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Effect of infection level of sesame (*Sesamum indicum* L.) seed by *Alternaria sesami* on severity of *Alternaria* leaf spot

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ABSTRACT

***Alternaria sesami* infection in sesame seed samples collected from farmers in Busia, Kakamega and Siaya districts of Kenya was detected by oat meal agar method. Infection levels in seed samples varied from 8.96% in Kakamega to 24.20% in Siaya with an infection level of 11.69% at Busia. Alternaria leaf spot was monitored in plots having six seedborne infection levels (0-8%) to determine the effect of transmission of the fungus by seed on disease severity. Increase in percent leaf area blighted and percent defoliation fitted the Gompertz model more closely than the logistic model. Rates of disease increase in blighted leaf areas and defoliation as well as areas under disease progress curves (AUDPC) varied among the six infection levels. Seed inocula levels with larger AUDPC generally had faster rates of disease progress. Disease was most severe on plants from seeds with highest infection level of 8% and least on plants from seeds with no infection. Severity of the disease increased with increase in seed infection.**

Key words: Alternaria leaf spot, *Alternaria sesami*, infection rate, seed infection, sesame, *Sesamum indicum*

INTRODUCTION

Among the oil crops currently grown in Kenya, sesame (*Sesamum indicum* L.) has the best adaptation to marginal agroecological zones. The production of the crop is restricted to the lower midlands in Western Kenya (Western and Nyanza provinces) and coastal lowlands in Coast province. Due to the low and unreliable rainfall, parts of agroecological zones in coastal lowlands are considered marginal for crop production. This is also true for Western Kenya during the short rainy season which receives 300 - 550 mm of annual rainfall (Anon. 1982). Sesame is considered drought resistant (Weiss 1971) and can give a crop with as little as 300 mm of rain. It is grown by small-scale farmers mainly in a crop mixture with a cereal for subsistence. Approximately 182,000 ha of land is currently under the crop (Anon. 1995). In Kenya, yields of upto 2,230 kg ha^{-1} have been reported from experimental fields, but average yield on farmers fields is only 80-400 kg ha^{-1} (W'Opindi 1980). In addition, there are no commercial certified varieties available for planting. Farmers therefore use landrace varieties for sowing (Gichuki and Gethi 1988).

The major constraints to sesame production in Kenya are diseases and insect pests (Ayiecho and Nyabundi 1995). Observations at Siaya Farmers Training Centre (FTC) in Western Kenya showed that Alternaria leaf spot caused by *Alternaria sesami* was the most severe disease on sesame. The disease was first reported on sesame in Kenya by Gatumbi (1986). A decline in photosynthetic area due to leaf damage is often the initial effect of the fungus; premature defoliation soon follows, and this adversely affects growth and yield (Elston *et al.* 1976). *A. sesami* also causes considerable damage to sesame capsules (Berry 1960). Yield losses ranging from 18 to 55% were attributed to the fungus (Barboza *et al.* 1966). Occasionally seedlings and young plants are killed exhibiting pre and post-emergence damping-off and losses of 55 to 59% have been attributed to the fungus (Yu *et al.* 1987).

The Alternaria leaf spot pathogen *A. sesami* is seed transmissible (Yu *et al.* 1981) and has been reported to be of worldwide distribution (Leppik and Sowell 1964), occurs in epidemic proportions in El Salvador (Weiss 1971), India (Desphande and Shinde 1976) and the United States (Culp and Thomas 1964). The pathogen can survive between cropping seasons or unfavourable conditions as an infectant in the seeds (Kolte 1985). Seedlings raised

Abbreviations: AUDPC-DF- Area under disease progress curve due to defoliation; AUPDC-DL- Area under disease progress curve due to disease severity; OMA- Oat meal agar

from infected seeds become primary source of inoculum for infection to other plants in the field (Neergaard 1979). Due to the lack of commercial certified seeds, farmers in Kenya plant their own seeds from a previous harvest. In some cases, they buy such seeds from other farmers (W'Opindi 1981). Although *A. sesami* is seedborne, no studies have been conducted to determine infection levels of the fungus in Kenyan seeds and the effect of such levels of infection on disease development. This study was carried out to assess sesame seeds commonly used for planting by small-scale farmers in Kenya for infection by *A. sesami* and to determine the effect of transmission of the fungus by seed on *Alternaria* leaf spot development under field conditions.

