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Effects of *Dothistroma* Needle Blight on Growth and Disease Tolerance of *Pinus Radiata* D. Don, Progenies in the Rift Valley, Kenya

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Abstract: ***A study on two 19-year-old half-sib open pollinated Pinus radiata (D. Don) trial plots, at Timboroa and Nabkoi areas in the Rift Valley Province of Kenya was carried out to determine the effects of Dothistroma needle blight on height and dbh growth and disease tolerance levels among 45 families raised from "plus" mother trees selected from New Zealand and Kenya based on growth and Dothistroma resistance. The Timboroa trial had 25 progenies, only one of which was Kenyan, while the Nabkoi trial had 43 progenies, 6 of which were from local populations. Visual ranking of disease severity was carried out by trained and qualified technical personnel based on percent crown attack (yellowing and browning) severity scores of 1-5: 1 = 0 – 25%; 2 = 25–50%; 3 = 50–75%; 4 = 75–100% attack; and 5 = Dead. Height and dbh data were subjected to analysis of variance to identify progenies with high growth for further improvement. Disease severity data was subjected to descriptive statistical analyses using graphs and charts of percent attack. Further, percent attack data was arcsine transformed to enable ANOVA tests. Disease severity was thereafter modelled against height and dbh using the Arcsine transformed data. The results showed that severely attacked progenies had poor height and dbh.Further, both mean height and dbh showed a negative linear relationship with disease severity. The results indicated that the genetic constitution of some families may have influenced their height and dbh growth more than the disease severity alone as some progenies had elevated disease scores but still recorded higher height and dbh sizes. This finding may be especially important in the backdrop of climate change as superior genotypes may be able to withstand many growth stressors. It is recommended that research on resistance be continued and further testing of the selected superior families be undertaken.***

Keywords: *Pinus radiata, Dothistroma* needle blight, disease, tree growth

1. Introduction

P. radiata (D. Don) was one of Kenya's major plantation species accounting for 25% of the total softwood plantations. The species has high growth rate, good quality timber for construction, high wood density, long fiber length and low chemical content making it the prime choice for high grade pulp and paper in Kenya (Gibson, 1972). In fact, radiata pine used to be grown worldwide on large scale in plantations (Lamprecht, 1989). However, in the early 1960's further planting of *P. radiata* on large scale in Kenya was suspended due to its high susceptibility to *Dothistroma* needle blight disease (Arap Sang 1987).

Dothistroma needle blight is a foliar disease caused by a fungal pathogen, *Dothistroma pini* Hulbery. The disease causes defoliation, decreased productivity and, in extreme cases, tree death (Carson, 1989, Gibson *et al*. 1964).The history and pathogenicity of the disease are well described by Gibson (1972). In Kenya, the disease is associated with areas experiencing high rainfall, low temperatures and mist; sites where most *Pinus radiata* plantations were initially established (Gibson 1974). The disease, also called Red band needle blight is found in other countries and attacks other pine species e.g. Corsican pine (*Pinus nigra* ssp. *Laricio*), lodgepole pine (*Pinus contorta* var. *Latifolia*) and Scots pine (*Pinus sylvestrisI*). *(*Bulman *et al*., 2013, Brown and Webber 2008).

In Kenya, like elsewhere where the species was raised, initial control measures were based on aerial application of copper-based fungicides which though effective proved to be expensive. By 1968, planting of *P. radiata* on large commercial scale was suspended. In the present time, aerial spraying would be unthinkable especially considering the potential negative effects on non-target organisms and to the environment. The tree breeding programme of the Kenya Forestry Research Institute (KEFRI) initiated trials combining local selections and introduction of new resistant germplasm from other countries, in particular New Zealand where the species is one of the major plantation species and disease research is far advanced. The main objective was to screen progenies which combine pest and disease resistance with fast growth and high yields as part of an Integrated Pest Management (IPM) strategy (Mutito pers comm. 2019). In retrospect, is it possible to identify families with natural resilience to be able to withstand vagaries of nature especially those which may be exacerbated by climate change (e.g. Woods *et al*. 2005).

