New Disease (*Balansia cyperi*) of Purple Nutsedge (*Cyperus rotundus*)

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ABSTRACT

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Purple nutsedge (*Cyperus rotundus*) in Louisiana is often infected by the systemic fungus *Balansia cyperi* (Ascomycetes, Clavicipitaceae). Infected plants often produce diseased inflorescences, and the fungus can be transmitted via tubers. It is related to endophytic fungi of grasses that are toxic to herbivores.

Purple nutsedge (PNS) (*Cyperus rotundus* L.) is considered by many to be the world's worst weed (12,13,25). Its perennial habit, rapid growth, and prolific production of underground tubers make it a serious weed pest in warm climates. As a result, much research has been devoted to its growth and reproductive biology as related to weed control (11,14,21,23,28). Recent observations have revealed that plants in many populations of PNS in Louisiana are infected by the fungus *Balansia cyperi* Edg. (Ascomycetes, Clavicipitaceae), previously reported only from two other species of *Cyperus* (5,7,8,24). Closely related fungi infecting grasses are known to have toxic effects on mammalian (1,2,15) and insect herbivores (4,9,16). This paper reports the occurrence and effects of *B. cyperi* on PNS and possible implications for weed control.

MATERIALS AND METHODS

Observations. In spring 1984, a specimen of PNS from Lafourche Parish, LA, was observed with a deformed inflorescence densely covered with white mycelium. Populations of PNS around the Louisiana State University (LSU) campus were subsequently examined for similar diseased inflorescences. Casual observations of PNS were made at more distant locations around Louisiana, although no attempt was made to search systematically for plants with diseased inflorescences. Local populations with infected plants were monitored through two growing seasons for a total of 18 mo.

The aerial shoots of PNS grow from a basal bulb that may be interconnected by rhizomes with other basal bulbs and tubers (following the terminology of Wills and Briscoe [29]). To investigate the association between PNS and the fungus, about 20 shoots with diseased inflorescences and 20 shoots with nondiseased inflorescences were collected in August 1984 from a site on the LSU campus for observations in the greenhouse and laboratory. Each shoot was cut back to its basal bulb, and each basal bulb was planted individually in a 6-cm plastic pot filled with a sterile soil mixture (two parts sand, two parts peat moss, two parts topsoil, and one part vermiculite) in the greenhouse. Inflorescences produced by plants that regrew from the basal bulbs were examined for symptoms of infection. In addition to collecting individual shoots with diseased or nondiseased inflorescences, clusters of shoots were dug up and the soil was carefully removed to see whether shoots bearing diseased inflorescences were connected by rhizomes.

A second collection of PNS was made in September 1985. About 50 shoots with diseased inflorescences were collected from each of two sites on the LSU campus, and in parallel, 50 shoots with nondiseased inflorescences were collected from each of two additional sites where diseased inflorescences had never been observed. The shoots were cut back to their basal bulbs and planted as before. At the same time, tubers connected by rhizomes to the shoots were separated, cleaned of soil, washed in distilled water, and planted in the same manner as the basal bulbs. After new shoots from tubers and basal bulbs had regrown and flowered in the greenhouse, the inflorescences were classified as diseased or nondiseased.

Microscopic examination. To confirm that PNS was systemically infected, a microscopic examination for fungal hyphae was conducted. Hyphae of related fungi infecting grasses have been detected in ovaries, seeds, leaf sheaths, and pith tissue (2,7). These tissues (except seeds) were examined as well as tissue from tubers, basal bulbs, and rhizomes of PNS. Freehand sections were cut under a dissecting scope using a single-edge razor blade and stained with lactophenol cotton blue. The stained sections were examined under a microscope at ×100. Apical meristems were dissected from basal bulbs, fixed in FAA, and dehydrated in a tertiary butyl alcohol series. The tissues were imbedded in Paraplast Plus, and 7-μm longitudinal sections were cut with an American Optical Rotary Microtome. The sections were stained in toluidine blue and examined under a microscope for fungal hyphae. Similar tissues from plants with diseased inflorescences, plants with nondiseased inflorescences, and nonflowering plants were examined.

RESULTS AND DISCUSSION

Mycelium-covered, aborted inflorescences were the only external symptoms of infection, identical to the symptoms of *B. cyperi* infecting *C. virens* (5,7,8). Conidia from infected PNS were similar in size and shape to conidia from *B. cyperi* infecting *C. virens*. Thus, all available evidence suggests that the fungus infecting PNS is *B. cyperi*. Specimens have been deposited in the National Mycological Herbarium in Beltsville, MD.

Diseased and nondiseased inflorescences are readily distinguished. Diseased inflorescences are aborted and covered with mycelium (Fig. 1A), whereas nondiseased inflorescences consist of an expanded panicle of reddish spikelets (Fig. 1B). Occasional intermediate inflorescences are observed that are only partly diseased (Fig. 1C). Ascomata have not been observed on plants in the field or greenhouse. However, I have never observed ascomata on *B. cyperi* infecting *C. virens* either.