MATERIALS AND METHODS

Seed sampling

Sesame seed samples were collected from small-scale farmers in Busia, Kakamega and Siaya Districts, Kenya. From each of these districts eight sampling areas were randomly chosen and in total 24 sampling areas for the three districts were selected. Twelve farmers were chosen from each sampling area. A sub-sample of 50 to 70 g of seed was collected from each farmer; the sub-samples were then mixed to form a representative sample of 0.60 - 0.84 kg seed.

Determination of seed infection levels

A. sesami was enumerated in sample seeds using the oat meal agar plate method (Lee 1978). All seeds were surface sterilized in a solution of 1% NaOCl containing 1% W/V available chlorine for 5 minutes, followed by draining-off the surplus liquid. Oat meal agar (OMA) was prepared by sterilizing the ingredients (oat 20g, agar 20g, water 1 litre) by steaming for 15 minutes at 121°C and 1.2 kg cm^{-2} pressure. Bacterial growth was checked by addition of 200 ppm of streptomycin sulphate to the molten OMA cooled to 45°C, immediately before aseptically dispensing into 9 cm diameter petri-plates (25 ml per plate). Development of saprophytes was prevented by plating sterilized seeds without rinsing. Four hundred seeds were aseptically plated in 20 plates for each sample, keeping a distance of 1 cm between seeds. Identification of *A. sesami* on seeds was based on characteristic growth of colonies on OMA by use of a low power microscope as described by Lee (1978) and Mehta and Prasad (1976).

Determination of effect of seed infection on disease severity

Sesame seeds with five infection levels 2, 4, 5, 7 and 8% as determined by the oat meal agar test were used in the study to determine the effect of seed infection on disease severity. Sesame accession SPS SIK 110 obtained from the germplasm collection of the Sesame Improvement Project of the University of Nairobi was used as a control (0% infection). The seeds were planted in a randomized complete block design with three replicates at the Kibwezi Institute for Dryland Research and Development on 21st March and 29th July 1996. Plots of each seed infection level consisted of five 4 m rows spaced 0.5 m apart with plants at a spacing of 0.20 m within rows. Plots were arranged perpendicular to the direction of the prevailing wind to reduce inter-plot interferences and were separated from each other at the ends and sides by 2 m strips of susceptible sesame accession SPS SIK 013.

Percent leaf area blighted and percent defoliation were assessed for severity of Alternaria leaf spot from April 1996 to June 1996 for the first season (long rainy season) and August 1996 to October 1996 for the second season (short rainy season). Average blighted leaf areas in the plots were obtained by selecting 10 representative plants at random on each sampling date. Leaf areas (one surface only) were calculated by multiplying average width by length measurements for various linear proportions of the leaves with the triangular leaf apices calculated separately. Lesion counts and lesion areas were recorded for 20 selected and taged plants every 10 to 14 days. Mean percent disease was calculated by lesion numbers times average lesion area divided by total leaf area. To determine percent defoliation, each row within the plot was divided into 0.6 m segment per row prior to each assessment date. One segment per row was randomly selected every 10 to 14 days in each plot and subdivided into four 0.15 m sub-segments. One such sub-segment was randomly selected every 10 to 14 days and the number of nodes and missing leaves counted on each stem. Percent leaf area blighted and percent defoliation were used in determination of the area under disease progress curve (AUDPC) due to disease severity (AUDPC-DL) and due to defoliation (AUDPC-DF) using the formulae of Shaner and Finney (1977).

Data analysis

Gompertz and logistic models were fitted to percent leaf area blighted and percent defoliation data.

