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# Response of Intermediate Ripening Tobacco (*Nicotiana tabacum*) Cultivars to Frog Eye (*Cercospora nicotianae*) and Alternaria Leaf Spot (*Alternaria alternata*) in the Medium Growing Areas of Zimbabwe

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## ABSTRACT

**Background:** Alternaria leaf spot (*Alternaria alternata)* and Frog eye leaf spot (*Cercospora nicotianae)* are foliar diseases of tobacco (*Nicotiana tabacum*) which occur mainly in mature tobacco. Hence, the diseases are often associated with maturity of cured tobacco leaf at the tobacco sales floor in Zimbabwe. Where they occur, they can result in significant reductions in yield and quality of cured tobacco leaf.

**Methods:** A study was carried out in Trewaney, Mashonaland West province in Zimbabwe to investigate the response of newly developed ten flue cured tobacco varieties to the two foliar diseases Alternaria leaf spot (*Alternaria alternata)* and frog eye leaf spot (*Cercospora nicotianae).* The experiment was laid out as a randomized complete block design (RCBD) with three replicates. Standard agronomic practices in tobacco farming were followed and disease assessments were done 18 weeks after planting.

**Result:** Significant differences ($p<0.05$) for resistance to alternaria leaf spot were observed with the experimental hybrid T60 showing the highest resistance to the disease with a disease score of 1.6 under a 0-6 disease scale that ranges from 0 (no disease) to 6 (severely affected). However, there were no significant ($p>0.05$) differences among the varieties for frog eye leaf spot resistance, yield (kg/ha) and quality as measured by the grade index, however, K 326 showed the highest resistance to frog eye leaf spot. All the varieties, however, showed resistance to the two diseases. It was concluded that K 326 was the source of the resistance to frog eye leaf spot in all the experimental hybrids evaluated and that T60 had the highest Alternaria leaf spot resistance obtained from its parents which are both classified as resistant to the disease.


**Key words:** *Alternaria alternate, Cercospora nicotianae,* Foliar diseases, *Nicotiana tabacum*, Resistance.

## INTRODUCTION

Performance of genotypes is dependent largely on the environment in which they are grown and managed, especially, the extent of the availability of disease causing pathogens and the fertility of the soils on which they are grown (Kumar *et al.* 2014). Occurrence of foliar diseases in crops whose commercial products are leaves or fruits have been reported to severely reduce the economic performance of crops (Bakshi, *et al.* 2012). Alternaria (*Alternaria alternata)* and frog eye leaf spot (*Cercospora nicotianae)* (Fig 1) are foliar tobacco diseases that occur across the whole world with varying extents of severity in different countries but causing severe quality and yield losses to the famers. In the US, Alternaria leaf spot is known as brown spot but in Zimbabwe and other parts of the world the disease is commonly referred to as alternaria leaf spot. Both are fungal pathogens which attack tobacco (*Nicotiana tabacum*) causing yield and quality losses (Fravel *et al*. 1977). Alternaria and frog eye leaf spots are diseases of economic importance in tobacco farming. Where they occur, these foliar diseases introduce blemishes on leaves reducing yield and quality and eventually the price and income fetched on the market falls. Frog eye and Alternaria are diseases associated with ripening in tobacco and are common in warm moist environments. The two diseases have been reported in Southern China, Malawi, Zimbabwe, Argentina and Northern Brazil Although the occurrence of these diseases in these countries does not hinder tobacco growing out rightly, in France, Alternaria is a limiting factor to tobacco production.

The disease causing pathogen for Alternaria, *Alternaria alternata,* is saprophytic allowing it to remain in the soil on lignified plant debris from cultivated plant previously infected

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with the pathogen (Wang *et al*. 2015). The fungus is also reported to overwinter as mycelium which readily invades dead plant tissues making it able to survive for many months in the soil. Invasion occurs when the fungus gets into contact with lower leaves of the plant via soil or water splashes.

In Zimbabwe, Alternaria becomes epidemic in conditions of incessant rainfall which creates a humid microclimate that favours disease development in the low veld tobacco growing areas characterized by high temperatures.

