CONIDIAL GERMINATION OF TWO BIOTYPES OF *NOMURAEA RILEYI*

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The entomopathogenic fungus *Nomuraea rileyi* has a worldwide distribution and has been reported from many different insects (Ignoffo 1981). Although geographical isolates of *N. rileyi* from different insects have very similar isozyme patterns (Boucias and Ignoffo unpub.), they exhibit widely different host spectra (Ignoffo 1981, Boucias et al. 1984, Ignoffo & Boucias 1991). As a specific example, a Mississippian biotype (Ms) will topically infect larvae of *Heliocoverpa* (*Heliothis*) *zea* and *Heliothis virescens*, while an Ecuadoran biotype (Ec), at equivalent rates, will infect only larvae of *H. zea* (Ignoffo & Garcia 1985). Infectivity and/or growth of *N. rileyi* may also be influenced by environmental conditions, nutritional factors, and/or intrinsic genetic factors (Gardner et al. 1977, Roberts & Campbell 1977, Boucias & Pendland 1984, Ignoffo & Garcia 1985). The objective of our research was to determine whether differences in germination might be used to further distinguish between these two morphologically identical biotypes. Differences in germination also might help explain why larvae of *H. virescens* are more resistant than *H. zea* to the Ec biotype.

The Ms and Ec isolates of *N. rileyi* were selected because of their differential pathogenicity for these two species of closely related larvae and because earlier observations revealed differences in germination patterns. The source of the Ec and Ms biotypes of *N. rileyi* was previously described (Ignoffo & Garcia 1985). Conidia were produced from cultures grown on Sabouraud maltose agar fortified with 1% yeast extract (SMAY) after one passage through a susceptible host (*Trichoplusia ni*). The harvested conidia were then stored at –70°C prior to experiments described herein. The following procedure was used to determine the extent and rate of germination of conidia of both biotypes of *N. rileyi*. A series of disposable petri dishes (50 x 12 mm) containing SMAY were individually seeded with 10^7 conidia of either biotype using 50 ul per plate. Conidia were dispensed in sterile distilled water containing equal parts (0.01% ml/ml) streptomycin, + penicillin, and gentamycin. Inoculated petri plates were then incubated at 25 ± 1°C. Three replicated plates of each biotype were removed at 24, 48, 72, 96, and 120 h of incubation. Each plate was washed with 3 successive rinses of 3 ml of sterile distilled water (a sterilized, angled-glass rod was used to aid in the removal of conidia), and the rinses from the 3 plates were pooled. A subsample of each pooled rinse was placed on a glass slide, and germinated and nongerminated conidia were counted under phase microscopy. A range of 250 to 750 conidia per biotype per incubation period was counted for each of three replicates. *Heliothis* larvae that had been dusted with conidia of either biotype were also examined with a scanning electronic microscope to determine whether germination had occurred on the cuticular surface.

The average percent germination of conidia for each of the incubation periods for the Ec biotype ranged from ca. 18 to 35% (Table 1). Germination of conidia for the Ms biotype ranged from ca. 3 to 92% (Table 1). Initial germination was most rapid in the Ec biotype; however, total germination was almost 3 X less than that of the Ms biotype. The rapid rate but low total germination seems to be an inherent trait of the Ec biotype. Other culture media (e.g. potato-dextrose-agar, Sabouraud-dextrose-agar, oatmeal-agar, cuticle + SMAY) did not increase total germination of the Ec biotype. The highest germination of conidia for the Ec biotype (34.9 ± 6.9%) occurred after only 24 h of

TABLE 1. Average percent germination[1] of an Ecuadoran and Mississippian biotype of *Nomuraea Rileyi*.

Incubation (Hours)[2]	Number Conidia Counted[3]	Percent Germination ($\bar{X}$ ± SEM)[4]
	Ecuadoran Biotype	
24	2204	34.9 ± 6.9 a
48	1605	24.3 ± 7.4 a
72	885	29.4 ± 5.1 a
96	761	32.5 ± 7.4 a
120	1031	18.2 ± 4.8 a
	Mississippian Biotype	
24	1889	3.4 ± 0.4 a
48	1618	16.6 ± 6.8 b
72	2184	56.6 ± 8.8 c
96	1792	91.8 ± 5.8 d
120	1078	89.7 ± 3.3 d

[1] ± Standard error of mean.
[2]Post-inoculation and incubation at 25 ± 1°C on SMAY media.
[3]250 to 750 spores counted per incubation period per replicate: 3 replicates per incubation period.
[4]Means with same letter are not significantly different (P>0.05). Separate analysis for each biotype. Means were compared using an ABSTAT™ program and Scheffe's test of significance at the 0.05 probability level (Anderson-Bell, Parker, CO 80634).

incubation at 25 ± 1°C: the highest percent germination of conidia for the Ms biotype (91.8 ± 5.8%) occurred after 96 h of incubation (Table 1). Mycelia were initially observed in the Ec and Ms biotypes after 64 ± 8 and 88 ± 8 h of incubation, respectively. Of those conidia that germinated, 10% and 90% germinated within 3 and 96 h, respectively, for the Ec biotype, and within 48 and 110 h for the Ms biotype.