Apparent rates of disease increase were obtained by regressing Gompertz transformed data against time, expressed as days after planting. The most suitable model for assessing infection and defoliation rates was determined by comparing coefficients of determination obtained using each model as applied by Campbell and Madden (1990). Rates of increase were obtained by regressing Gompertz-transformed disease/defoliation data against time (days after planting) using the equation:

$$K = [\text{gompit}(Y\text{max}) - \text{gompit}(Y\text{min})]/(t_2 - t_1)$$

in which, gompit = -ln [-ln(Y)], (Ymin) and (Ymax) being proportion of disease/defoliation observed at the beginning (t_1) and at the end (t_2) (Luke and Berger 1982). Student's *t* test was carried out separately for AUDPC-DL, AUDPC-DF, infection and defoliation rates for the two seasons. Within each season, AUDPC-DL, AUDPC-DF, infection and defoliation rates for each treatment were compared using analysis of variance and significant differences between treatment means were identified using Duncan's Multiple Range Test (DMRT) as applied by Luke and Berger (1982).

RESULTS

Alternaria sesami was detected in all the sampled seeds with infection levels being dependent on the district. The highest infection level was 24.2% in Rarieda of Siaya district and the least infection was7.5% in seed samples from Mumias and Navakholo in Kakamega district (Table 1). On overall basis, Siaya district recorded the highest mean infection of 19.9%, whereas the lowest level was recorded in Kakamega with a mean of 9.0% infection. Busia district recorded a mean infection level of 11.7%. Infection levels in Siaya ranged from 18.1% in Usingu to 24.2% in Rarieda. Busia district had infection levels ranging from 10.0% in Mayenje to 14.0% in Bugengi. Infection levels in Kakamega district ranged from 7.5% in Mumias to 11.0% in Majengo (Table 1).

Area under disease progress curve for percent leaf area blighted (AUDPC) due to Alternaria leaf spot were significantly larger during the first season than in the second season at Kibwezi (t = 3.08, P = 0.01). In both seasons, highly significant differences in disease severity were observed among the six seedborne inocula levels. Plants from seeds with 8% infection levels of *A. sesami* exhibited the highest AUDPC - DL in both experimental seasons (Table 2). The other inocula levels studied had significantly lower AUDPC-DL except 5% and 7% in the first season and 7% during the second season. Judged by AUDPC - DL, disease was least severe in plots of 0% seedborne *A. sesami* infection.

Table 1. Mean percent infection levels of *A. Sesami* in sampled seeds from three districts in Kenya.

Sampling area	Percent infection[w]
Siaya District	
Alego	20.0
Bondo	19.0
Boro	18.1
Hawinga	19.0
Rarieda	24.2
Ugunja	21.1
Ukwala	19.5
Usingu	18.1
Mean	19.9
Busia District	
Alupe	11.0
Amagoro	10.1
Amukura	13.1
Angoromo	13.1
Bugengi	14.0
Butula	11.1
Mayenje	10.0
Roadblock	11.1
Mean	11.7
Kakamega District	
Butere	10.1
Khwisero	8.1
Lurambi	10.0
Majengo	11.0
Mumias	7.5
Municipality	9.5
Navakholo	7.5
Shinyalu	8.0
Mean	9.0

[w]Each value is an average of 3 replicates.

Like AUDPC - DL, area under disease progress curves for percent defoliation (AUDPC - DF) due to seedborne *A. sesami* infection were also significantly larger in the first season than in the second season at Kibwezi (t = 2.30, P = 0.05). There were also highly significant differences in AUDPC - DF among the six seedborne inocula levels in both experimental seasons (Table 2). Inocula levels of 8% exhibited a significantly larger AUDPC - DF than did other inocula levels tested except 4%, 5%, and 7% during both seasons of the study. The smallest AUDPC - DF was observed in plots of 0% seedborne inoculum in both seasons.