This paper reports the results of a study carried out in Timboroa and Nabkoi areas of the Rift Valley, Kenya to elucidate the effects of *Dothistroma* needle blight on height and dbh growth and tolerance levels among 45 families raised from "plus" mother trees selected from New Zealand and Kenya.

2. Material and Methods

2.1 The study area

The study was conducted in two sites(Nabkoi and Timboroa) measuring 1 ha each, which were among the favourite sites for growing radiata pine in Kenya. The trials were put up by KEFRI in 1991 (Mbinga 2002). The areas are located in Uasin Gishu, in the Rift valley of Kenya. Timboroa is found at latitude 0°04' north and longitude 35°33' east at an altitude of 2, 743 m above sea level (masl.). Meanwhile, Nabkoi is at an altitude of 2, 591 masl. and latitude 0°8' north and longitude 35°28' east. Both sites have low temperatures with Nabkoi having a range of 14-16°Cwhile Timboroa has a range of 12-14°C. Mean annual rainfall ranges between 1000-1600 mm for Nabkoi and 1100-1700 mm for Timboroa with both sites experiencing dry spells from October to March (Mbinga 2002). A detailed description of the sites' geology and soils can be found in Musyoka (2010).

2.2 Methods

2.2.1 Plant material

The Timboroa trial comprised of 25 progenies, 24 of which were obtained from New Zealand while the Nabkoi site comprised of 43 progenies of which 37 were from New Zealand. The remainder were from Kenyan local populations. The experimental design used for both trials, was a Complete Randomized Block Design (CRBD), with four blocks in each trial. Plots of 6x6 trees per progeny were used. The trees were spaced at 2.75 m x 2.75 m giving 1320 stems per ha. The plots were raised through the shamba system (Taungya or PELIS, where trees were raised together with agricultural crops by communities. As the communities tended their crops, they also cared for the tree seedlings until canopy closure when they moved to another area), (Witcomb and Dorward, 2009, Agevi *et al*., 2016).

2.2.2 Tree growth Assessment

Total tree height, dbh, stem form, crown characteristics (i.e. crown shape, size, density, height) and cone production capacity were assessed although for this paper only height and dbh data were used.

2.3 Assessment of Dothistroma blight

Subjective approach to disease scoring by visually quantifying the crown percent affected by the disease was used (Ivory 1968, Gibson, 1971, 1972), (figure 1). Qualified technical staff from the Kenya Forestry Research Institute (KEFRI) carried out this exercise. The method is cost effective, easy and quick to execute and favourable for large area disease assessment but its subjectivity is its main drawback. However, if used by qualified personnel it has been reported to be reliable.

Table 1: Disease scoring categories used during the study

Disease severity Score (class)	Level (%) of Browning of foliage	Description
1	0-25	Trees with little or no symptoms of disease. Affected whorls with up to 25% needle blight.
2	25-50	Few symptoms evident. Affected whorls with 25 to 50% needle blight.
3	50-75	Disease is obvious, affected whorls with 50 to 75% needle blight.
4	75-100	Disease is obvious covering most of the crown, affected whorls with 75 to 100% needle blight.
5	Tree dead	Dead standing or dead fallen

[a]The scoring protocol was aimed to grade the range of crown conditions observed across the stand as evenly as possible. Source: Raymonds and Cotterill, 1990.

