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Full Length Research Paper

Pestalotia spp. causes leaf spot of *Vitellaria paradoxa* in Ghana

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Attempts were made at Bole, Ghana to determine the incidence and severity of leaf spot of *Vitellaria paradoxa* (shea), identify the causal pathogen and alternative hosts, and assess the impact of disease incidence on shea nut yield. Each of 4960 shea trees and surrounding herbs, shrubs or trees were inspected for symptoms of leaf spots. Disease severity was scored on a scale of 0 to 4 on each of 30 leaves randomly sampled from the lower, middle and upper canopy of each of 64 selected trees categorised into eight different classes on the basis of age and leaf characteristics. Out of the 4960 *V. paradoxa* plants inspected, 2976 (60%) was infected with the disease. A range of fungi was isolated from the infected leaves but only *Pestalotia* spp. was found to be the causal pathogen of leaf spot of *V. paradoxa*. *Nauclea latifolia* Smith, a Rubiaceae and *Daniellia oliveri* Hutch and Dalz, a Caesalpiniaceae were found to be alternative hosts. The disease was most severe in the lower portion of the canopy and it adversely affected yield. This is the first report of *Pestalotia* leaf spot disease on *N. latifolia* and *D. oliveri*.

Key words: *Vitellaria paradoxa*, alternative hosts, leaf spot, *Nauclea latifolia*, *Daniellia oliveri.*

INTRODUCTION

The shea nut tree, *Vitellaria paradoxa*, C.F. Gaertn., a Sapotaceae, is indigenous to the Guinea savannah belt of West Africa. It occurs on an estimated 1 million km^2 between Senegal and north-western Uganda (Salle et al., 1991). Shea tree populations are wild and diverse in phenotype and genotype (Fontaine et al., 2004; Lovett and Haq, 2000), and hence, nut production fluctuates from year to year. In Ghana, shea occurs in the northern part of the country and it is estimated that the tree population is about 9.5 million (Abbiw, 1990). However, annual collection of the nuts ranges from 4,000 to 8,000 tons (Hall et al., 1996). The fresh pulp of its fruits and the butter extracted from the kernels play an important social and economic role in rural areas in Northern Ghana. The fat is used locally as oil, for soap making, as an ointment; cosmetic and for waterproofing house walls. The sweet pulp is a source of energy; the wood is used for making tools while the roots and bark have medicinal applications. Africa earned 10.5 million dollars from 56,000 tonnes of shea nuts exported in 1998, of which 60% came from Ghana (Anon, 2008). *V. paradoxa* is affected by many pests (Dwomoh, 2003; Dwomoh et al., 2004) and diseases. Dakwa (1986) reported of the incidence of leaf spot of shea nut trees in Northern Ghana and consistently isolated *Pestalotia* and *Botryodiplodia (Lasiodiplodia)* spp. from the infected materials. In spite of the local and national importance of shea, there is little information on diseases of the plant. The objective of this study was to assess the incidence and severity of leaf spot disease of *V. paradoxa*, identify the causal organism, alternative host(s) and the effect of the disease on nut yield in Ghana.

MATERIALS AND METHODS

The study was conducted on a 24.3 ha plot containing wild growing *V. paradoxa* trees of varying ages and spacing at the Cocoa Research Institute of Ghana's (CRIG) substation at Bole (9°01' N, 2°29' W, 309 m above mean sea level from 2004 to 2007. Each of the 4960 *V. paradoxa* trees and the surrounding herbs, shrubs and other trees were inspected for symptoms of leaf spot (Figure 1). Tissue segments (c. 2 mm^2) of the advancing margin of lesions were excised, surface sterilized by a 3 min. immersion in 10% $NaClO_3$, rinsed twice in sterile distilled water, blotted dry with sterile tissue paper, and plated on potato dextrose agar. The cultures were incubated for 6 d at 26 ± 2°C under 12 h day/night regime. Pure cultures of the isolates and the infected shrubs were identified

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Figure 1. Typical *Pestalotia* leaf spots symptom on *V. paradoxa* at Bole, Ghana.

Table 1. Mean severity of leaf spot (lesions/leaf) in different canopy regions and cumulative yield (kg) of *Vitellaria paradoxa* at Bole, Ghana.

