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Infection of sesame seed by Alternaria sesami (Kawamura) Mohanty and Behera and severity of Alternaria leaf spot in Kenya

(*Keywords:* Alternaria leaf spot, infection rate, Kenya, seed infection, sesame)

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Abstract. Infection levels of *Alternaria sesami* in sesame (*Sesamum indicum* L.) seeds collected from farmers in western districts of Kenya were detected using the oatmeal agar plate method. Infection levels varied from 9% in Kakamega to 24% in Siaya. Samples from Busia had a mean infection level of 11.69%. Alternaria leaf spot was monitored in plots planted with *Alternaria sesami* infected seed at six different infection levels at Kibwezi to determine the effect of transmission of the fungus by seed on disease severity. Increase in per cent leaf area blighted and per cent defoliation fit more closely the Gompertz model than the logistic model. Rates of disease increase in blighted leaf areas and defoliation, and areas under disease progress curves (AUDPC), varied among the six seed infection levels. Infection levels with larger AUDPC generally had faster rates of disease progress. Disease was most severe on plants established from seeds with 8% infection and least on plants established from seeds with 0% infection. Disease severity increased with increased seed infection level.

1. Introduction

Among the oil crops currently grown in Kenya, sesame is best adapted to marginal agroecological zones. Crop production is restricted to the lower midlands in western Kenya (Western and Nyanza provinces) and coastal lowlands in Coast province. Due to 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 rain season. Sesame is considered drought-resistant (Weiss, 1971) and can produce 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 crop production (Anon., 1995). Yields of up to 2230 kg/ha have been reported from experimental fields (W'Opindi, 1980), but average yield on farmers' fields is only 80–400 kg/ha. In Kenya, there are no commercial certified varieties available for planting. Farmers, therefore, use a genetic mixture, referred to as 'landraces', for sowing (Gichuki and Gethi, 1988). The major constraints to sesame production in Kenya are diseases and insect pests (Ayiecho and Nyabundi, 1995). Based on observations at Siaya Farmers Training Centre (FTC) in 1996, Alternaria leaf spot caused by *Alternaria sesami* was the most severe disease on sesame. Initial effects of infection by the fungus include a decline in photosynthetic area due to leaf damage; premature defoliation soon follows which adversely affects growth and yield. The fungus also causes considerable damage to sesame capsules. Yield losses ranging from 18 to 55% were attributed to the fungus (Barboza *et al.*, 1966). Occasionally, seedlings and young plants exhibiting pre- and post-emergence damping-off are killed with losses of 55–59% attributed to the fungus (Yu *et al.*, 1987).

The Alternaria leaf spot pathogen, *A. sesami*, is seed transmissible (Yu *et al.*, 1981). The pathogen can survive between cropping seasons or unfavourable conditions as seed infectant. Seedlings raised from infected seeds become primary inocula sources for infection of other plants in the field (Neergaard, 1979). Due to a lack of commercial certified seeds, farmers plant their own seed from previous harvests. In some cases, they purchase seed from other farmers (W'Opindi, 1980). No studies have been conducted to determine infection levels of the causal fungus in Kenyan seeds and the effect of such infection levels on disease development. This study was conducted 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 seed transmission of the fungus on Alternaria leaf spot development under field conditions.

2. Materials and methods

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

A. sesami was enumerated in a seed sample using the oatmeal agar plate method. All seeds were surface sterilized in a solution of 1% NaOCl for 5 min, followed by draining of the surplus liquid. Oatmeal agar (OMA) was prepared by steam

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sterilization of the ingredients (oat 20 g, agar 20 g, water 1 litre) for 15 min at 121°C and 1.2 kg/cm^2. Bacterial growth was controlled by the addition of 200 ppm of streptomycin sulphate to molten OMA cooled to 45°C just 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 from each sample were aseptically plated onto 20 plates 1 cm apart. Identification of *A. sesami* on seeds was based on characteristic colony growth on OMA by using a lower power microscope as described by Lee (1978) and Mehta and Prasada (1976).

Sesame seeds with infection levels of 2, 4, 5, 6, 7 and 8%, determined by the oatmeal agar test, were used in the field experiments. 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. Seeds were planted in a three replicate randomized complete block design at the Kibwezi Institute for Dryland Research and Development (IDRDU) on 21 March, and 4 October, 1996. Plots, planted with seed of each infection level consisted of five 4 m rows spaced 0.5 m apart with plants 0.20 m within rows. Plots were arranged perpendicular to the direction of the prevailing wind to reduce inter-plot interference and were separated from each other at the ends and sides by 2 m strips of susceptible sesame accession SPS SIK 013.