Breeding efforts have been made to identify sources of resistance to Alternaria leaf spot in flue cured tobacco in Zimbabwe. The source of the resistance was the cigar type of tobacco Beinhert 1000 -1 which was found to possess monogenic resistance for the trait. This route was, however, abandoned in virginia tobacco after it was discovered that the gene in Bainhert was linked to delayed maturity and reduced leaf quality when it occurs as a homozygote. Heterozygote varieties have, however, been found to be promising in Zimbabwe. Varieties with quantitative resistance have been developed in Zimbabwe but the challenge is to get strong resistances under these situations. Gradual pyramiding of the resistance genes is one approach that is being used and has resulted in the available batch of varieties which show a complete gradation in Alternaria leaf spot resistance which is not absolute.

Studies have shown that Alternaria leaf spot is associated with fast ripening in susceptible varieties and frog eye is associated with maturity in many areas of its prevalence. However, it has also been shown that when the conditions are favorable, the two diseases can become an epidemic. Yield losses as a result of Alternaria leaf spot and frog eye leaf spot have not been quantified yet but the losses can be severe warranting studies targeting their control (Mamgain *et al*. 2013). The common approach used in controlling these diseases is the use of fungicides, balanced fertilizer, use of less N fertilizer and more Potash but phosphates and reduced plant populations and for some time it has worked in situations of less severe epidemics (Slavov *et al*. 2004). Gveroska and Ziberoski (2012) have reported the possibility of biological control of the alternaria using *Trichoderma harzianum in vitro* but the approach has not been put to field practice. During periods of pronounced outbreaks chemical control has also proved not to be quite effective and it has worked. Large quantities of chemicals were applied increasing the cost of controlling the diseases. This has prompted breeders to breed for resistance to the diseases. Resistance has been successfully incorporated into elite breeding lines and populations. Zhang *et al*. (2008) emphasized the reliability of incorporation of resistance to alternaria leaf spot in tobacco cultivars. However, there are no varieties on the other hand with frog eye resistance on the market to date. After crosses and selections had been made for resistance to alternaria and frog eye had been made, evaluations on their response to the two diseases under natural infestations were done.

## MATERIALS AND METHODS

### Genotype development

A breeding program was initiated using the recurrent selection approach to develop lines resistant to alternaria and frog eye leaf spot. After making the initial intercrosses and growing the segregating populations under naturally infected lands, lines resistant to the two diseases were identified and used to make hybrids.

### Raising the trial plants

Seedlings used were raised in float trays and transplanted on to the lands when they were pencil thick and 15 cm tall. Transplanting was done on 25 October 2013 using the water planting approaches where a seedling is planted with 5 liters of water. A basal fertilizer application was done at a rate of 800kg/ha Compound C (6:15:12) at planting and a topdressing of ammonium nitrate applied 4 weeks after planting at a rate of 120 kg/ha. The trial was set out under rainfed conditions. Assessments for incidence and severity of the frog eye and alternaria was done at 14 weeks after planting using scales of 0 (no disease) to 6 (heavy infestation). Thirty randomly selected plants were selected randomly and had their leaves rated per plot. The data generated was subject to analysis of variance (ANOVA) using Genstat version 14.

### Evaluation of disease resistance

Ten experimental hybrids were evaluated for resistance to Alternaria (*Alternaria alternata)* and frog eye leaf spot (*Cercospora nicotianae)* in the lands at Trelawney after they had been selected for acceptability of growth habit and type.


**Fig 1:** Typical *Alternaria alternata* infected tobacco leaf (a), stem (b) and Frog eye (*Cercospora nicotionae*) spot (c) on burley tobacco leaf.

The experiment was set out as a randomized complete block design with four replicates in a piece of land inherently infested with alternaria and frog eye. Each plot consisted of two rows each of 32 plants to make up a total plot size of 64 plants. An inter-row spacing of 120 cm and intra-row spacing of 56 cm was used. Only 60 plants were assessed for disease severity and incidence and eventually used in estimating yield performance. Table 1 below shows the scales used for scoring for the two diseases.