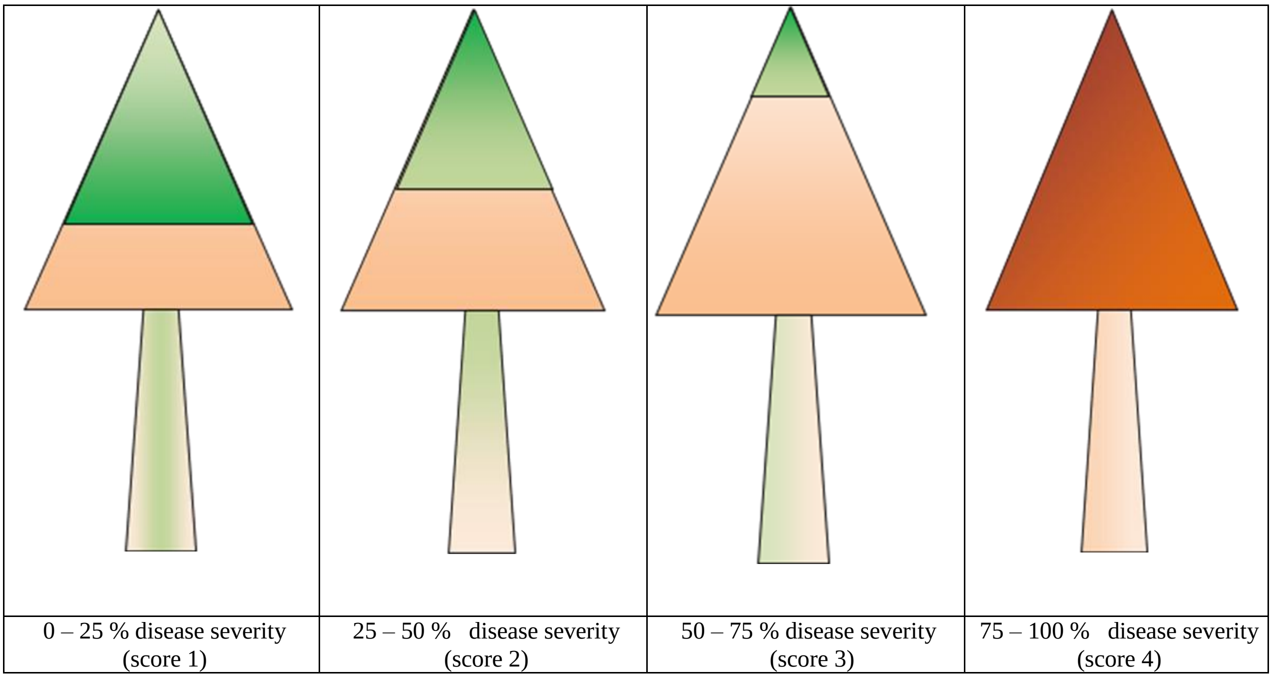


Figure 1: Categories of visual disease scoring used in the study

Data Analyses methods

Height and dbh data were subjected to analysis of variance to find out superior progenies. Disease scoring values were summed and averaged to give mean scores for each progeny. Plots were then drawn of height and dbh versus the mean disease scores. The mean scores were Arcsine transformed and ANOVA tests run on the transformed data. Scatter diagrams of mean disease scores and mean height and dbh were plotted to visualise the trends between disease severity scores and height and diameter of the progenies. Appropriate models describing the trends were fitted.

3. Results and Discussion

Height

Significant height differences (p = 0.0001) were observed both in Timboroa and Nabkoi.For Nabkoi, the best 5 progenies in height were F20, F34, F33, F50, and F9, with mean heights ranging between 26.8m to 31.54m. F22 and F41 were the poorest progenies with mean heights of 13.76m and 11.14m respectively, this being only about 50% that attained by the best performing progenies (Figure 2).