The Gompertz model generally produced slightly higher coefficients of determination (R^2) than did the logistic model. Rates of increase in Alternaria leaf spot were thus estimated and compared using the Gompertz model. Goodness of fit of the models to disease progress data, however appeared to vary from one seedborne inoculum level to another. The data in Table 3 show the mean rates of disease increase on the six seedborne levels of *A.sesami* in either season as was computed using the

Table 2. Mean area under disease progress curves[w] for percent leaf area blighted (AUDPC - DL) and percent defoliation (AUDPC - DF) from field tests conducted on six levels of seedborne *A. sesami* during two seasons at Kibwezi, Kenya.

	Season I March-June 1996		Season II July-October 1996	
Inoculum level (%)	AUDPC-DL	AUDPC-DF	AUDPC-DL	AUDPC-DF
0	0.18cde	0.35de	0.10bcde	0.20cde
2	0.35bcde	0.56bcde	0.33bcde	0.35bcde
4	0.59bcd	0.60abcde	0.60bcd	0.40abcd
5	1.41abc	0.80abc	1.39bc	0.48abc
7	1.95ab	0.89ab	1.59ab	0.55ab
8	2.30a	0.99a	2.26a	0.66a
Mean	1.13	0.70	1.04	0.44

[w]Average of 3 replications; Within each column, means followed by the same letter do not differ significantly at P = 0.01.

Table 3. Rates of increase in percent leaf area diseased and percent defoliation due to Alternaria leaf spot on six levels of seedborne *A. sesami* during two seasons at Kibwezi, Kenya.

	Season I March-June 1996		Season II July-October 1996	
Inoculum level (%)	Infection rate[w]	Defoliation rate[w]	Infection rate	Defoliation rate
0	0.032d	0.027c	0.032bcde	0.020bcde
2	0.042bcd	0.027c	0.040bcde	0.019bcde
4	0.078abcd	0.028bc	0.056bcd	0.021bcd
5	0.086abc	0.032abc	0.061abc	0.022bc
7	0.088ab	0.042ab	0.108a	0.030ab
8	0.092a	0.043a	0.069ab	0.034a
Mean	0.070	0.033	0.061	0.024

[w]Values shown represent average of 3 replications. Within each column, means followed by the same letter do not differ significantly at P = 0.01

Gompertz model.

Rates of increase in percent leaf area diseased (infection rates) due to *Alternaria* leaf spot were statistically similar in both experimental seasons (t = 0.59, p = 0.05). There were however, highy significant differences in infection rates among the six levels of inocula in both seasons (Table 3). Maximum infection rates were observed in plots with 7 and 8% seed infection in the second and first season respectively. The rate of increase in percent leaf area blighted were significantly slower on the other inocula levels studied except 4, 5 and 7% in the first season, and 5 and 8% in the second season. Although the least rate of disease increase was observed in plots of 0% infection in both seasons, it did not differ significantly from that of other inocula levels studied except 7 and 8%.

Table 4. Meterological data for March - October, 1996 at Kibwezi, Kenya.

Parameter	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct
Temperature (°C)								
Minimum	10.1	18.6	18.3	17.6	17.8	18.3	17.5	16.7
Maximum	28.4	30.2	29.9	28.4	28.8	30.3	31.1	31.3
Mean	21.3	24.4	24.1	23.0	23.3	24.3	24.3	24.0
Humidity								
Minimum	56.7	N/A[t]	58.0	N/A	N/A	N/A	50.0	50.0
Maximum	90.5	N/A	69.0	N/A	N/A	N/A	81.0	79.0
Mean	73.6	N/A	63.5	N/A	N/A	N/A	65.5	64.5
Rainfall (mm)	109.0	181.7	79.0	53.7	63.0	13.0	6.0	38.0

Source: Meterological station at Institute for Dry Land Research and Development, Kibwezi, Kenya.
[t]N/A Not available.

Rates of increase in percent defoliation due to *Alternaria* leaf spot as a result of seedborne inocula were significantly faster in the first season than in the second season (t = 2.23, p = 0.05). Differences in defoliation rates among the six levels of inocula during both seasons were highly significant (Table 3). In both seasons, 8% seed infection exhibited a significantly faster rate of defoliation than did other levels except 5 and 7% in the first season and 7% in the second season. The slowest rate of increase in percent defoliation was observed in plots of 0% seed infection.