Severity of Alternaria leaf spot was assessed as per cent leaf area blighted and per cent defoliation from April to June 1996 for the first season and from November to February 1997 for the second season. Average blighted leaf areas in the plots were estimated using 10 plants selected at random on each sampling date. Leaf areas (one surface only) were calculated by multiplying average width times length measurements for various linear proportions of the leaves with the triangular leaf apices calculated separately. Lesion counts and lesion areas were recorded every 10–14 days for the 10 plants that were tagged throughout the study for disease progression. Mean per cent disease was calculated by lesion numbers times average lesion area divided by total leaf area. To determine per cent defoliation, each row within the plot was divided into 0.5 m segments per row prior to each assessment date. One segment per row was randomly selected every 10–14 days in each plot, and the number of nodes and missing leaves were counted on each main stem. Per cent leaf area blighted and per cent defoliation were used in determination of the area under disease progress curve (AUDPC-DL and AUDPC-DF, respectively) using the formula of Shaner and Finney (1977).

Gompertz and logistic models were fitted to per cent leaf area blighted and per cent defoliation data. The most suitable model for assessing infection and defoliation rates was identified by comparing the coefficients of determination obtained using each model as applied by Campbell and Madden (1990). Apparent rates of disease increase were obtained by regressing Gompertz transformed data against time (expressed as days after planting). Student's *t*-test was carried out separately for AUDPC-DL, AUDPC-DF, and apparent infection and defoliation rates in the first and second season to determine season effect on these parameters. Within each season, all the parameters for each of the treatments were compared using ANOVA. The Least Significant Difference (LSD) test (Steel and Torrie, 1980) was used to separate group means where ANOVA indicated significant difference ($P = 0.05$).

3. Results

Alternaria sesami was detected in all seed sampled with the levels being dependent on the district. The highest seed infection level detected was 24% in samples from Rarieda in the Siaya district and the lowest infection level was 8% in seed samples from Mumias and Navakholo in Kakamega district. The highest infection levels were detected in Siaya district. The lowest level was detected in Kakamega and Busia district had an intermediate infection level. Seed infection levels in Siaya ranged from 18% in Usingu and Boro to 24% in Rarieda. Busia district had infection levels ranging from 10% in Amagoro and Mayenje to 14% in Bugengi. Infection levels in Kakamega district ranged from 8% in Mumias and Navakholo to 11% in Majengo (table 1).

Areas under disease progress curves for per cent leaf area blighted (AUDPC) due to Alternaria leaf spot were significantly larger during the first season than in the second season at Kibwezi IDRDU ($t = 3.08$, $P = 0.01$), although there was no significant season by treatment interaction. In both seasons, highly significant differences in disease severity were observed among the six seed infection levels. Plants established from seeds with 8% infection level had the highest AUDPC-DL in both seasons (table 2). Area under disease progress curves for per

Table 1. Seed infection levels of A. sesami in sample from three districts in Kenya as determined by oatmeal agar plate method

Sampling Area	Infection (%)
Sampling Area	
Alego	20[a]
Bondo	19
Boro	18
Hawinga	19
Rarieda	24
Ugunja	21
Ukwala	20
Usingu	18
Mean ($LSD_{0.05}$)	19.88 (1.08)
Busia District	
Alupe	11
Amagoro	10
Amukura	13
Angoromo	13
Bugengi	14
Butula	11
Mayenje	10
Roadblock	11
Mean ($LSD_{0.05}$)	11.69 (1.13)
Kakamega District	
Butere	10
Khwisero	8
Lurambi	10
Majengo	11
Mumias	8
Municipality	9
Navakholo	8
Shinyalu	8
Mean ($LSD_{0.05}$)	8.96 (1.01)

[a]Each value is an average of three replications.

cent defoliation (AUDPC-DF) due to seed infection by *A. sesami* were also significantly larger in the first season than in the second season ($t = 2.30$, $P = 0.05$). There were also highly significant differences in AUDPC-DF among the six seed infection levels in both seasons (table 2). The 8% seed infection level had significantly larger AUDPC-DF than did other infection levels tested except 5% and 7% in both seasons. The smallest AUDPC-DF was observed in plots established from seed with 0% infection.

Rates of increase in Alternaria leaf spot were estimated and compared using the Gompertz model since it generally produced slightly higher coefficients of determination ($R^2 = 0.95$) and smaller error mean squares (EMS = 0.14) than did the logistic model ($R^2 = 0.89$, EMS = 0.21). Goodness of fit to disease progress data, however, varied among seedborne inocula levels. Rates of increase in per cent leaf area diseased (infection rates) due to Alternaria leaf spot were statistically similar in both seasons ($t = 0.59$, $P = 0.05$). There were, however, highly significant differences in infection rates among the six treatments in both seasons (table 3). Maximum infection rates were observed on plants established from seeds with 8 and 7% seed infection in the first and second season, respectively. The rate of increase in per cent leaf area blighted was significantly slower for other infection levels studied except 5 and 7% in the first season, and 5 and 8% in the second season. The slowest rate of disease increase was observed on plants established from seed with 0% infection in both seasons. Rates of increase in per cent defoliation due to Alternaria leaf spot as a result of seed infection were significantly faster in the first than in the second season ($t = 2.23$, $P = 0.05$). Differences in defoliation rates among the six levels of infection in both seasons were highly significant (table 3). In both seasons, the treatment with seed infected at 8% had a significantly faster rate of defoliation than did other levels except 5 and 7% in both seasons. The slowest rate of increase in per cent defoliation was observed on plants established from uninfected seed.