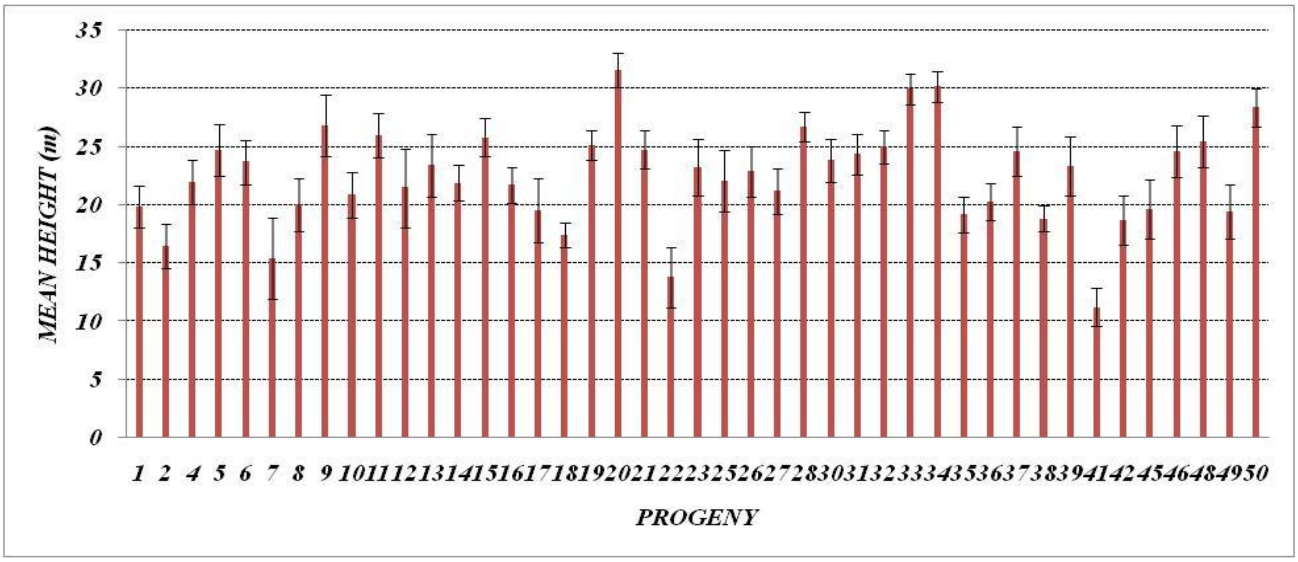


Figure 2: Mean height of progenies in Nabkoi

Table 2: Analysis of Variance (ANOVA) for Height for Nabkoi progenies

Source of Variation	DF	Sum of Squares	MS	F	Pr>F
Block	3	269.30	89.77	2.07	0.1036
Progeny	42	6516.71	155.16	3.58	0.0001
Error	422	18303.56	43.37		
Total	467	25424.98			

Tukey's Studentized Range (HSD) Test results for height showed that; progeny F20 was significantly better compared to progenies F17, F49, F35, F38, F42, F18, F27, F22 and F41.

For Timboroa the best five progenies with respect to height were F34, F26, F20, F33 and F28 with a mean height range of 25.16 m – 30.53 m. Progenies F34, F26, F20 and F33 stood out as the dominant families with regard to height in Nabkoi as well. Tukey's Studentized Range (HSD) Test for mean height showed that progenies F34, F26, F20, F33, F28, F15, F25, F11, F21, F9, F30, F24, F17, F23, F42, F36 and F6 were not significantly different, while progeny F34 differed significantly from progenies F16, F4, F1, F3, F13, F18, F10 and F27.

Dbh

Results for dbh showed significant differences (p=0.0001) among the progenies with 3 of the best 5 (F20, F33, F34, F9 and F48) progenies also being the best in height in Nabkoi. The mean range for dbh was 12.84 cm to 32.62 cm (Figure 3; Table 3). Analysis of variance showed that there were significant differences in dbh between progenies (Table 3).