Although the mean temperature was similar during both seasons, the rainfall was greater in season I (Table 4).

DISCUSSION

The seed health test revealed that most of the sesame seeds used for planting by small-scale farmers in Kenya are infected by *A. sesami*. Seed samples from Siaya and Kakamega districts had the highest and least infections respectively, with Busia district having intermediate infection levels. The disease severity and infection of seeds by the fungus are known to be dependent on prevailing environmental conditions and pathogen population. Ngabala and Zambettakis (1970) reported that wet conditions favour the development of *Alternaria* leaf spot. This results in increased seed infection by *Alternaria* species (Degenhardt *et al.* 1982). This may also

explain the differences in *A. sesami* infection levels in the districts studied. Observations made in sesame plants during the sampling revealed that *Alternaria* leaf spot was more severe and widespread in Siaya as compared to Busia and Kakamega districts. Sesame is produced in Kakamega only during the short rainy season that is drier and less humid. In contrast, both the short and long seasons are used for production of sesame in Siaya. The long season is wetter and there is prolonged humidity. Such conditions favour *Alternaria* leaf spot (Kolte 1985). These conditions maintain higher leaf surface humidity throughout the growing season favouring *A. sesami* infection. This leads to high infection of seed by the fungus (Gray and Guthrie 1977). Similar effects of environmental conditions on infection of onion by *Alternaria porri* have been reported by Everts and Lacy (1996).

Although there was previous evidence that *A. sesami* was seedborne (Yu *et al.* 1987) and the fungus reported to occur in sesame seed, information on the effect of transmission of the fungus by seed on disease development was lacking. This study has shown that *Alternaria* leaf spot severity is directly related to use of infected seed. Similar results have been reported by Maude and Presly (1977) in onions infected by *Botrytis allii*. In the present study, a high degree of relationship between seed infection and disease severity was established. Apart from the percentage of infection in sesame seed, the environmental conditions during the growth of the crop may have affected transmission and disease development. Although the same infection levels were studied in both seasons, the progress of the disease was more rapid during the first season. The long periods of high humidity and spore dispersal in frequent rain showers during this season, were more suitable for infection by *A. sesami* (Table 4).

The Gompertz model was superior to the logistic model in linearizing the six levels of seedborne *A. sesami* disease progress curves, though both models did not fit the disease data equally well. Although the Gompertz model provided statistically significant fits to disease progress data for all the seedborne inocula tested, coefficients of determination of Gompertz transformed disease data varied across the inocula levels. This problem could have been avoided by using more mathematically explicit models such as the Weibull model (Pennypecker *et al.* 1980), but such models are often too computationally complex to use especially when evaluating more than a few inocula levels. Apart from making it possible for avoidance of problems associated with imperfect fits of disease data and being relatively easy to calculate, AUDPCs appeared also to be better descriptors of Alternaria leaf spot severity due to seed transmission than estimates of infection or defoliation rates. Rates of disease increase were calculated from averages of disease over 10 to 14 days intervals. AUDPCs may thus reflect timing of disease increase more accurately than rates of disease increase. Johnson *et al.* (1986) also made similar observation while studying early leaf spot of peanuts caused by *Cercospora arachidicola*. However, as defoliation due to greater severity can be confounded with effects of plant senescence and environmental stress, AUDPC - DF is a less reliable tool than AUDPC - DL. The accurate leaf area calculations were valuable for the determination of disease severity progress. The leaf areas would understandably depend upon variety, crop nutrition and weather. Such measurements provided a better understanding of leaf spot progress than would have been possible otherwise.

CONCLUSION

Use of sesame seeds infected by *Alternaria sesami* for planting greatly affect the level of severity of Alternaria leaf spot disease. The control of the disease is therefore highly dependent on the use of disease free seed. The availability of certified sesame seeds to farmers is an integral part in the management of the disease, particulary in the major sesame growing areas.

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