Comparative Spore Morphology and Pathogenicity of Four Florida Isolates of *Nectria galligena*

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ABSTRACT

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Perithecia of *Nectria galligena* were observed in the field in association with 1) stem galls on *Cercis canadensis*, 2) hypertrophied, roughened, and fissured bark in branch axils of *Swietenia mahagoni*, and 3) stem cankers on *Quercus laurifolia* and *Acer rubrum*. In the greenhouse, canker symptoms developed on seedlings of all four hosts in response to artificial wound inoculations with mass isolates from each host. Symptoms produced on *S. mahagoni* by the *S. mahagoni* isolate, however, were notably restricted in comparison with those produced on the other three hosts. Observations and measurements performed on ascospores, as well as on conidia of the *Cylindrocarpon heteronema* anamorph, revealed no distinct differences in spore sizes or septation patterns among isolates. *C. canadensis*, *S. mahagoni*, and, possibly, *Q. laurifolia* represent new host and suscept records for *N. galligena*.

Nectria cankers are common and widespread on hardwood species in Europe, and North America, (2,7,8,13, 15,17). In Florida, we have observed perithecia typical of *Nectria* spp. in association with targetlike cankers on laurel oak (*Quercus laurifolia* Michx.) and red maple (*Acer rubrum* L.), as well as burllike galls on stems of eastern redbud (*Cercis canadensis* L.) (Fig. 1) and areas of hypertrophied, roughened, and fissured bark in branch axils of West Indies mahogany (*Swietenia mahagoni* Jacq.) (Fig. 2). In this paper we report results of studies to identify these fungi and to evaluate their pathogenicity on each of these hosts. A preliminary report has been published (3).

MATERIALS AND METHODS

Isolation, culture maintenance, and inoculum preparation. Mass isolates (one per host) were obtained from ascospores exuded from *Nectria* perithecia removed from bark tissues collected from the four hosts (Fig. 3). Cultures were maintained at room temperature (25 ± 2 C) under normal laboratory lighting on potato-dextrose agar (PDA) (11). Inoculum for artificial inoculations consisted of 4-mm-diameter agar plugs cut with a cork borer from 2-wk-old PDA-plate subcultures. Each plug supported fungus mycelium and conidia of the *Cylindrocarpon* anamorph.

Morphological comparison and identification of isolates. Perithecia of the *Nectria* teleomorph were induced on carnation leaf-water agar (12) after 3–4 wk in a growth chamber at 26.5 ± 0.5 C with alternating 12-hr light/dark periods (approximately 3,000 lx generated by GE F20T12/CW fluorescent tubes). Ascospores, as well as microconidia and macroconidia from these cultures, were observed and measured under oil immersion at ×1,000. In addition, subcultures of each isolate were forwarded to C. Booth at the C.A.B. International Mycological Institute, Kew, Surrey, England for verification.

Artificial inoculations and evaluation of pathogenicity. First-year seedlings of the four source host species with stem diameters of 0.5–1.0 cm were used for

Fig. 1. Burllike galls on stems of *Cercis canadensis*.

Fig. 2. Hypertrophied, roughened, and fissured bark in a branch axil of *Swietenia mahagoni*.

Fig. 3. Location of hardwood hosts from which *Nectria* isolates were obtained for study.

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artificial stem inoculations. Seedlings were potted in plastic pots 15 cm in diameter and maintained on a greenhouse bench under natural lighting, with temperatures fluctuating diurnally between a minimum of 22 C and a maximum of 47 C. Inoculations were performed at 10 and 20 cm above the soil line on each of four seedlings per host species with each of the four test isolates. Single inoculum plugs (above) were inserted beneath the bark into 5- to 7-mm vertically oblique incisions made with a sterile scalpel. Four control seedlings for each host species were similarly inoculated with sterile PDA plugs.

Test seedlings were observed periodically for development of canker symptoms. After 4 mo, test seedlings were harvested and stems were dissected longitudinally through the center of each inoculation point. The longitudinal extent of xylem discoloration and/or necrosis was measured to the nearest millimeter. Isolations were performed from tissues at the margins and center of the discolored xylem to confirm the presence of the test organism.

Fig. 4. Droplets of clear to amber-colored gum exuded by stem of *Swietenia mahagoni* in response to artificial inoculation with *Nectria galligena*.