## RESULTS AND DISCUSSION

### Response of genotypes to *Alternaria alternata* leaf spot

There were significant differences among the varieties evaluated for their resistance to Alternaria leaf spot (Fig 2). Of the genotypes evaluated, T60 was the least infected by *Alternaria alternata* with a score of 1.6 while K 326 and the control variety K RK26 were the most infected both with a score of 3.1. However, these varieties can be grouped into three clusters based on their resistance to alternaria leaf spot. The first group contains T60; the second group has T69, T73, K 326R × XM26R, K 326R × XZR, T68 and T70 while the last group has K 326, K RK26 and T69. The resistance trend exhibited by the entries above (Fig 4) comes from the resistances of parents constituting these varieties. T60 is a hybrid of the lines BAZR and XM26R. Both of these lines have high resistance to Alternaria leaf spot and this resistance is exhibited in their F1 hybrid T60. Combining the two parents both with high resistance to alternaria pyramid their resistance in the resultant hybrid making it even higher hence T60 has the highest resistance among the genotypes tested.

The second group consisting of T69, T73, K 326R × XM26R, K 326R × XZR, T68 and T70 is K 326 based. That is the female parent in these hybrids is K 326 which is susceptible to alternaria leaf spot and the male parents in these hybrids have moderate resistance to *Alternaria alternata*. The moderate resistance to Alternaria leaf spot can therefore be coming from the male parents in this group. On the other hand, the last group consisting of K 326, K RK26 and T69 has K 326 as the common parent. K 326 is susceptible to *Alternaria alternata*. The male parents RW and RWR in the case of K RK26 and T69 have slight resistance to *Alternaria alternata*. When K 326 and either of the parents with slight resistance are combined to form a hybrid, the resistance obtained is not very strong.

### Response of genotypes to frog eye leaf spot (*Cercospora nicotianae*)

There were no significant differences among the genotypes evaluated for resistance to *Cercospora nicotianae* (Fig 3). Response to the disease ranged from 1.3 to 1.9 with K 326 exhibiting the best resistance. As has been mentioned earlier, these hybrids evaluated are K 326 based. Since K 326 is showing relatively high resistance to frog eye leaf spot, it can be concluded that this resistance is the one

**Table 1:** Alternaria leaf spot (*Alternaria alternata*) and Frog eye leaf spot (*Cercospora nicotianae*) disease assessment scales.

| Alternaria leaf spot (*Alternaria alternata*) | | Frog eye leaf spot (*Cercospora nicotianae*) | |
|---|---|---|---|
| Category | Area of leaf damaged | Category | No. of lesions/leaf |
| 0 | 0% leaf area damaged | 0 | 0 lesions |
| 1 | <0.33% leaf area damaged | 1 | 1-10 lesions |
| 2 | 0.34-1.0% leaf area damaged | 2 | 11-50 lesions |
| 3 | 1.1-3.3% leaf area damaged | 3 | 50-100 lesions |
| 4 | 3.4-10% leaf area damaged | 4 | >100 lesions |
| 5 | 10.1-33% leaf area damaged | | |
| 6 | >33% leaf area damaged | | |

NB: *Alternaria alternata* score ranges from 0-6 while that for *Cercospora nicotianae* ranges from 0-4.