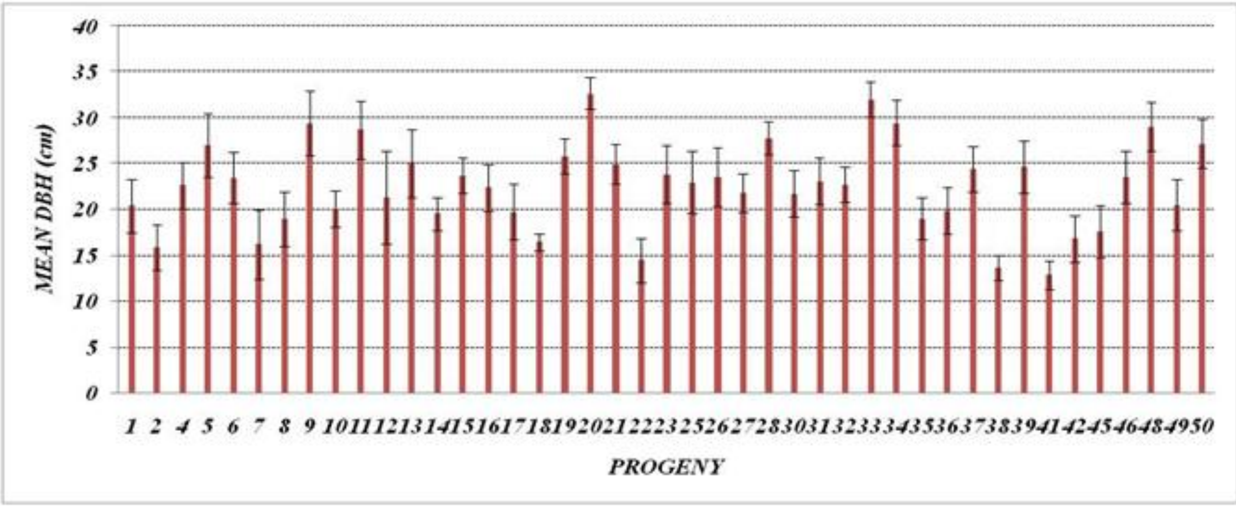


Figure 3: Mean dbh of progenies in Nabkoi

Table 3: Analysis of Variance (ANOVA) for dbh in Nabkoi

Source of Variation	DF	Sum of Squares	MS	F	Pr>F
Block	3	168.12	56.04	0.77	0.5123
Progeny	42	8353.24	198.87	2.73	0.0001
Error	416	30346.66	72.95		
Total	461	39342.19			

Tukey's Studentized Range (HSD) Test showed that progeny F20 was better and differed significantly from progenies F18, F7, F22, F2, F38 and F41 in dbh.

For Timboroa, the dbh (ANOVA) results were similar (results not shown) with progenies showing significant differences (p=0.05) amongst themselves. Progenies with the largest mean dbh were F26, F34, F20, F15 and F33. It is worth noting that progenies F20, F33 and F34 were also superior in terms of dbh in Nabkoi trial site and were among the best in terms of height in both sites. While progenies with the least mean dbh were F27, F10, F18 and F1, with mean dbh < 15cm. Based on the results of Tukey's Studentized Range (HSD) Test for mean dbh, progenies F26, F34, F20, F15, F33, F25, F21, F9, F28, F11, F17, F30, F23, F36, F24, F42, F16 and F3 were not statistically different from each other; while progeny F26 was significantly different from progenies F6, F13, F4, F1, F18, F10 and F27. Progenies F10 and F27 were significantly poor in dbh compared to progenies F20 and F34.

Disease scores

Dothistroma needle blight severity

Progenies with the highest disease tolerance i.e. low mean disease score, were F34, F26, F11, F33, F20, F28 and F15, with disease mean score < 2. This mean score is within the range of 0 – 25% crown infection levels. Whereas, progenies with the highest disease severity were F27, F10, F18, F3, F1, F6, F17 and F4, with mean score > 3, which translates to affected whorls with more than 50% needle blight. It was observed that as disease severity increased, mean height and dbh growth decreased (Figure 5).Results of scatter plots of mean disease scores versus mean height and/or dbh showed strong linear trends with negative slope showing a strong inverse relationship between disease severity and tree height and diameter growth for Nabkoi (Figure 4&5). Timboroa had similar linear trends with negative slope as shown in figures 6 & 7.