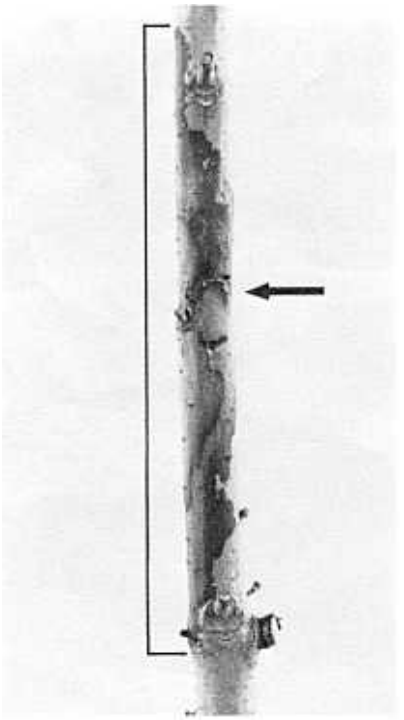

Fig. 5. Water-soaking/staining of bark on stem of *Quercus laurifolia* above and below point of artificial inoculation (arrow) with *Nectria galligena*. Extent of water-soaking/staining delimited by bracket at left of stem.

RESULTS

Morphological comparison and identification of isolates. Spore measurements revealed similarities among the *Nectria* isolates from the four hosts (Table 1). Spore dimensions were in close agreement with those published by others (2,5,6,10, 11,15,16) as descriptive of *N. galligena* Bres. In addition, Booth (*personal*