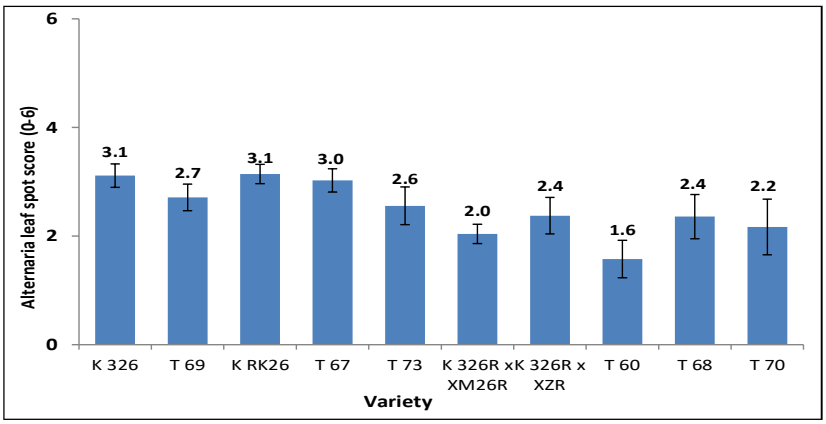


**Fig 2:** Response of genotypes to *Alternaria alternata* leaf spot.

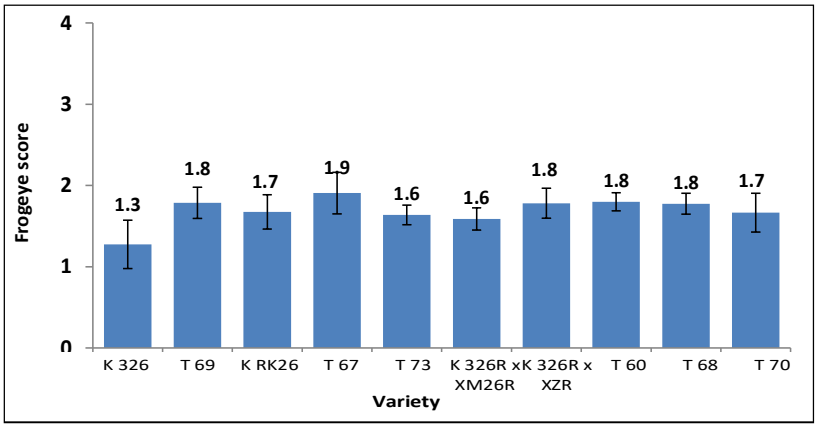


**Fig 3:** Response of genotypes to Frog eye leaf spot (*Cercospora nicotianae*).

exhibited in the hybrids constituted using lines which are K 326 based as the females.

**Yield (kg/ha) and grade index response**

Yield ranged from 2868 kg/ha for the variety T70 to 3985 kg/ha for the variety K 326R × XM26R (Fig 5). These varieties are medium maturing and ripening hence the yield performance of not more than 4 000 kg/ha are expected. K 326R × XM26R had the highest yield because of the male line XM26R which has the ability to accumulate relatively high dry matter within a short space of time. The high yield performance exhibited by these varieties point out that the two diseases for which their resistance to was evaluated did not negatively affect their dry matter accumulation. This could be because these diseases mostly affect the crop at later stages of their development when most of the dry matter accumulation has already taken place. Yield lasses as a result of these diseases are usually associated accelerated leaf senescence and degradation. Because of the presence of the disease in susceptible varieties, handling of the cured leaf usually becomes a challenge as most of it breaks down to form chaff. If the farmer harvests the crop affected relatively early these problems are reduced. This could have been the case in this trial.

Grade index is a measure of the acceptability of a cured tobacco leaf for marketing purposes. The grade index value

**Fig 4:** Frog eye leaf spot (*Cercospora nicotianae)* and Alternaria leaf spot resistant variety in Virginia tobacco.

is a measure of the sum total of all the blemishes the cured leaf starting from those factors which are a result of field management inadequacies and the presence or absence of diseases as well as general leaf handling properties after curing. If all the other management activities are done properly them disease can be a factor that reduces the quality of a cured leaf. In this study, there were no significant differences among the varieties evaluated on grade index