Although morphologically identical, these two geographical isolates of *N. rileyi* have distinctly different germination patterns. The Ecuadoran isolate reaches maximum germination (ca 30%) within 24 h. In contrast, maximum germination (90%) of the Mississippian isolate occurs 72 h later. Even though the biotype will not infect *H. virescens* its conidia will germinate and develop penetration hyphae on the cuticular surface of living larvae. Thus, the inability of the biotype to infect *H. virescens* can not be attributed to a slower rate of germination *per se* and factors other than germination rate must be operative. Since conidia can germinate on intact cuticle, and larvae of *H. virescens* are susceptible to intrahemocoelic injections of *N. rileyi* (Ignoffo & Garcia 1985), resistance of *H. virescens* probably resides within the cuticle *per se*.

References Cited

BOUCIAS, D. G., AND J. C. PENDLAND. 1984. Nutritional requirements for conidial germination of several host range pathotypes of the entomopathogenic fungus *Nomuraea rileyi*. J. Invertebr. Pathol. 43: 288-292.

BOUCIAS, D. G., D. L. BRADFORD, AND C. S. BARFIELD. 1984. Susceptibility of the velvetbean caterpillar and soybean looper (Lepidoptera: Noctuidae) to *Nomuraea rileyi*: Effects of pathotype, dosage, temperature, and host age. J. Econ. Entomol. 77: 247-253.

GARDNER, W. A., R. M. SUTTON, AND R. NOBLET. 1977. Persistence of *Beauveria bassiana*, *Nomuraea rileyi*, and *Nosema necatrix* on soybean foliage. Environ. Entomol. 6: 616-618.

IGNOFFO, C. M. 1981. The fungus *Nomuraea rileyi* as a microbial insecticide, *in* H. D. Burges [ed.], Microbial control of pests and plant diseases 1970-1980. Academic Press. 914 pp.

IGNOFFO, C. M. AND C. GARCIA. 1985. Hosts spectrum and relative virulence of an Ecuadoran and Mississippian biotype of *Nomuraea rileyi*. J. of Invertebr. Pathol. 45: 346-352.

IGNOFFO, C. M., AND D. G. BOUCIAS. 1991. Relative activity of geographical isolates of *Nomuraea* bioassayed against the cabbage looper and velvetbean caterpillar. J. Invertebr. Pathol. (in press).

ROBERTS, D. W., AND A. S. CAMPBELL. 1977. Stability of entomopathogenic fungi. Misc. Publ. Entomol. Soc. Am. 10: 19-76.

END NOTE

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OUTBREAK OF VARIABLE OAKLEAF CATERPILLAR, *LOCHMAEUS MANTEO* (LEPIDOPTERA: NOTODONTIDAE) AT THE KATHARINE ORDWAY PRESERVE-SWISHER MEMORIAL SANCTUARY, PUTNAM COUNTY, FLORIDA

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The Katharine Ordway Preserve-Swisher Memorial Sanctuary is located in northwestern Putnam County near the southern end of a deep sand formation known as the Trail Ridge. The preserve is a complex of xeric upland, mesophytic, and wetland communities (Franz & Hall 1990). During early August 1990, I observed large numbers of variable oakleaf caterpillars (*Lochmaeus manteo* Doubleday) at several sites on the Ordway Preserve. This common eastern North American moth undergoes sporadic population outbreaks that may result in the extensive defoliation of deciduous forests (Hooker 1908, Staines 1977, and Wilson & Surgeoner 1979).

The caterpillars were most abundant in mesophytic hardwood forests adjacent to the larger lakes and in the Mill Creek Swamp. At Tucker's Pond, frass falling through the foliage sounded like light rain and formed a thin layer on the ground. Laurel oaks (*Quercus hemisphaerica* Bartram) in the Tucker's Pond hammock were in various stages of defoliation. Many canopy-level laurel oaks 12 to 15 meters tall were completely defoliated. Smaller (1-2 m) understory laurel oaks ranged from nearly uneaten to completely defoliated. The caterpillars seemed to avoid tender new leaves, but readily fed upon mature foliage. Laurel oaks and water oaks (*Quercus nigra* L.) were similarly defoliated in the hammock along the Mill Creek Swamp.

Variable oakleaf caterpillars were also found on laurel oaks and water oaks in the hammock along the eastern edge of Ross Lake. This infestation was limited mostly to canopy-level trees in a few areas of the hammock. Although the oaks at Ross Lake were not noticeably defoliated, the sound of falling frass indicated that large numbers of caterpillars were present. Outside of the preserve but in the same general area of