Studies on Alternate Hosts of the Rust *Puccinia canaliculata*, a Potential Biological Control Agent for Nutsedges

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ABSTRACT

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Puccinia canaliculata is a potential biological control agent for purple (*Cyperus rotundus*) and yellow (*C. esculentus*) nutsedges. In 1905, *Xanthium canadense* L. was shown to be an alternate host for *P. canaliculata*. After extensive surveys, the aecial stage has not been found occurring naturally on *X. strumarium* in Georgia. Inoculation studies demonstrated that *X. strumarium* and *Helianthus annuus* are alternate hosts. Inoculation studies were also carried out on *Cosmos bipinnatus* with inconclusive results. Inoculations on alternate hosts may offer a simple method for developing more universally virulent rust strains to overcome variations in susceptibility among nutsedge ecotypes.

In 1942, *Puccinia canaliculata* (Schw.) Lagerh. was suggested as a potential biological control agent for purple nutsedge (*Cyperus rotundus* L.) (4). Actual success in controlling yellow nutsedge (*C. esculentus* L.) with this rust was reported in 1983 (8). A high degree of host specificity is considered a requirement for the use of rusts in weed control (5). However, this high degree of specificity or variability in host susceptibility may be an equally important constraint in the use of rust fungi for biocontrol (W. L. Bruckart, *personal communication*). This paper describes a simple method with possible application in the development of more virulent rust strains through gene recombination.

Using naturally infected *Xanthium canadense* L. (cocklebur), Arthur (1) demonstrated that *P. canaliculata* was a macrocyclic, heteroecious rust on *Cyperus* spp., with cocklebur as an alternate host. The significance of this finding for biological control of yellow nutsedge is apparent through the opportunity for genetic recombination in *P. canaliculata* on the alternate host. Extensive surveys have failed to show the aecial stage occurring naturally on *X. strumarium* L. in Georgia. Other alternate hosts including *Bahia dissecta* (Gray) Britt., *Cosmos parviflorus* (Jacq.) H.B.K., *Engelmannia pinnatifida* Nutt., *Helianthus annuus* L., *Heliopsis parviflorus* Gray, *Rudbeckia serotina* Nutt., and *Senecio ampulaceus* Hook. have been implicated from aecial collections on herbarium specimens (9). *Ambrosia trifida* L. has been implicated by pycnial and aecial observations on the host (2). These hosts have never been verified by inoculation studies. Sunflower (*Helianthus annuus* L.) is the only alternate host among the herbarium specimens that is currently of commercial value. The purpose of this paper was to determine whether sunflower or *X. strumarium* were alternate hosts of *P. canaliculata* in greenhouse inoculation studies. *Cosmos bipinnatus* Cav. also was inoculated as part of the study.

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MATERIALS AND METHODS

Cocklebur. For alternate host studies, leaves of yellow nutsedge bearing telia of the rust *P. canaliculata* were collected in 1982 from the Blackshank Farm near Tifton, GA, and in 1983 from Gopher Ridge and Old Ocilla Road, near Tifton. In 1983, teliospores were also collected near Fayetteville, AR. Leaves were stored at room temperature until spring 1984, when telia were used to inoculate 16 plants of greenhouse-grown *X. strumarium* from Georgia. Four plants were inoculated with each of the four *P. canaliculata* collections. One drop of Triton B 1956 was blended for 15 sec in 50 ml of high performance liquid chromatography (HPLC) grade water and sprayed on the cocklebur leaves immediately before inoculation. Teliospores were scraped from the nutsedge leaves with a razor blade onto a microscope slide. Three or four leaves per cocklebur plant were inoculated by brushing the teliospores onto the adaxial leaf surface with a camel's-hair brush. Pots were placed four to a bag in clear plastic bags humidified with HPLC water. Bags were then sealed and placed out of direct

Fig. 1. Pycnia and aecial cups on cocklebur leaf (about ×17). Arrows: A = aecial cups and B = pycnia.