Table 2. Effect of A. sesami seed infection on mean area under disease progress curves for per cent leaf area blighted (AUDPC-DL) and per cent defoliation (AUDPC-DF) due to Alternaria leaf spot

Infection level (%)	AUDPC-DL	AUDPC-DF
0	0.14[a]	0.28
2	0.34	0.46
4	0.60	0.50
5	1.40	0.64
7	1.77	0.72
8	2.28	0.83
Mean	1.09	0.57
CV (%)	15.93	32.54
$LSD_{(0.05)}$	0.95	0.20

[a]Average of three replications. Ranking of treatments was the same in both seasons and data presented for AUDPC-DL and AUDPC-DF are combined for the two seasons.

4. Discussion

Based on the seed assay, all 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 lowest infections, respectively. Infection of seeds by this fungus is 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, and this results in increased seed infection by *Alternaria* spp. Sesame is produced in Kakamega only during the short rainy season which is drier and less humid. This short rainy season is also the main season for sesame production in Busia. Both the short and long seasons are used for production of sesame in Siaya. The long season is wetter and there is prolonged humidity. Alternaria leaf spot is favoured by conditions which maintain higher leaf surface humidity throughout the growing season and promote *A. sesami* infection. This leads to high infection levels of seed by the fungus. Similar effects of environmental conditions on infection of onions by *Alternaria porri* have been reported by Everts and Lacy (1996).

A. sesami is seed transmissible (Yu *et al.*, 1987), but information on the effect of seed transmission on disease development is lacking. Based on results of this study, Alternaria leaf spot severity is directly related to use of infected seed. Similar results have been reported by Maude and Presly (1977) on onions infected by *Botrytis allii*. In this study disease severity

Table 3. Effect of A. sesami seed infection on rate of increase in per cent leaf area diseased and per cent defoliation due to Alternaria leaf spot

	Season I		Season II	
Infection level (%)	Infection rate[a]	Defoliation rate	Infection rate	Defoliation rate
0	0.032[b]	0.027	0.032	0.020
2	0.042	0.027	0.040	0.019
4	0.078	0.028	0.056	0.021
5	0.086	0.032	0.061	0.022
7	0.088	0.042	0.108	0.030
8	0.092	0.043	0.069	0.034
Mean	0.070	0.033	0.061	0.024
CV (%)	34.280	42.560	35.670	45.340
$LSD_{(0.05)}$	0.013	0.015	0.046	0.012

[a]Rates of increase were obtained by regressing Gompertz-transformed disease/defoliation data against time (days after planting) using the equation $K = [\text{gompit}\ (Y_{max}) - \text{gompit}\ Y_{(min)}]/(t_2 - t_1)$ in which $\text{gompit} = -\ln[-\ln(Y)]$, (Y_{min}) and (Y_{max}) being proportion of disease/defoliation observed at the beginning (t_1) and the end (t_2) (Luke and Berger, 1982). [b]Values shown represent average of three replications.

increased with increased seed infection which could be the result of increased transmission of the fungus by infected seed. Environmental conditions during the growth of the crop may also have affected transmission and thereby influenced disease development. Although similar infection levels were studied in the both seasons, disease progress was more rapid during the first season than the second season. The long periods of high humidity characteristic of the first season, and spore dispersal during frequent rains, were more suitable for *A. sesami*. These conditions were also more favourable for infection and subsequent disease development.

The Gompertz model was superior to the logistic model in linearizing disease progress curves, though neither model fits the disease data very well. The Gompertz model provided statistically significant fits to disease progress data for all seed infection levels tested, although the coefficients of determination of Gompertz transformed disease data varied with infection level. This problem could have been avoided by using more mathematically explicit models such as the Weibull model (Pennypecker *et al.*, 1980). However, such models are often computationally complex and difficult to use, especially when evaluating more than a few seed infection levels. AUDPCs were better descriptors of Alternaria leaf spot severity due to seed transmission than estimates of infection or defoliation rates, were relatively easy to calculate and avoided the problems associated with imperfect fits of disease data. Rates of disease increase were calculated from averages of disease over 10–14 day intervals. AUDPCs may, thus, reflect timing of disease increase more accurately than rates of disease increase. Johnson *et al.* (1986) also made similar observations while studying early leaf spot of peanuts caused by *Cercospora arachidicola* However, because 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. 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 accurate measurements provided a better understanding of Alternaria leaf spot progress on sesame.

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