At least 20 sites in Louisiana have been found with large numbers of diseased PNS, most in the vicinity of the LSU campus, where more time searching was spent. However, populations of PNS on Avery Island, Vermillion Parish, and in Thibodeaux, Lafourche Parish, containing diseased plants have also been observed. These two locations are more than 100 km from LSU and each other. On the basis of the preliminary surveys, it seems likely the fungus may be widespread on PNS. *C. virens* was the only confirmed host of *B. cyperi* (7,8) until recently, when the sedge *C. pseudovegetus* was found infected by the same fungus in a site where infected *C. virens* was also found. Sites with diseased PNS are all within the geographical range where *B. cyperi* infects *C. virens*.

Sites with diseased plants have been monitored through two growing seasons. Diseased inflorescences were observed most commonly in the fall and in the same locations as in the previous year. At

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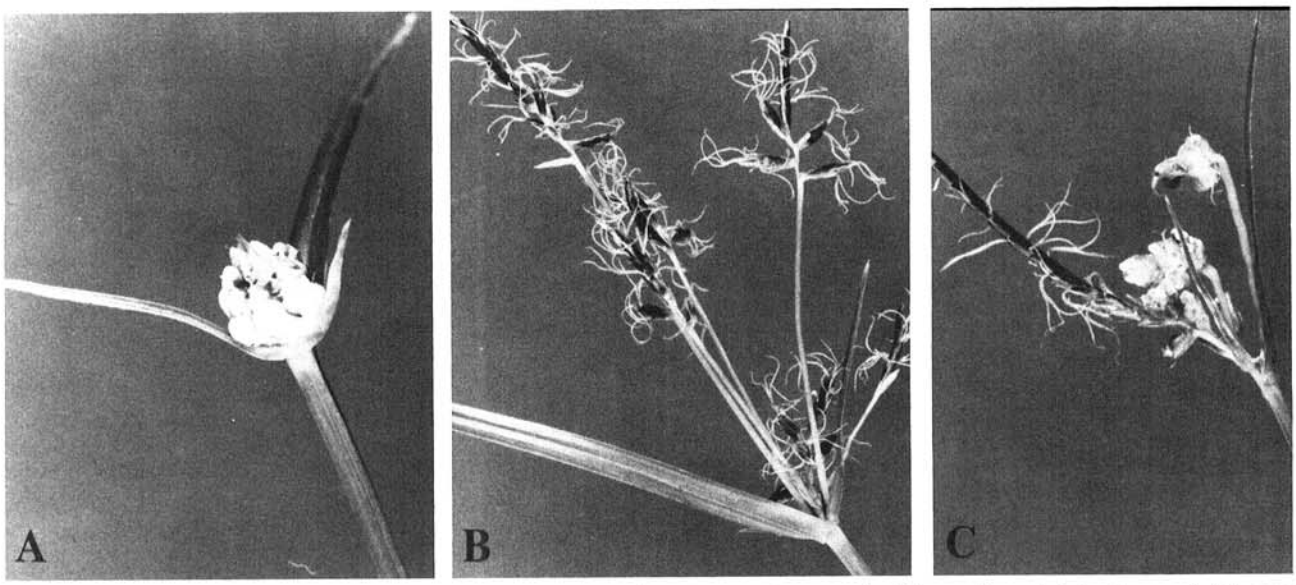

Fig. 1. Inflorescences of purple nutsedge: **(A)** Aborted inflorescence of purple nutsedge covered with mycelium of *Balansia cyperi*; **(B)** normal, nondiseased inflorescence; and **(C)** partly diseased inflorescence.

other times of the growing season, most inflorescences were nondiseased. Excavations demonstrated that neighboring shoots with diseased inflorescences are often interconnected by rhizomes and tubers. However, shoots with diseased inflorescences were also connected by rhizomes to shoots with nondiseased inflorescences. When the initial collection of shoots with diseased and nondiseased inflorescences was observed in the greenhouse, both diseased and nondiseased inflorescences were produced by shoots that were initially diseased. Similarly, both diseased and nondiseased inflorescences were produced by shoots that were initially nondiseased.

The second collection of PNS was from four sites, two of which had never been observed with diseased inflorescences. Most of the shoots that resprouted from initially diseased basal bulbs produced infected inflorescences in the greenhouse (Table 1). Sometimes, both diseased and nondiseased inflorescences were produced from the same basal bulb (Table 1). In contrast, shoots from nondiseased basal bulbs produced only nondiseased inflorescences (Table 1).

The results from tubers generally paralleled those from basal bulbs. Shoots raised from tubers from initially diseased shoots produced mostly nondiseased inflorescences, but a few shoots produced diseased inflorescences (Table 2). No diseased inflorescences were produced from tubers collected from the two nondiseased populations (Table 2).