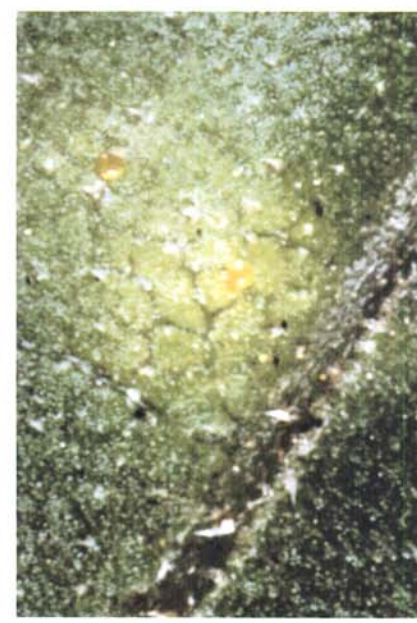

Fig. 2. Close-up of pycnia on cocklebur leaf (about ×22).

sunlight under a greenhouse bench. Plants inoculated with different teliospore collections were bagged separately. After 72 hr, plants were removed from the bags and replaced on a greenhouse bench the next overcast day. One to 2 days after the appearance of the pycnia, a camel's-hair brush was brushed over the pycnia to ensure fertilization and to overcome the possibility of self-incompatibility. Cross-fertilization procedures were continued at 1- to 2-day intervals until exudation of the pycnia had ceased. A second experiment was carried out using eight plants and three of the four teliospore collections. Procedures were the same as for the first inoculation.

Sunflower. Plants were inoculated in two separate experiments. In the first, an unidentified, large-seeded cultivar was inoculated with teliospores from the Blackshank Farm and Old Ocilla Road collections. Two plants were inoculated with each teliospore collection.

In the second inoculation, the ornamental cultivar Double Sun Gold (Ferry-Morse Seed Co., Fulton, KY) was inoculated with teliospores from the Blackshank Farm and Arkansas collections. Three plants were inoculated with the Blackshank Farm collection and one plant with the Arkansas collection. Procedures were the same as those described for cocklebur.

Cosmos. With the procedures described for cocklebur, three plants of *Cosmos bipinnatus* 'Sensation' (Ferry-Morse Seed Co., Fulton, KY) were inoculated with teliospores from the Blackshank Farm and one plant with the Arkansas collection.

Nutsedge. Three procedures were investigated for the inoculation of nutsedge with aeciospores from cocklebur.

1. Abaxial leaf surfaces (location of all stomata) (11) were painted with a solution of Triton B 1956 and HPLC water (0.1%, v/v). Aeciospores were then transferred from aecia on the cocklebur to the wet nutsedge leaf surface using a camel's-hair brush. Inoculated nutsedge plants were placed out of direct sunlight in clear plastic bags humidified with HPLC water for 72 hr. Various ecotypes of *C. esculentus* and *C. rotundus* were inoculated (Table 1).

2. Aeciospores were shaken from intact cocklebur leaves onto two susceptible ecotypes of nutsedge. The inoculated plants were then placed in clear, plastic bags and treated as in procedure 1.

3. Two susceptible ecotypes of nutsedge were placed on the greenhouse bench under cocklebur leaves containing sporulating aecia. Cocklebur leaves were occasionally shaken above the nutsedge plants.

RESULTS

Cocklebur. Leaf crinkling appeared within 3–4 days of inoculation. The first pycnia appeared after 8 days in both the first and second inoculations. Pycnia appear on the abaxial leaf surface as small, raised, orange spots surrounded by a chlorotic halo (Figs. 1 and 2). There were differences between the four teliospore collections (Table 2). Sporulating aecia appeared in the first and second inoculation groups after 23 and 18 days, respectively (Figs. 1 and 3). This proves that lack of susceptibility is not the reason for the failure of extensive surveys to show the aecial stage occurring

Table 1. Nutsedge ecotypes, aecial inoculation procedures, and susceptibilities

Ecotype	Inoculation date (1984)	Inoculation procedure(s)[a]	Susceptibility
Cyperus esculentus			
Washington State	20 April		
	4 May		
	5 June		
	8 June	1	Very high
C. esculentus			
Gopher Ridge, GA	20 April		
Tifton, GA	4 May		
	5 June		
	8 June	1	High
C. rotundus			
Tifton, GA	4 April		
	20 May	1	Resistant
C. rotundus			
Painter, VA	4 April		
	20 May	1	Resistant
C. rotundus			
Thomasville, GA	8 June	1	Resistant
C. esculentus			
Washington State	31 May		
	13 June	2,3	Very high
C. esculentus			
Gopher Ridge, GA	31 May		
Tifton, GA	13 June	2,3	High

[a] 1 = Triton B 1956 brush application; 2 = aeciospores shaken onto nutsedge plants, plants bagged; and 3 = aeciospores shaken onto nutsedge plants on greenhouse bench.