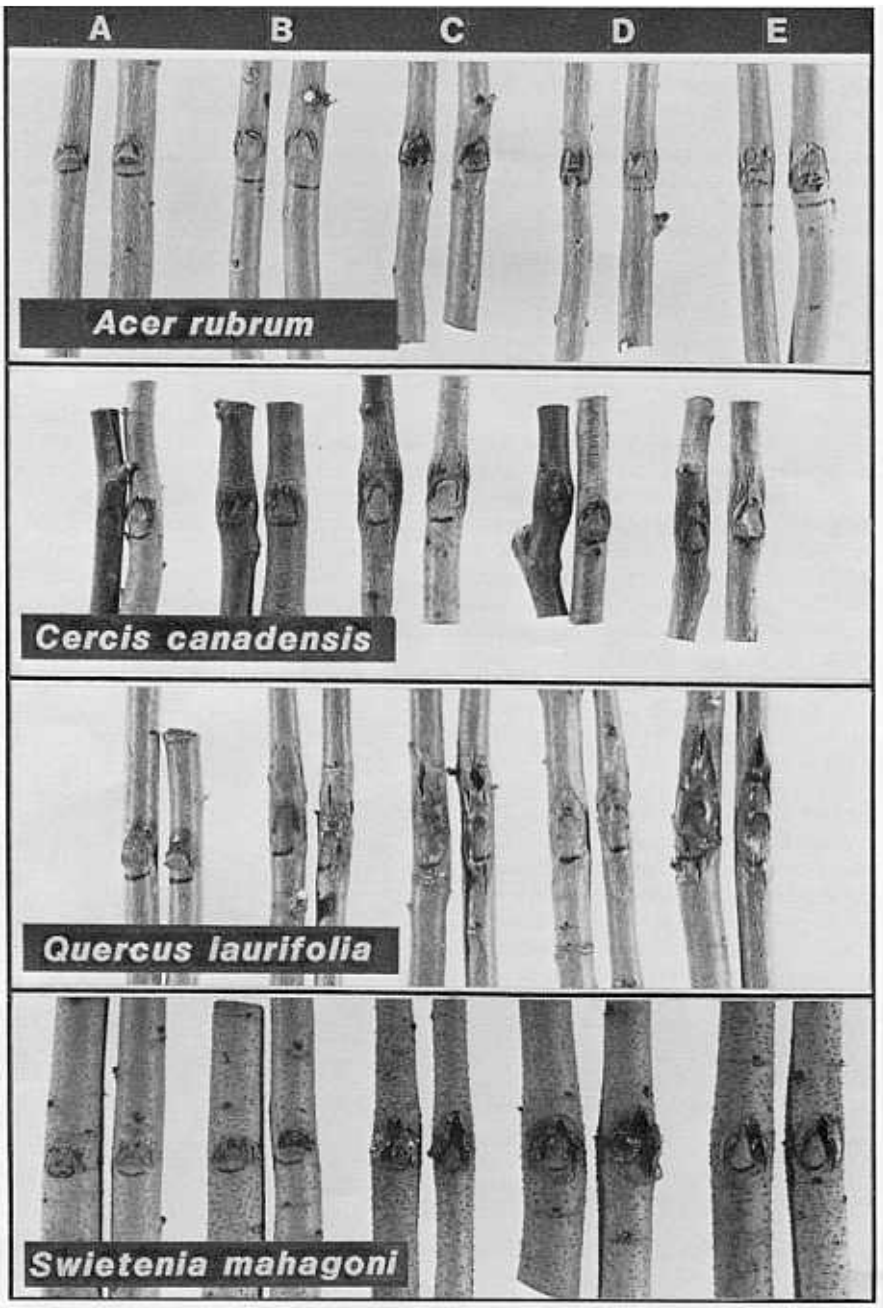

Fig. 6. External appearance of hardwood seedling stems 4 mo after artificial inoculation with Florida isolates of *Nectria galligena*. Source of isolates: (A) control, (B) *Swietenia mahagoni*, (C) *Quercus laurifolia*, (D) *Cercis canadensis*, (E) *Acer rubrum*.

communication) supported placement of our isolates within the current concepts of this species and its *Cylindrocarpon heteronema* (Berk. & Br.) Wollenw. anamorph.

Artificial inoculations and evaluation of pathogenicity. Within 7–10 days after inoculation, most mahogany seedlings began to exude clear to amber-colored gum droplets at the points of artificial inoculation (Fig. 4). This host reponse was exhibited by seedlings inoculated with each of the four *N. galligena* isolates, but was notably lacking in the control inoculations. Within a few weeks, the majority of inoculated laurel oaks displayed a distinct water-soaking/staining of the bark for considerable distances above and below the points of inoculation (Fig. 5). Again this host response was exhibited by seedlings inoculated with each of the four isolates, but was not present in seedlings receiving control inoculations. Over time, all hosts displayed varying degrees of bark tissue discoloration/necrosis, callus tissue development, bark fissuring, and/or stem deformation at or near the points of artificial inoculation. Comparable symptoms were lacking in control inoculations (Fig. 6).

Internally, inoculated stems exhibited varying degrees of cambial necrosis and vertically extensive zones of discolored/necrotic sapwood in all four hosts after 4 mo in the greenhouse (Fig. 7). These symptoms were consistent across all host-isolate combinations, with one exception: mahogany stems inoculated with the mahogany isolate developed neither. These results held consistent, even in a second round of mahogany stem inoculations using the mahogany *Nectria* isolate. Although substantial, the zone of discolored/necrotic sapwood in inoculated laurel oak stems was not coincident with the external water-soaking/staining of the bark (above). Typically, the external water-soaking/staining on these stems extended well above and below the internally altered sapwood. *N. galligena* was consistently