Tree age class and leaf characteristics*	Mean disease severity (lesions/leaf) N = 8			Yield Kernel Weight (kg) N = 4
	Lower canopy	Middle canopy	Top canopy	
YpLGSL	1.38±0.16	1.20±0.13	1.52±0.51	6.87± 5.93
MpLGSL	1.36±0.18	1.33±0.43	1.34±0.41	7.18± 3.92
YpLGnSL	1.80±0.38	1.69±0.66	1.52±0.11	2.10± 1.40
MpLGnSL	1.54±0.43	1.32±0.47	1.46±0.68	16.18±14.29
YpDGSL	1.57±0.06	1.43±0.43	1.59±0.53	4.56± 3.48
MpDGSL	1.75±0.34	1.33±0.25	1.35±0.59	8.37± 3.44
YpDGnSL	1.58±0.32	1.41±0.30	1.55±0.56	3.28± 1.57
MpDGnSL	1.76±0.44	1.39±0.33	1.52±0.66	7.43± 4.59
$LSD_{(0.05)}$ 21df	0.25	1.00	0.18	7.41

*YpLGSL ,young plants with light serrated leaves; MpLGSL, mature plants with light green serrated leaves; YpLGnSL, young plants with light green non-serrated leaves; MpLGnSL , mature plants with light green non-serrated leaves; YpDGSL, young plants with deep-green serrated leaves; MpDGSL, mature plants with deep green serrated leaves; YpDGnSL, young plants with deep-green non-serrated leaves and MpDGnSL, mature plants with deep-green non-serrated leaves.

at the Botany Department, University of Ghana, Legon. Pathogenicity of isolates was tested by spraying 5 ml conidia suspension ($2x10^5$ conidia/ml) of the isolated fungi onto the adaxial surface of leaves of 12-month old *V. paradoxa, Nauclea latifolia* and *Daniellia oliveri* seedlings. The seedlings were placed in a shed at $28±2^0C$ and 80% RH. There were 10 seedlings per isolate. Control plants were sprayed with sterile distilled water.

To determine the within-tree distribution of disease severity in both young plants (Yp), plants with diameter at breast height (dbh) < 15 cm and mature plants (Mp), plants with dbh>15 cm with different leaf morphologies, 64 *V. paradoxa* trees were randomly selected and categorised into eight groups (Table 1). The canopy of each tree was further subdivided into three levels: lower (below 2.5 m), middle (2.5 to 5.0 m) and upper regions (above 5 m). Bimonthly assessment of disease severity in September and November during the infection season (July to December) was done on individual trees for three consecutive years. The mean number of lesions per leaf for 30 leaves randomly sampled from the three levels of the canopy of each selected tree was scored on a scale of 0 to 4 (0=no lesion; 1=1 to 25%; 2=26 to 50%; 3= 51 to 75% and 4= 76 to 100% infected leaf surface).

Four trees were randomly sampled from each group and all nuts produced by the sampled trees were harvested and air-dried. Yield (dry weight of nuts) from the sampled trees in each group was recorded. The yield data for the three years were pooled together because no difference was observed between years.

RESULTS AND DISCUSSION

The study found all stages of infection on infected plants, from pinhead points to severely blighted leaves. The sequence of infection and lesion development were similar in all categories. Infections started as small pinhead points from which the lesions enlarged and coalesced. Lesion first appeared as reddish-wine spot, which was later surrounded by hallow bands of concentric rings and followed by a wide water-soaked pale band, representing the active growing front of the lesion. The distribution of the lesions on the leaves did not follow any regular pattern. However, lesions traversed all ribs on the lamina, except the midrib. Lesions appeared on abaxial leaf surfaces of the *V. paradoxa* too*.* Similar symptoms were reported earlier by Dakwa, (1986).

The disease occurred on young and matured plants as well as on some shrubs. Out of the 4960 *V. paradoxa* plants inspected, 2976 (60%) were infected. Seventy six of the infected trees were totally defoliated. The uninfected trees of different ages were intermixed with the infected ones. The apparent lack of spread of the pathogen to the uninfected trees suggests that the spread of the fungus can be slow or the healthy trees are genetically different. Observations from other farms also corroborate this (Akrofi, personal observation). Genotypic and phenotypic variations have been reported in shea trees in Africa (Fontaine et al., 2004; Lovett and Haq 2000). This variation may partially account for the absence of the disease on some trees within the same location.