Table 2. Susceptibility of *Xanthium strumarium* to sporidia of *Puccinia canaliculata* from four locations

Origin of teliospores	Plants inoculated (%)	No. plants with pycnia (%)	Infected plants (%)
Blackshank Farm, Tifton, GA	4	2	50
Old Ocilla Road, Tifton, GA	4	0	0
Gopher Ridge, Tifton, GA	4	0	0
Fayetteville, AR	4	3	75

Fig. 3. Sporulating aecia on cocklebur leaf (about ×22).

Fig. 4. Sporulating aecia on sunflower leaf (about ×17).

naturally on *X. strumarium*. Inoculated leaves 5 cm or shorter showed no symptoms of infection on any plants.

Sunflower. Pycnia appeared 9–11 days after inoculation of each sunflower cultivar. Sporulating aecia appeared within 15 days of inoculation on Double Sun Gold (Fig. 4). Aecia did not develop on the unidentified, large-seeded cultivar because necrotic areas formed and enlarged around the pycnia, resulting in pycnial necrosis. Again, inoculations using teliospores from the Blackshank Farm and Arkansas collections resulted in infection. No leaves shorter than 5 cm became infected.

Cosmos. Sunken, white lesions were observed 11 days after inoculation. Pycnia failed to develop.

Nutsedge. With procedure 1, no uredia were found on any of the ecotypes inoculated. With procedure 2, uredia became evident within 17 days of inoculation on the Washington State ecotype and within 19 days on the Gopher Ridge ecotype. Visually, the former ecotype showed a greater pustule frequency. With procedure 3, uredia were visible within 26 days on both ecotypes, although the Washington State ecotype showed a greater pustule frequency.

DISCUSSION

Cocklebur. When Arthur (1) inoculated yellow nutsedge with aeciospores from naturally inoculated *X. canadense*, uredia were observed 16–22 days later. This is comparable to the interval of 26 days for uredial development observed in these tests with *X. strumarium*. *P. canaliculata* has been collected from *C. esculentus* ecotypes over a wide geographical area (2,3,6,7,10). It has also been collected from an even more internationally troublesome weed, *Cyperus rotundus* (2,4,7). The strains of *P. canaliculata* currently under investigation as biological control agents have exhibited specificity to collections of *C. esculentus* and do not attack collections of *C. rotundus* studied to date (W. L. Bruckart, *personal communication*; S. C. Phatak, *unpublished*). By using alternate hosts, methods of sexual recombination similar to those described above may be useful in efforts to develop a more universally virulent strain.

Differences between teliospore collections were noted. These differences may be due to differences in viability, satisfaction of dormancy requirements, specificity, or a combination of these factors.

Sunflower. This study represents the first published report verifying sunflower as an alternate host of *P. canaliculata*. Leaf tissue necrosis observed in the large-seeded cultivar may have resulted from trichome damage during brush application of the teliospores or from a resistance reaction.

Cosmos. Aecial collections of *P. canaliculata* on herbarium specimens of *Cosmos parviflorus* have been reported previously (9). *C. bipinnatus*, rather than *C. parviflorus*, was chosen because of its availability and commercial value as an ornamental. Inoculation studies to verify *C. bipinnatus* as an alternate host were inconclusive. The pattern and time of appearance of sunken, white lesions on the leaves suggests that germination of teliospores may have occurred. Pycnia failed to develop. Further studies with other *Cosmos* spp. and/or cultivars seem warranted.

Nutsedge. Results suggest that unlike uredospores (9), aeciospores may be sensitive to Triton B 1956 at the 0.1% concentration used. Simple techniques, as described in procedures 2 and 3, were found to be efficient inoculation methods when aeciospores are abundant.

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