Microscopic examinations of plants with diseased inflorescences revealed no fungal hyphae in the pith or leaf sheaths. Hyphae were observed in ovules of nondiseased inflorescences produced by shoots from initially diseased basal bulbs. However, the hyphae did not appear to be internal, rather they were enclosed between the ovules and the integuments near the micropyle. Abundant hyphae were observed around apical meristems and leaf primordia of basal bulbs and tubers but again did not appear to penetrate host tissues (Fig. 2). Dense mats of hyphae were found on the surfaces of young leaves. Similar mats of hyphae were found on plants producing nondiseased inflorescences and nonflowering plants in addition to plants producing diseased inflorescences. How-

Table 1. Comparison of numbers and types of inflorescences produced by 6-wk-old plants grown from initially diseased or nondiseased basal bulbs

Collection site	Sample size	Percent flowering	Percent diseased[a]	Mean inflorescence number per plant[b]	
				Nondiseased	Diseased
Nondiseased (no. 1)	50	84[c]	0	1.8[d]	0.0
Nondiseased (no. 2)	54	78	0	1.7	0.0
Diseased (no. 1)	59	92	93	0.4	2.1
Diseased (no. 2)	52	85	68	0.8	0.9

[a] Percentage of flowering plants producing at least one diseased inflorescence.
[b] Nonflowering plants not included.
[c] Percentage of plants flowering did not differ significantly among populations (χ^2 test, $P<0.05$).
[d] Total inflorescence number per plant did not differ significantly among populations (ANOVA, $P<0.05$).

Table 2. Comparison of numbers and types of inflorescences produced by 6-wk-old plants grown from tubers from initially diseased or nondiseased shoots

Collection site	Sample size (no.)	Percent germinated	Percent flowering[a]	Percent diseased[b]	Mean inflorescence number per plant[c]	
					Nondiseased	Diseased
Nondiseased (no. 1)	48	71[d]	74[e]	0	1.3[f]	0.0
Nondiseased (no. 2)	50	68	74	0	1.4	0.0
Diseased (no. 1)	27	85	82	21	1.3	0.4
Diseased (no. 2)	40	75	57	6	1.1	0.1

[a] Nongerminated tubers not included.
[b] Percentage of flowering plants producing at least one diseased inflorescence.
[c] Nonflowering plants not included.
[d] Germination of tubers did not differ significantly among populations (χ^2 test, $P<0.05$).
[e] Percentage of plants flowering did not differ significantly among populations (χ^2 test, $P<0.05$).
[f] Total inflorescence number per plant did not differ significantly among populations (ANOVA, $P<0.05$).

Fig. 2. Longitudinal section illustrating location of fungal hyphae around meristem and leaf primordia (×380).

ever, hyphae were never observed on plants collected from sites where diseased inflorescences were never observed. It appears therefore that *B. cyperi* is epiphytic rather than endophytic, as other species of *Balansia* (7). Luttrell and Bacon (18) have described a similar situation in the fungus *Myriogenospora atramentosa* (Berk. & Curt.) Diehl, which is in the same tribe as the genus *Balansia*.

The results from field, greenhouse, and microscopic observations provide evidence that *B. cyperi* is sytemic in PNS. Seasonal variation in disease symptoms, variation in symptoms among shoots interconnected by rhizomes, and variation in symptoms among shoots growing from the same basal bulb suggest that infected plants are often asymptomatic. Microscopic detection of hyphae around meristems and on young leaves of shoots that otherwise are asymptomatic provide further support for the idea that infected plants can be either diseased or nondiseased. Alternatively, the observed variation in symptoms may represent escape from infection. PNS can grow very rapidly; it may outgrow the fungus, resulting in new uninfected shoots and tubers. This phenomenon has been documented in grasses infected by related endophytic fungi (7,20). The fact that shoots from infected basal bulbs produced diseased inflorescences more frequently than shoots from infected tubers provides support for this possibility.

Related endophytic and epiphytic fungi of grasses are probably transmitted through seeds (2,6,7,9). In the grasses *Festuca arundinacea* Schreb., *Lolium perenne* L., and *Danthonia spicata* (L.) Beauv., fungal hyphae may grow directly into the ovule from the maternal host tissue (2,6,9). When infected hosts do not produce flowers or seeds, windborne or waterborne conidia and ascospores may land on a grass stigma and germinate and subsequently infect the ovule (7). PNS rarely produces seed (11,23), so this mechanism would appear unlikely; instead, growth of hyphae from the parent plant into or onto rhizomes, tubers, and basal bulbs appears to be the main mode of transmission.

PNS has proven difficult to control by conventional methods and can cause substantial crop losses in many areas (19,26,27). Biocontrol techniques have controlled some weeds (10,22), but it is doubtful that *B. cyperi* represents a potential biocontrol agent. The mode of infection by species of *Balansia* is not well understood, and artificial innoculations largely have been unsuccessful (20). More importantly, it is not clear whether infection is detrimental to the host. Infected shoots produce diseased inflorescences but otherwise appear quite healthy. PNS rarely produces viable seed, so diseased inflorescences may have little effect on its reproductive potential (14,23). Previous studies have demonstrated that grasses infected by related fungi have higher growth rates and greater competitive abilities than comparable noninfected grasses (3,17). Increased resistance to insect pests also has been found in several infected grasses (4,9,16) and in two *Cyperus* species infected by *B. cyperi* (5). Greater vigor and resistance to insect pests, if present in infected PNS, would exacerbate crop losses caused by this weed.

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