2 had a mean disease rating of 1.26 and a mean yield of 4.03 t/ha. Treatment 3 had a significant highest mean disease rating of 3.49 and lowest yield with a mean of 3.90 t/ha.

**Results from the quick screening lab based method on seedlings**

A disease index was created based on the method used by van Rensburg, *et al*. (2013). The number of seeds showing infection was counted and the amount of kernel covered by the charcoal rot was given a % value. This data was used to calculate a resistance index on a rating scale of 1 to 5.

[(number of infected seeds) + (disease rating)] / total number of seeds.

Mean disease index and mean disease ratings of common cultivars (Table 1) was used to conduct Pearson correlations. Correlation results between the quick screening results and those of mean disease scores of plants inoculated in die field with toothpicks at flowering had a moderate positive correlation (r=0.59 and P-value =0.03). Correlation results between the quick screening results and those of mean disease scores of plants left to naturally infect had a moderate positive correlation (r=0.69 and P-value = 0.01).

**Table 1: Mean disease index and disease ratings of common cultivars tested in the quick screening lab based method and field methods (toothpick inoculation at flowering and plants left to be naturally infected).**

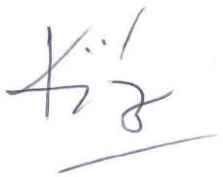
| Cultivar | Mean disease index of the quick screening method | Toothpick inoculations disease ratings 2019/20 season | Natural infection disease ratings 2019/20 season |
|---|---|---|---|
| 2 PAN6R-710BR | 4.11 | 2,40 | 2,80 |
| 4 KKS8410BR | 2.82 | 2,07 | 1,33 |
| 5 PAN4A-172 | 2.82 | 2,27 | 2,80 |
| 7 DKC68-58BR | 1.52 | 2,86 | 2,13 |
| 8 US9616 | 2.93 | 2,73 | 1,93 |
| 10 IMP52-12BR | 0.90 | 2,73 | 2,60 |
| 12 LS8542 | 4.44 | 2,87 | 2,07 |
| 14 VP8405BR | 4.40 | 2,27 | 1,33 |
| 18 DKC75-65BR | 4.80 | 1,46 | 1,20 |
| 20 LS8541BR | 4.81 | 1,60 | 1,40 |
| 22 PAN5R-785BR | 4.43 | 1,73 | 1,26 |
| 23 US9777 | 4.88 | 2,93 | 2,00 |
| 26 PAN5R-591R | 5.00 | 1,47 | 1,26 |
| 27 DKC78-45BGREN | 1.43 | 1,93 | 2,07 |
| 30 P2880WYR | 1.50 | 2,27 | 1,00 |

**PROBLEMS ENCOUNTERED**
None.

**References**
KENDIG, S.R., RUPE, J.C. & SCOTT, H.D., 2000. Effect of irrigation and soil water stress on densities of *Macrophomina phaseolina* in soil and roots of two soybean cultivars. *Plant Disease*, 84: 895-900.

TANDZI, L.N. & MUTENGWA, C.S., 2020. Estimation of maize *(Zea maydis* L.) yield per harvest area: appropriate methods. *Agronomy*, 10, 29; DOI:10.3390/agronomy10010029

VAN RENSBURG G.D.J., GILIOMEE, J.H. & PRINGLE, K.L., 2013. Resistance of South African maize hybrids to maize streak virus. *South African Journal of Plant and Soil*, 8: 38-42.



Handtekening van Snr Navorsingsb*estuurder*
*Signature of Senior Manager Research*

30 September 2020
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*Date*