

# Didymosphaeria Leaf Spot of Cluster Yam (*Dioscorea dumetorum*)

S. A. EMUA and A. O. FAJOLA, Department of Botany, University of Ibadan, Ibadan, Nigeria

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## ABSTRACT

Emua, S. A., and Fajola, A. O. 1981. *Didymosphaeria* leaf spot of cluster yam (*Dioscorea dumetorum*). Plant Disease 65:443-444.

A new leaf spot disease of cluster yam (*Dioscorea dumetorum* Pax) caused by *Didymosphaeria donacina* is described. The pathogenicity of both the perfect and imperfect stages of the causal organism was established. Symptoms developed 4 days after inoculation, and sporulation occurred 2 days later. Although the perfect stage of the fungus was found commonly on infected leaves, only sterile perithecia were observed in culture.

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*Didymosphaeria* species cause diseases of many plants. Wolf (8) reported a severe blight of asparagus fern (*Asparagus plumosus* Baker) caused by *D. brunneola* Niessl in Florida. In France, Goidanich and Vivani (2) reported *D. populina* Vuill. as a parasite causing lesions on the leaves of vigorously growing poplar plants (*Populus* sp.). Yen and Chi (9) reported a leaf blast disease of sugarcane (*Saccharum officinarum* L.) caused by *D. taiwanensis* Yen & Chi in Formosa. A severe blotch of peanut (*Arachis hypogaea* L.) caused by *D. arachidicola* (Chochrjakov) Alcorn, Punithalingam &

McCarthy was reported by Alcorn et al in Queensland (1). However, the fungus has not been previously recorded on *Dioscorea*. We describe a leaf spot disease of *Dioscorea dumetorum* Pax caused by *D. donacina* (Niessl) Sacc. in Nigeria.

## MATERIALS AND METHODS

Yam farms were surveyed for disease in 12 locations in Nigeria: Agbor, Asaba, Auch, Benin, Ekpoma, and Issele-Uku in Bendel State; Ibadan, Iseyin, and Oshogbo in Oyo State; and Ifon, Ado-Ekiti, and Ondo in Ondo State. Disease incidence was assessed as the percentage of stands (staked yam plants) affected in randomly selected rows on each farm.

Severity was expressed in terms of infection indexes similar to those of McCarter and Littrell (3), where 0 = no infection and 5 = very severe infection. Only the 10 lowermost leaves of each stand were evaluated.

Diseased leaves were collected in polyethylene bags for microscopic examination in the laboratory. Isolations were made by a method similar to that employed by Waddel and Weber (7). Diseased portions were washed, cut into small pieces, surface-sterilized in 0.1% mercuric chloride for 1 min, and rinsed in several changes of sterile, distilled water. The pieces were plated vertically instead of horizontally (7), thereby exposing both leaf surfaces to the medium. Our initial identification of the isolated organisms was confirmed by the Commonwealth Mycological Institute.

Four solid media were used for the growth and sporulation of the causal organism: potato-dextrose agar, potato-nutrient agar, vegetable juice agar, and yam leaf nutrient agar. To prepare the yam leaf nutrient agar, mature yam leaves (250 g) were chopped in a Waring Blender, boiled for 1 hr in 500 ml of



Fig. 1. *Didymosphaeria donacina* leaf spot disease on *Dioscorea dumetorum* leaflet.

distilled water in an autoclave without pressure, cooled, and filtered. Oxoid agar (20 g) and nutrient broth (10 g) (Oxoid Limited, London) were added to the filtrate, and the mixture was made up to 1,000 ml and sterilized at 1 kg/cm<sup>2</sup> for 15 min.

Pathogenicity tests were carried out following the methods of Ogundana (4). For each test, 10 carefully labeled healthy leaves on each plant (6 mo old) in the greenhouse were washed with sterile, distilled water to remove soil particles and sprayed with 10 ml of a conidial or ascospore suspension ( $5 \times 10^4$  spores per milliliter) or with sterile, distilled water (control). Conidia were obtained from 7-day-old cultures on yam leaf nutrient agar; ascospores were obtained from perithecia harvested from naturally infected leaves.

The sprayed leaves were incubated at 95–98% relative humidity and 28 C for 48 hr before being exposed to the normal variations of relative humidity (70–85%) and temperature (25–31 C) in the greenhouse. Disease was assessed at regular intervals for 15 days and expressed as percentage infection (ie, number of infected leaves divided by number of inoculated leaves times 100).