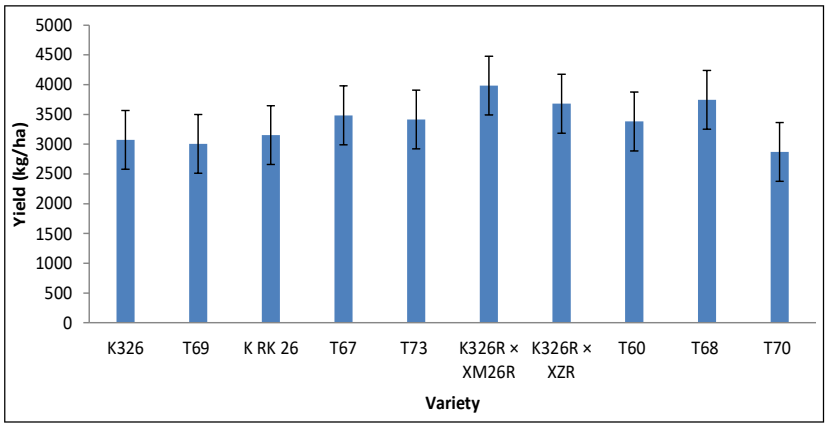


**Fig 5:** Yield response of genotypes to the presence of the *Alternaria alternata* and *Cercospora nicotianae.*

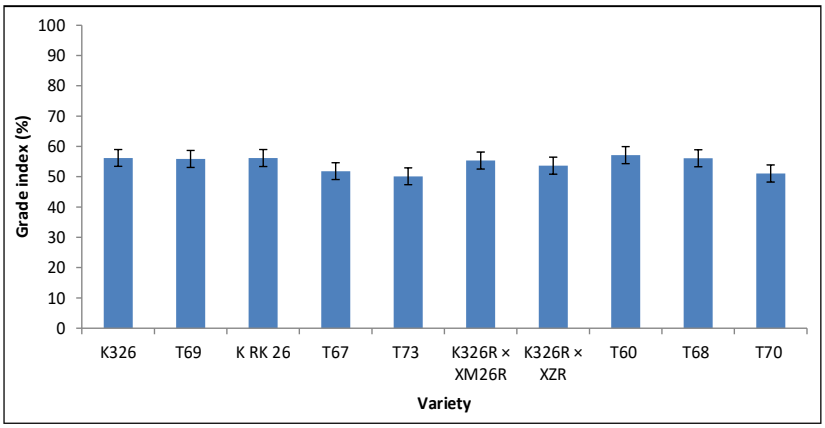


**Fig 6:** Yield response of genotypes to the presence of the *Alternaria alternata* and *Cercospora nicotianae.*

(Fig 6). It means that disease did not reduce the quality of the cured leaf. This is expected since the varieties exhibited resistance to the two foliar diseases for which they were evaluated.

## CONCLUSION

All the virginia tobacco varieties evaluated exhibited resistance to both Alternaria leaf spot (*Alternaria alternata*) and frog eye leaf spot (*Cercosprora nicotianae*). T60 had the highest resistance to alternaria leaf spot while K 326 had the highest resistance to frog eye leaf spot. The experimental hybrid T60 had the highest resistance to alternaria since both of its parents have relatively high resistance to the disease. All the hybrid varieties evaluated have their female parent as K 326 so their resistance to frog eye can be said to originate from this line. There were, however, no significant ($p>0.05$) differences among the varieties in yield performance and quality as indicated by their grade index. Quality and yield were not negatively affected since the varieties had resistance to the two foliar diseases tested.

## REFERENCES

Bakshi, P., Kumar, R., Jasrotia, A. and Wali, V.K. (2012). Growth and yield performance of mango varieties under rainfed areas of Jammu. Indian Journal of Agricultural Research. 46: 281-285.

Fravel, D.R. and Spurr, H.W., Jr. (1977). Bio-control of tobacco brown-spot disease by *Bacillus cereus subsp. mycoides* in a controlled environment. Phytopathology. 67: 930-932.

Gveroska, B. and Jovancev, P. (2011). Biological characteristics of *Alternaria Alternata* in tobacco. Tobacco. 61(1-6): 41-48.

Gveroska, B. and Ziberoski, J. (2012). *Trichoderma harzianum* as a bio-control agent against *Alternaria alternata* on tobacco. ATI-Applied Tech. Innovations. 7(2): 67-76.

Kumar, R., Prasad, B.K., Singh, M.K., Verma, A. and Tyagi, B.S. (2014). Genetic analysis for phenological and physiological traits in wheat (*Triticum aestivum* L.) under heat stress environment. Indian Journal of Agricultural Research. 48: 62-66.

Mamgain, A., Roychowdhury, R. and Tah, J. (2013). Alternaria pathogenicity and its strategic controls. Research Journal of Biology. 1: 01-09.

Slavov, S., Mayama, S. and Atanassov, A. (2004). Some aspects of epidemiology of *Alternaria Alternata* tobacco pathotype, Biotechnology and Biotechnological Equipment. 18(3): 85-89.

Wang, H., Huang, Y., Xia, H., Wang, J., Wang, M., Zhang, C. and Lu, H. (2015). Phenotypic analysis of *Alternaria alternata*, the causal agent of tobacco brown spot. Plant Pathology Journal. 14(2): 79-85.

Zhang, H.Y., Yang, Y.M., Li, F.S., He, C.S. and Liu, X.Z. (2008). Screening and characterization a RAPD marker of tobacco brown-spot resistant gene. African Journal of Biotechnology. 7(15): 2559-2561.