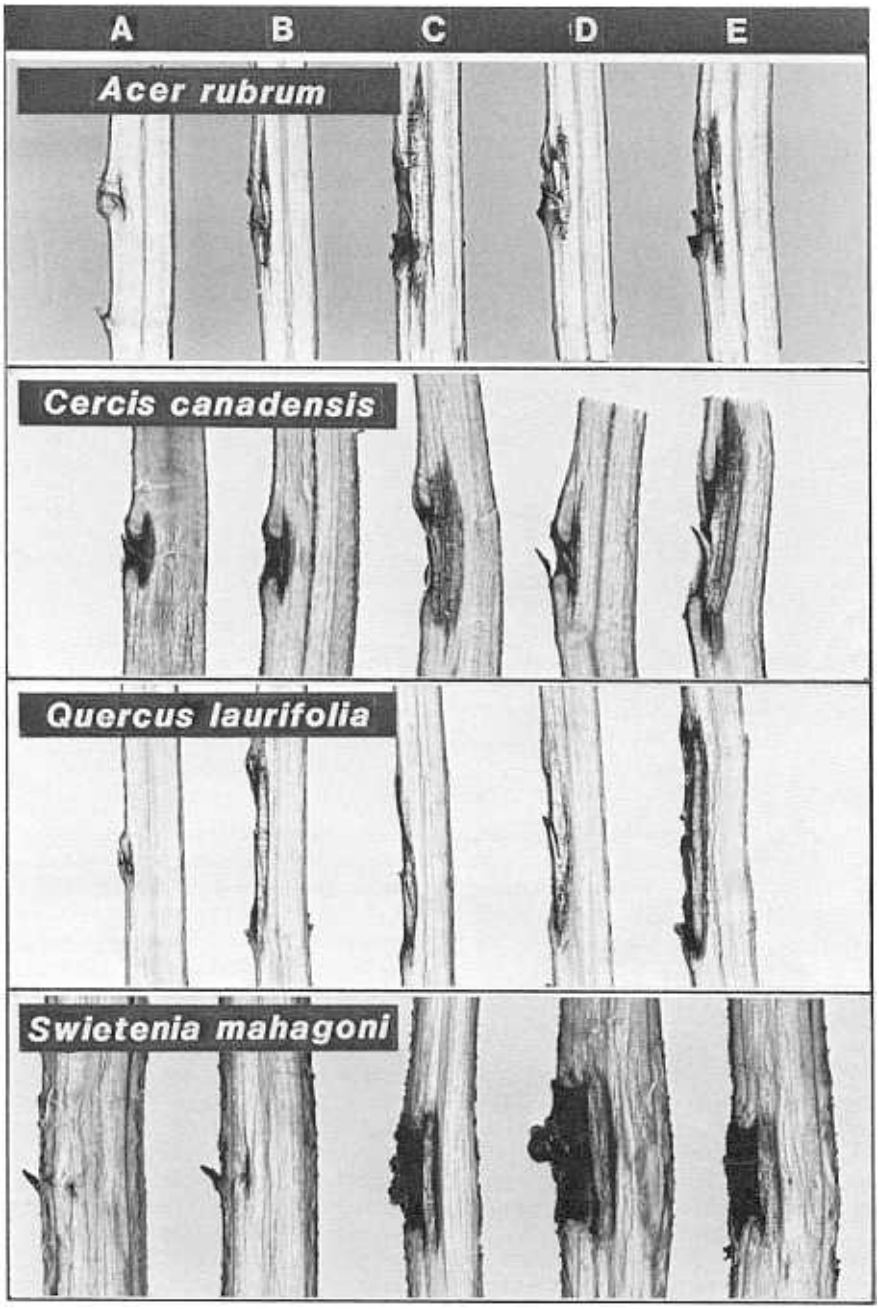

Fig. 7. Discoloration/necrosis of cambium and sapwood of hardwood seedling stems 4 mo after artificial inoculation with Florida isolates of *Nectria galligena*. Source of isolates: **(A)** control, **(B)** *Swietenia mahagoni*, **(C)** *Quercus laurifolia*, **(D)** *Cercis canadensis*, **(E)** *Acer rubrum*.

Table 1. Comparative measurements[a] of ascospores and conidia produced on carnation leaf-water agar by four Florida isolates of *Nectria galligena* and its *Cylindrocarpon heteronema* anamorph

Isolate source	Spore type	No. observed	No. septa[a]	Length (μm)	Width (μm)[b]
Acer rubrum	Ascospores	24	1	(16.8-)18.5(-21.3)	(4.0-)4.6(-5.5)
	Macroconidia	60	5	(53.5-)65.1(-74.3)	(5.7-)6.8(-7.7)
	Microconidia	46	0	(4.0-)6.9(-14.4)	(1.5-)2.2(-4.0)
Cercis canadensis	Ascospores	40	1	(16.8-)19.2(-21.9)	(4.0-)5.1(-6.4)
	Macroconidia	43	5	(40.6-)58.5(-73.3)	(5.0-)6.5(-7.4)
	Microconidia	50	0	(4.5-)6.8(-12.4)	(1.5-)2.4(-4.5)
Quercus laurifolia	Ascospores	50	1	(14.9-)17.2(-19.3)	(4.0-)5.0(-6.2)
	Macroconidia	56	5	(44.6-)58.8(-73.4)	(5.2-)6.1(-7.2)
	Microconidia	56	0	(4.0-)6.9(-12.2)	(1.5-)2.4(-5.0)
Swietenia mahagoni	Ascospores	222	1	(13.9-)18.3(-23.8)	(3.7-)4.9(-6.9)
	Macroconidia	25	5	(59.4-)70.6(-79.7)	(5.9-)7.0(-7.4)
	Microconidia	54	0	(3.5-)6.5(-13.2)	(1.5-)2.4(-5.3)

[a](Min-)mean(-max) dimension based on number of spores indicated.
[b]Macroconidia with 1–6 septa observed in all isolates (1–7 in two); 5 septate conidia predominating.
[c]Ascospore width measured at the central septum.