## RESULTS

The disease was found at all 12 survey locations. Disease incidence was high in all locations (54–94%; mean 81%). Severity ranged from very light to very severe. Severe infection resulted in loss of about 25% of the total leaf area.

Disease symptoms were observed on both young and mature leaves but were more common on mature leaves. Lesions started as dark brown spots less than 1 mm in diameter. As lesions expanded,

Table 1. Percentage of *Dioscorea dumetorum* plants infected after inoculation with conidia or ascospores of *Didymosphaeria donacina*<sup>a</sup>

Days after inoculation	Type of inoculation		
	Ascospores from diseased leaves	Conidia from culture	Sterile, distilled water (control)
2	0	0	0
5	30	61	0
7	49	79	0
9	67	83	0
11	75	93	0
13	75	93	0
15	75	93	0

<sup>a</sup>Data are means of three determinations.

they became yellowish green at the margin but remained brown at the center. At maturity, spots measured 10–20 mm in diameter and had three distinct zones: a dirty white or pale brown center with minute black specks surrounded by a dark brown region, with a yellowish green transition zone (Fig. 1).

Microscopic examination of the scrapings and isolations from diseased material consistently yielded a fungus identified as *Didymosphaeria donacina* (Niessl) Sacc. (IMI 231943). However, a *Fusarium* species was found occasionally.

Pathogenicity tests (Table 1) established *D. donacina* as the causal organism. The incubation period was 4 days, and sporulation occurred about 2 days later. Inoculation of leaves with a conidial suspension of *Fusarium* sp. or sterile, distilled water did not result in disease. Ascospores from naturally infected leaves and conidia from culture were pathogenic. Although the same inoculum concentration was used of both spore types, conidia induced a higher infection percentage.

The black specks in the center of mature leaf spots were found to be aggregates of smooth-walled perithecia. Each perithecium ( $16.8\text{--}84.2 \times 53.0\text{--}67.7 \mu\text{m}$ ; mean  $75.5 \pm 7.1 \times 60.6 \pm 5.3 \mu\text{m}$ ) contained 8–15 hyaline asci. Each ascus ( $27.1\text{--}61.2 \times 14.1\text{--}21.6 \mu\text{m}$ ; mean  $41.0 \pm 10.9 \times 17.0 \pm 2.4 \mu\text{m}$ ) contained eight irregularly arranged ascospores. Ascospores ( $14.1\text{--}21.6 \times 7.0\text{--}10.8 \mu\text{m}$ ; mean  $16.6 \pm 1.9 \times 8.7 \pm 1.2 \mu\text{m}$ ) were one-celled and hyaline, with three or four conspicuous oil droplets.

Conidia were rarely found on naturally infected leaves but were produced abundantly in culture. They were hyaline and two-celled, measuring  $14.4\text{--}21.1 \times 3.5\text{--}3.9 \mu\text{m}$  (mean  $17.6 \pm 1.9 \times 3.7 \pm 0.1 \mu\text{m}$ ). Conidiophores were highly branched.

In culture, *D. donacina* grew readily on yam leaf nutrient agar (4 mm/day) and

vegetable juice agar (3.6 mm/day) but slowly on potato-nutrient agar (2.0 mm/day) and potato-dextrose agar (1.3 mm/day). The colony on each culture medium was at first gray and appressed to the medium. Conidia were produced abundantly in young (5-day-old) cultures. Later, the cultures turned a darker color, and conidial production declined. Some eruptive perithecia were produced after 2 wk on each medium, but they were sterile even in very old (4–6 wk) cultures.

## DISCUSSION

Yen and Chi (9) found that both the perfect and imperfect stages of *D. taiwanensis* were pathogenic on sugarcane. We established *D. donacina* as a pathogen of a species of *Dioscorea* for the first time. A higher infection percentage was obtained with conidia, indicating that conidia may have greater viability, germ tube vigor, or virulence than ascospores.

*Dioscorea dumetorum* is an important cultivated yam. In Nigeria, it follows *Dioscorea rotundata*, *Dioscorea cayenensis*, and *Dioscorea alata* in importance (6). Loss in functional leaf area from disease may lead to loss in tuber production. Parham (5) reported that Gloeosporium leaf spot of *Dioscorea* sp. caused the failure of tuber production in Fiji. We observed that severe infection of *Dioscorea dumetorum* with *D. donacina* resulted in the loss of about 25% of total leaf area. This disease, therefore, may be of great economic importance, and attention should be directed toward its control.

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