In general, infection on younger plants was more severe on lower leaves closer to the soil than on those further away in the canopy (1.49 ± 0.18 lesions/leaf) . In the mature trees also, the disease intensity was most severe in the lower canopy (1.59 ± 0.16 lesions/leaf) followed by the top canopy (1.49 ± 0.09 lesions/leaf) and middle canopy (1.34 ± 0.14 lesions/leaf) in that order (Table 1). The disease was also most severe (1.67 ± 0.12 lesions / leaf) in young trees with light green non-serrated leaves and least (1.34 ± 0.01 lesions/leaf) on mature plants with light green serrated leaves. (Sanou et al. 2006) reported of a very low genetic relation between shea trees, leaves and fruits but found a significantly positive correlation between leaf and fruit size traits.

Data from the present study suggest that tree age partially determines yield. The mature plants with dbh>15 cm had a relatively higher kernel weight irrespective of leaf characteristic. The consistent yield pattern observed in this study is in contrast to the phenomenon of variable nut production reported in shea (Lovett and Haq 2000). This may be due to the location of the experimental trees, which were not exposed to the annual bush fires characteristic of shea in the wild. The plot was also grazed by cattle whose droppings served as manure for the shea trees.

The lowest yield was recorded on young plants with light green, non-serrated leaves which also had the greatest disease severity (Table 1). The adverse effect of the disease on yield may be due to the reduction in photosynthetic capacity and efficiency of infected leaves. As leaf spot increases in plants, effective leaf surface area decline. This implies that heavily infected leaves contribute very little to the development of the plant and hence the negative impact on nut yield observed in *V. paradoxa* observed in this study.

The infected *V. paradoxa* trees were not killed, but appeared debilitated. The number of spots resulting from infection is an important measure of the economic importance of a disease. It is reported that during kernel development in trees, nuts serve as high- energy sinks, drawing some of the photosynthates from leaves on nut-bearing terminals (Davis and Sparks, 1974). A similar response may be occurring in *V. paradoxa* and this could account for the reduction in yield of the infected trees.

The infected shrubs in the vicinity were identified as *Nauclea latifolia* Smith, a Rubiaceae and *D. oliveri* Hutch and Dalz, a Caesalpiniaceae. Five *N. latifolia* and seven *D. oliveri* were infected. The lesions on *V. paradoxa* were slightly different from those on these shrubs. On *V. Paradoxa*, the lesions were relatively smaller and the outer ring of concentric lesions darker than the inner ones, whereas, on *N. latifolia*, the concentric rings were few, large and conspicuous. On *D. oliveri,* the lesions were joined by light brown tissues originating from the midrib. *N. latifolia* and *D. Oliveri* are native to tropical Africa and Asia and in Northern Ghana, these plants form part of the natural flora. Various parts of these plants are traditionally used to treat ailments such as diabetes mellitus, malaria, gastrointestinal tract disorders and sleeping sickness and hypertension (Boye, 1990; Maduabunyi, 1995),

From the 90 samples collected, 35 isolations of *Pestalotia* spp. was made. A range of other saprophytic and parasitic fungi was also consistently isolated. These included Mucorales (*Absidia, Syncephalastrum*) (6), *Paecilomyces puntonii* (Vuil.). Nannizzi (5), *Acrospeira laevis*, Wiltshire (7), *Pleospora herbarum* Pers ex Fr. Rabenh (=*Stemphylium botryosum* Wallr.) (8), *Trichocladium asperum*, Harz (3), Mycelia sterilia (4), *Alternaria* (3), *Lasiodiplodia* spp (7) and other unidentified species (12). This suggests that the leaves support a variety of microorganisms.

In the pathogenicity tests, leaf spot symptoms similar to that found in the field was observed in *V. paradoxa, N. latifolia* and *D. oliveri* inoculated with *Pestalotia* spp., and the fungus could easily be re-isolated from the diseased seedlings. Morphological characteristics of re-isolated microorganism as seen under the microscope were similar to those used in the inoculation of the seedlings. This confirms that the causal pathogen of the leaf spot was *Pestalotia* spp. Earlier reports had associated leaf spot of shea in Ghana with *Pestalotia* and *Botryodiplodia* (*Lasiodiplodia* spp) (Dakwa, 1986).

Conclusion

Pestalotia spp. has been identified as the causal pathogen of leaf spot of *V. paradoxa* at Bole in Ghana. *N. latifolia* and *D. oliveri* were found to be alternative hosts of the pathogen. This is the first report of *Pestalotia* leaf spot associated with *N. latifolia* and *D. oliveri* and this has implications for the management of the disease, because of the diverse uses of these plants in the study area. Further studies will assess the soil as a possible source of primary inoculum, how the pathogen is spread in the field and the role of the other fungi and alternative hosts in the epidemiology of the disease.

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