

regard, a neuraminidase activity has been detected in African trypanosomes, and the anemia of cattle experimentally infected with *T. vivax* has been related to this enzyme activity (14). Moreover, it is possible that the enzyme plays a role in adsorption to and penetration of the trypanosome into host cells and in the release of parasites from infected cells.

It is of interest that the highest level of neuraminidase activity was found in trypomastigotes (Table 1), the trypanosome form that infects host cells and is released from them into the circulation to spread the infection. A role for neuraminidase in infection was first described with the mixoviruses (15), and a correlation has been found between the protection of individuals vaccinated with influenza virus and the titer of antibodies to neuraminidase (16). It would therefore be interesting to determine whether patients with Chagas' disease, or experimental animals infected with *T. cruzi*, develop antibodies to neuraminidase and whether such antibodies have functional significance in the trypanosomal infection. The applicability of this system to other parasitic infections, such as malaria, should also be investigated.

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11. For the neuraminidase assay, 50  $\mu$ l of a suspension of trypanosomes ( $1 \times 10^6$  bloodstream or tissue culture trypomastigotes and  $1 \times 10^7$  epimastigotes or amastigotes) was incubated at 37°C with 20  $\mu$ l of 1M sodium phosphate buffer (pH value indicated in each experiment), 50  $\mu$ l of an aqueous solution of substrate containing 100 nmole of bound sialic acid, and 80  $\mu$ l of distilled water. At the end of the incubation period, 20 to 50  $\mu$ l of reaction mixture was centrifuged in a Beckman microfuge and the supernatant was analyzed for free sialic acid by the periodate-thiobarbituric acid method [D. Aminoff, *Biochem. J.* **81**, 384 (1961)]. Enzyme and substrate controls were incubated concurrently and the corresponding readings subtracted from that obtained with the complete enzyme system. One unit of neuraminidase activity is defined as the amount of enzyme that releases 1 nmole of sialic acid per hour under the conditions of the assay. Specific activities are expressed as units of neuraminidase activity per  $10^6$  trypanosomes.

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## Biological Control of Yellow Nutsedge with the Indigenous Rust Fungus *Puccinia canaliculata*

**Abstract.** *Yellow nutsedge (Cyperus esculentus L.) is a serious weed problem in the United States and other countries. An indigenous rust fungus [Puccinia canaliculata (Schw.) Lagerh.], pathogenic on yellow nutsedge, was released in early spring as a potential biological control agent. The fungus inhibited nutsedge flowering and new tuber formation. The fungus also dehydrated and killed nutsedge plants. The successful control of yellow nutsedge by a rust epiphytotic under experimental conditions demonstrates the potential use of the rust in an integrated weed management system.*

Yellow nutsedge (*Cyperus esculentus* L.) is thought to be one of the world's most troublesome weeds (1). Hauser (2) described nutsedge as a worldwide plague. This weed has been a serious pest for many decades. In recent years it has spread rapidly throughout the United States and is now the most troublesome perennial weed in most of the Midwest (3).

Yellow nutsedge reproduces prolifically. One tuber planted in a field in Minnesota produced 1900 plants and 6900 tubers in 1 year (4). In Georgia 622 tubers were produced from one tuber in 17 weeks (5).

In spite of considerable effort, biocontrol methods for yellow nutsedge are not yet effective for field use (3). Rust caused by *Puccinia canaliculata* (Schw.) Lagerh. was first described in 1832. In 1906, Arthur demonstrated that *Xanthium* sp. was an alternate host, an indication that the pathogen is a macrocyclic heteroecious rust (6). Another possible alternate host is giant ragweed, *Ambrosia trifida* L. To our knowledge, no research has been reported on this rust except by us (7, 8). Researchers have observed the rust on yellow nutsedge in many locations in the United States and

Canada. However, it usually does not appear until August and does not increase substantially until September. By then, the nutsedge has produced new seed and tubers and its life cycle is unaffected by the rust.

The objective of our research program is to develop an integrated weed management system (IWMS) for yellow nutsedge by integrating biological, chemical, and cultural practices. Selective and nonselective chemical treatments are available for the control of yellow nutsedge (4), and cultural and chemical practices can be used to reduce this pest without reducing the yield of soybeans and corn (3). However, acceptable control of yellow nutsedge is difficult to achieve in horticultural crops. We present here data on the effect of this rust fungus on yellow nutsedge in southern Georgia from 1978 to 1981.

An epiphytotic of rust was observed on a dense stand of yellow nutsedge in September 1978 on a research farm near Tifton, Georgia, and in August-September 1979 a severe rust epiphytotic developed on this nutsedge. In 1980, we carried out weekly observations and found a few plants with rust in June. The infected nutsedge was located in vegetable re-

Table 1. Effect of rust fungus on nutsedge 60 days after rust release. Each mean is the average of four replications. In all cases listed, the means in each column were different at the 5 percent level (*t*-test).