Table 2. Results of artificial wound inoculations of four hardwoods with Florida isolates of *Nectria galligena* (anamorph = *Cylindrocarpon heteronema*) from each of the four respective hosts

Isolate source	Inoculated host	Symptoms[a]	Mean xylem discoloration (mm)[b]	Pathogen recovered
	A. rubrum		6.0	
	C. canadensis		7.0	
	Q. laurifolia		0-Trace	
	S. mahagoni		0-Trace	
Acer rubrum	*A. rubrum*		22.6	
	C. canadensis		16.8	
	Q. laurifolia		20.1	
	S. mahagoni		19.9	
Cercis canadensis	*A. rubrum*		36.3	
	C. canadensis		21.9	
	Q. laurifolia		32.4	
	S. mahagoni		18.8	
Quercus laurifolia	*A. rubrum*		25.3	
	C. canadensis		26.1	
	Q. laurifolia		23.3	
	S. mahagoni		19.0	
Swietenia mahagoni	*A. rubrum*		18.6	
	C. canadensis		23.0	
	Q. laurifolia		18.9	
	S. mahagoni		0-Trace	

[a] Includes canker, sap exudation, profuse callus development, and/or xylem discoloration; + = symptoms present, − = symptoms absent.
[b] Total vertical length above and below point of inoculation; $n = 8$.
[c] Symptoms limited to gummosis only.

reisolated from all inoculated stems, including the mahogany stems that failed to develop definitive canker symptoms. Control stems remained essentially symptomless and did not yield *N. galligena* upon isolation (Table 2).

DISCUSSION

Our data indicate no substantive intraspecific variability with respect to spore morphology among our four *N. galligena* isolates. Additionally, our data suggest little evidence of host specificity among isolates because all isolates were pathogenic to all four hosts. In this respect, our results parallel those of Ashcroft (2) who reported that isolates of *N. galligena* were capable of infecting a wide variety of hardwood species upon cross-inoculation. Others (13,14), however, have noted differences in both host range and symptoms produced by *Nectria* spp. from different source hosts. Flack and Swinburne (13) proposed the designation of formae speciales for morphologically similar, yet pathogenically distinct, isolates of *N. galligena* from ash and apple.

We do not understand why the isolate from mahogany produced such limited canker symptoms on mahogany, when this isolate was aggressive on each of the other three host species. This isolate may be physiologically or pathogenically distinct from the other three isolates tested, but more studies are required before firm conclusions are justified.

Although *Nectria* spp. occur commonly on *Acer* and *Quercus* spp., our search of the literature, including three host indices (1,14,18), revealed no specific report of association between *N. galligena* and *Q. laurifolia*. Therefore, our finding of this host/pathogen association may represent a new record.

Nectria spp. have been observed previously in association with canker or cankerlike diseases of *Swietenia* spp. Wan (19) described Nectria cankers on *S. mahagoni* and *S. macrophylla* King in Taiwan, but did not specifically identify the associated *Nectria* sp. Chen (9) later described *N. swieteniae-mahoganii* sp. nov. as the causal agent of cankers on *S. mahagoni* in Taiwan.

Barry and Anderson (4) recently have reported Nectria cankers on *S. macrophylla* in Puerto Rico. The *Nectria* sp. associated with these cankers has been tentatively identified as *N. haematococca* Berk. & Br., based on limited evaluations of its *Fusarium solani* (Mart.) Sacc. anamorph (D. F. Farr, *personal communication*).

To our knowledge, this is the first published report of *N. galligena* on both *C. canadensis* and *S. mahagoni*. Additionally, our work represents the first published evidence for proof of pathogenicity for *N. galligena* on these two hosts. However, we must emphasize that we did not, by way of artificial inoculation, duplicate the symptoms associated with *N. galligena* on these two hosts in the field (i.e., burllike galls and axillary hypertrophied, roughened, and fissured bark, respectively). Additional studies, perhaps on older trees or with longer incubation periods, are needed to determine whether the field symptoms we observed are caused by or are simply associated with *N. galligena*.

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