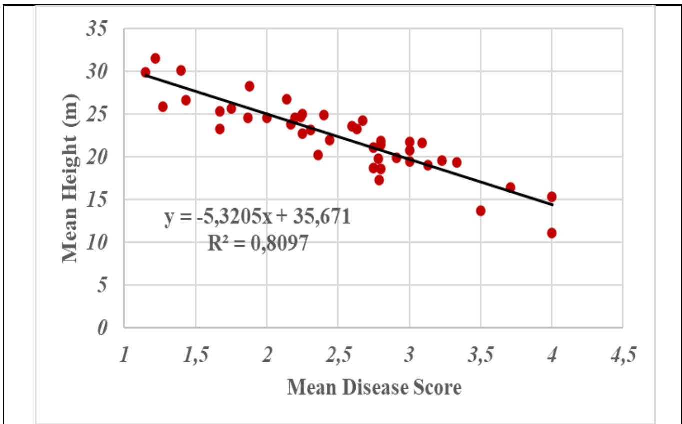
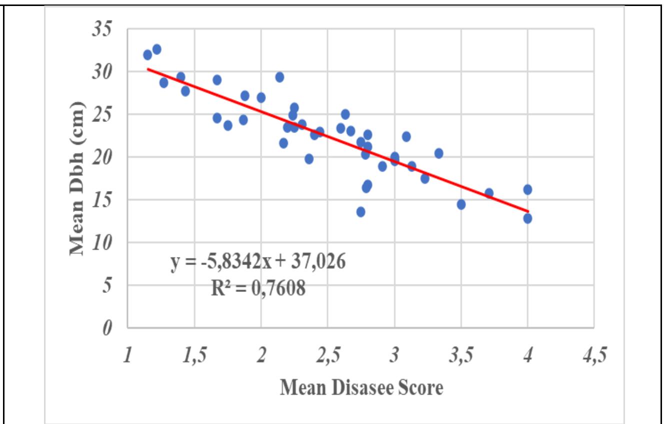


Figure 4: Scatter diagram of mean disease severity scores plotted against tree heights for Nabkoi progeny trial.



Figurer 5: Scatter diagram of mean disease severity scores plotted against mean Dbh for Nabkoi

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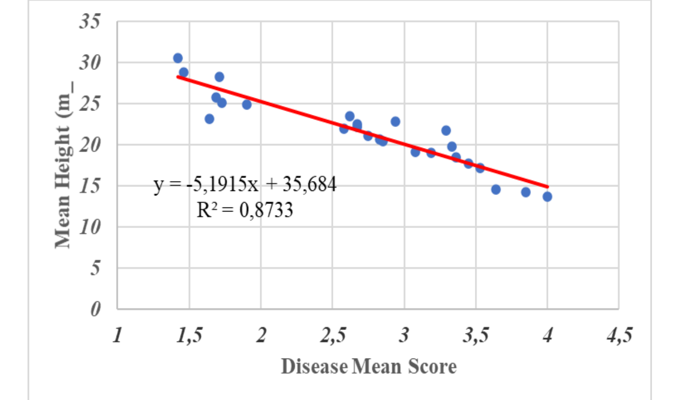

Figure 6: Scatter diagram of mean disease severity scores plotted against tree heights for Timboroa progeny trial.

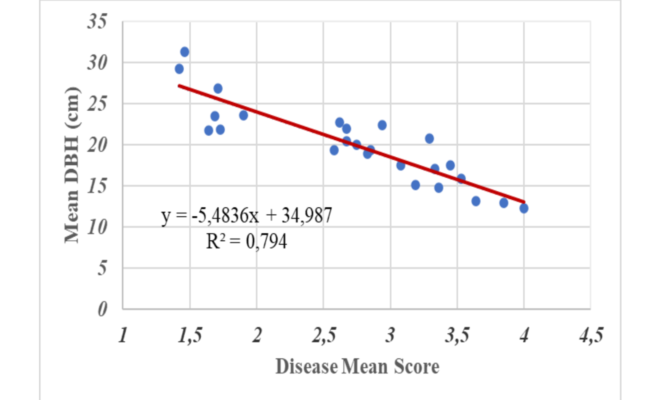

Figure 7: Scatter diagram of mean disease severity scores plotted against mean Dbh for Timboroa

Linear models of the general form; $Y = \alpha + \beta * X + \varepsilon$;(Y = mean height or dbh, X = mean disease score and α and β are coefficients, ε = error term) were then fitted with negative slopes (coefficients and R^2 values shown in Table 4).

Table 4: Fitted linear model coefficients and R^2 for height and Dbh.