Nutsedge sample	Leaf area diseased (%)	Root growth (%)	Fresh weight per plant (g)	Dry weight per plant (g)	Dry matter (%)
Rust-infected	78.5	18.5	5.9	1.8	29.8
Control*	10.5	87.0	13.7	2.9	21.1

\*Weekly application of chlorothalonil.

search plots that had been sprayed weekly with fungicides. The infected plants were potted and placed among a thick stand of yellow nutsedge (0.5 ha) without symptoms 7 km away. The new area was not sprayed with fungicides. Rust pustules were evident on plants at the new site within 12 days. An epiphytotic developed within 4 weeks over the entire area.

Our fungicide study (8) indicated that, with proper selection of fungicides, rust may be used in an IWMS for controlling yellow nutsedge. Chlorothalonil, a fungicide commonly used on a wide variety of crops, reduced the development of rust when the weekly spraying was started along with the rust inoculations (8). However, if chlorothalonil (2.9 liter/ha) sprays were delayed until an epiphytotic of rust developed, the fungicide had no effect on the efficacy of rust.

Rust was maintained on infected plants in a greenhouse during the winter. Pots of nutsedge plants with only one to two visible rust pustules were placed in four plots (13 by 13 m) on 1 May 1981 as soon as nutsedge appeared in the plots. Within 10 days pustules were observed on all nutsedge plants within 1 m of the original plants and within 7 m in 14 days. There was no visible infection for up to 17 days on the windward side. The major direction of spread was eastward, an indication of leeward spore movement. Twenty-eight days later rust was observed 130 m away on the leeward side of the original inoculum. By the first week of June, an epiphytotic developed with over 90 percent of the nutsedge leaves dead or dying. During this period, rust released in yellow nutsedge under a corn canopy moved only 3 m. This result demonstrates that wind is essential for the spread of rust. Weekly observations indicated that dehydration of nutsedge root occurs soon after a few pustules appear on the plant. General dehydration of the plant follows root dehydration; this is reflected in a higher percentage of dry matter in rust-infected plants (Table 1). Old parent tubers on infected plants were also dehydrated. Rust reduced the dry weight and fresh weight of nutsedge (Table 1). Flowering and tuber formation of nutsedge were inhibited in rust-infected areas for up to 16 weeks after inoculation, whereas chlorothalonil-sprayed nutsedge controls flowered profusely and produced tubers (Table 2). Rapid dehydration and substantial reduction or elimination of normal vegetative and sexual reproductive processes, even with moderate rust infection, suggests the production of a biologically active substance or substances. Further re-

Table 2. Effect of rust fungus on nutsedge tuber formation, flowering, and survival 90 and 120 days after rust release. The numbers in parentheses represent the percentages of the totals. Each mean is the average of four replications. Each pair of rust-control means is different at the 5 percent level (*t*-test).

Nutsedge	Number per 0.93 ha			
	90 days		120 days	
	Rust-infected	Control*	Rust-infected	Control*
Tubers				
Old	169	132	167	108
New	17 (9)	60 (31)	16 (9)	63 (37)
Plants				
Live	112	201	144	313
Dead	91 (45)	4 (2)	122 (46)	6 (2)
Inflorescences	0	23	0	83

\*Weekly application chlorothalonil.

search is needed to identify these chemicals and their possible function in controlling nutsedge through inhibition of the reproductive process.

The rust is adapted to a wide range of climatic conditions. Rust epiphytotic developed over several hectares from a series of releases throughout the growing season under all conditions where nutsedge was growing. The reported range of this rust is from Massachusetts to Nebraska, southward to Florida, and west to California; it is also found in Mexico, Central and South America, the West Indies, and Hawaii (6).

*Puccinia canaliculata* is specific on yellow nutsedge. It has been reported only on a number of weeds belonging to the genus *Cyperus* including yellow and purple nutsedge (6). However, the strain of rust we have was not observed on purple nutsedge (*Cyperus rotundus* L.) when released in an area where mixed stands of purple and yellow nutsedge were present. No rust was observed on corn, soybean, peanut, tomato, pepper, okra, snap bean, lima bean, cucumber, squash, watermelon, southern pea, and cotton in the areas where yellow nutsedge surrounding these crops was covered with rust. Thus, this rust organism satisfies all the requirements for biological control agents outlined by Shaw (9).

These results also indicate that an IWMS program could be developed for various crops, with rust being used as one of the components for controlling yellow nutsedge. Success of the IWMS strategies will depend on further research on several factors: (i) the ability to produce abundant rust spores for storage and for formulation of rust inoculum for field use; (ii) the development of the appropriate technology for rust application with pesticide delivery systems (sprayers, granular applicators, airplanes, and irrigation); (iii) the most effective scheduling of fungicide applica-

tion; (iv) the development of fungicides with reduced activity against the rust; and (v) the development of ways to combine rust use with appropriate herbicides or cultivation. Research in progress on some of these factors suggests that under both cropping and noncropping situations the rust caused by *Puccinia canaliculata* provides a valuable tool for the control of yellow nutsedge.

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