	α	β	R^2
TIMBOROA Height	35.684	-5.1915	0.8733
TIMBOROA Dbh	34.987	-5.4836	0.7940
NABKOI Height	35.671	-5.3205	0.8097
NABKOI Dbh	37.026	-5.8342	0.7608

4. Discussion

The results from this study showed that both tree height and dbh of radiata pine in Timboroa and Nabkoi were negatively affected by *Dothistroma* needle blight. Further, family variation in height and dbh were shown to be related to *D. pini* resistance (Figure 2 & 3). Healthy families had statistically larger mean dbh and mean total height than families with severe *Dothistroma* blight infection (Table 2 & 3). This level of correlation between these traits in radiata pine was also reported in earlier similar studies e.g. Beets and Jokela (1994). Bergmann and Stomp (1994), found that tree genotype influenced susceptibility over and above the effect on growth rate. Our findings support this thinking as well.

The findings have shown clearly that families with low infection levels (low disease mean scores) had the best mean height and dbh (figure 4, 5, 6 & 7). This shows that the disease actually reduced potential growth of the species. Mbinga (2002) reported similar findings while working on three different radiata pine populations in Timboroa – Kenya. Kirongo *et al*. (2002) while studying the interference mechanisms of pasture on the growth and fascicle dynamics of 3-year old radiata pine clones reported that trees reacted to any growth impediment by reducing growth especially if the impediment reduced the photosynthetic leaf area as was the case in this study where the *Dothistroma* needle blight caused yellowing, browning and death of the needles. Therefore, growth stresses whether from competition between trees (intra-specific) (e.g. Balozi *et al*. 2013) or from fungi attack as in this study will lead to reduced growth. This observation has important implications to forest management as it implies that managers have to ensure that trees grow in stress-free environments. This may also imply that trees will react by reducing growth when faced with stress brough about by climate change effects (e.g. increased drought, high disease incidence, severe fungi and pest attack).

The results in this study also suggested high resistance levels among the progenies, with some outstanding families having a mean disease score of < 2, i.e. 0 – 25% crown infection levels. These families which showed disease resistance can be selected for inclusion in future breeding programmes for *Pinus radiata* in Kenya. This observation is especially important in the backdrop of climate change as superior genotypes may be able to withstand many growth stressors (e.g. Wilcox 1989) compared to genotypes which do not have inherent ability to resist stressors. According to Woollons and Hayward, (1984) for an effective breeding programme a selection of trees with a score of 1 or 2 (i.e. 0 – 25% or 25 – 50% browning of foliage respectively) is recommended. In this study, using this criterion would result in 23 progenies in Nabkoi and 13 in Timboroa being selected for further improvement research.

Progenies with consistently low disease scores (i.e. percentage infection) can be assumed to be relatively resistant to the blight (Chagala, 1995). Meanwhile, some progenies showed high growth, at relatively high disease mean scores indicating that their resistance could be genetically predisposed. Zas *et al*. (2005) reported genetic variation of *Pinus pinaster* Ait. seedlings in susceptibility to *Hylobius abietis* L. In this study we saw evidence of genetic prowess in some progenies which made them to grow better even under considerable attack from the needle blight. While other studies have reported significant Genetic-by-Environment interactions (e.g. ZAS *et al*. 2004), we did not find significant evidence for this and similar families performed best in both Timboroa and Nabkoi sites.

While it may be argued that the subjective/visual approach to assessing disease severance may be a weakness to the study, many researchers have used subjective approaches successfully so long as the scoring is done by trained, qualified technicians/researchers. For example, Ivory (1968), and Gibson, (1971, 1972), successfully used subjective methods to quantify disease severity in *Pinus* *radiata*. This study has shownthat *Dothistroma* needle blight had detrimental effects on height and dbh growth and that disease severity was related to mean height and dbh by an inverse linear relationship. Further, the study also revealed that there are significant family differences in relation to *Dothistroma* needle blight resistance and that there are opportunities to select and breed for further resistance using the material in Nabkoi and Timboroa research plots.

5. Conclusions and Recommendations

Height and Dbh growth were significantly affected by Dothistroma needle blight. Family differences were evident and helpful in thwarting *Dothistroma* attack in radiata pine. Visual assessment methods of disease severity are reliable when done by qualified technicians.

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