Stabilization of Conidium Morphology in Cultures of Alternaria longipes

E. K. Sobers and B. Doupnik, Jr.

Associate Professor of Plant Pathology, University of Georgia College of Agriculture Experiment Stations, Coastal Plain Station, Tifton 31794; and Assistant Professor of Plant Pathology, University of Nebraska, South Central Station, Clay Center 68933, respectively.

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ABSTRACT

Five of 58 isolates of *Alternaria longipes* pathogenic to tobacco retained their morphological identity and pathogenicity after 4 to 5 years in culture. Conidia produced by most of the remaining 53 isolates changed from the long, wide body (39.1 × 15.2 μ), and long-beaked (20.6 μ) type exhibited by pathogenic isolates, to type II conidia with short, narrow bodies (25.3 × 10.8 μ), and short beaks (5.9 μ). The majority of 79 isolates that were initially nonpathogenic exhibited a similar change in morphology to type II conidia. It is assumed that at least in culture, type II conidia represent the most stable form of *A. longipes*.

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One hundred thirty-seven single conidium isolates of *Alternaria longipes* (Ell. & Ev.) Mason, causal agent of brown spot of tobacco, were acquired in 1966 and 1967. These isolates were studied morphologically from cultures, tested for pathogenicity to tobacco leaves, and for toxicity to experimental animals. The results of these studies indicated that an apparent relationship exists between conidium morphology and pathogenicity to tobacco leaves (4, 6), that isolates of *A. longipes* are toxic to chicks, ducks, and turkeys (1, 2, 3), and that a probable relationship exists between pathogenicity to tobacco leaves and toxicity to chicks (5, 7). We also found that nonpathogenic isolates grow more rapidly in culture than pathogenic isolates, that loss of pathogenicity of isolates was frequently accompanied by changes in conidium morphology, and that at least four forms, one pathogenic and three nonpathogenic, of *A. longipes* can be distinguished by their conidium morphology (6).

The purpose of this report is to present information on changes that have occurred in pathogenicity and conidium morphology among 137 pathogenic and nonpathogenic isolates of *A. longipes* after 4 to 5 years in culture.

MATERIALS AND METHODS.—The 137 cultures of *A. longipes* used were derived from single conidia. Each isolation was made at random from a single, typical brown spot lesion on a leaf of one of 137 tobacco plants that included 17 cultivars and four breeding lines of flue-cured tobacco, three cigar filler cultivars, three cigar wrapper cultivars, and one cultivar of burley tobacco. Cultures of all isolates were maintained at 4 to 8 C on slants of Difco potato-dextrose agar and were transferred at 4-month intervals. Incubation and preparation of inoculum for pathogenicity tests was the same as that described in a previous report (6).

Cultures were examined after 6-days' growth on V-8 juice agar. Designation of conidium type is based on the following descriptions: (i) Pathogenic conidia—bodies are mostly cylindric to obclavate, or rarely ellipsoidal, 21.8-66.6 × 9.5-23.1 μ, average 39.1 × 15.2 μ; longitudinal septa 0-6, average 3.6; and beaks 0-61.2 μ, average 20.6 μ. Beaks on the average represent one-third to one-half the total conidium length, although it is not uncommon to find conidia with beaks as long or longer than the conidium body. (ii) Type I conidia—bodies are mostly cylindric, 20.4-63.9 × 8.2-19.0 μ, average 36.7 × 10.2 μ; longitudinal septa 0-3, average 0.7; and beaks 0-19.0 μ, average 5.1 μ. (iii) Type II conidia—bodies mostly cylindric, 12.2-44.9 × 8.2-17.7 μ, average 25.3 × 10.8 μ; longitudinal septa 0-3, average 0.7; and beaks 0-20.4 μ, average 5.9 μ. (iv) Type III conidia—bodies mostly obclavate, 17.4-42.2 × 9.5-20.4 μ, average 27.3 × 15.5 μ; longitudinal septa 1-6, average 3.1; and beaks 0-13.6 μ average 4.2 μ.

RESULTS AND DISCUSSION.—After 4 to 5 years, only 5 of 58 pathogenic isolates were still able

to infect tobacco leaves. These five isolates continued to produce conidia characteristic of pathogenic isolates, whereas the 53 that lost their pathogenicity exhibited conidia (type I, II, and III) typical of nonpathogenic isolates (6). Two of the isolates had type I conidia, 36 had type II conidia, one had type III conidia, and 14 isolates were nonsporulating. Prior to becoming nonsporulating, however, 12 of the 14 isolates had already changed morphologically and were producing type II conidia. The two remaining isolates had exhibited type I conidia.

Similar changes were noted among 79 isolates that were nonpathogenic when isolated. Of 55 initially identified as having type I conidia, six remained type I, 30 isolates produced type II conidia, and 19 were nonsporulating. Fifteen of the 19 nonsporulating isolates exhibited type II conidia, and four had type I conidia before losing their ability to sporulate.

Eleven of 16 isolates with type II conidia initially continued to produce type II conidia. The remaining isolates had type II conidia prior to becoming nonsporulating.

All of the eight isolates originally with type III conidia changed to type II. Two of these isolates eventually became nonsporulating.

All nonpathogenic isolates remained nonpathogenic during the 4 to 5 year period of observation. In no instance was there any evidence of a change from nonpathogenic conidial types to that of the type associated with pathogenic isolates, or of a loss of pathogenicity without a change in morphology to one of the three nonpathogenic conidial types.

When initial conidium morphology of the 137 isolates is compared with that after 4 to 5 years in culture (Table 1), a distinct shift to cultures that produce type II conidia is noted. Whereas 11.7% (16) of the isolates were initially type II, 60.7% (83) were type II 4 to 5 years later, or 85.5% (117) when 34 of the 40 nonsporulating isolates that had changed to type II prior to losing their ability to sporulate were included.

Since such a large percentage of the isolates changed to cultures that produced type II conidia, and all of the cultures with type II conidia originally retained their morphological identity without change for 4 to 5 years, we conclude that cultures producing type II conidia represent the most stable sporulating form of *A. longipes*.

In attempting to explain these morphological changes, we are confronted with the fact that immediately after single conidium isolations were made, pathogenic cultures and, to a lesser extent, nonpathogenic cultures contained a small percentage of one or more of the nonpathogenic conidial types. That the presence of these forms represented more than expected morphological variation was not apparent until increasing numbers of these conidia (mostly type II) were discovered in pathogenic cultures that had decreased significantly in virulence. It was first thought that the increase in numbers of these conidia was due to the fact that mycelia from nonpathogenic conidia grew almost twice as fast as those from pathogenic conidia, and successive transfers from the periphery of these colonies inadvertently favored propagation of the nonpathogenic forms. When similar changes were found among nonpathogenic cultures, it was evident that changing morphology could not be explained entirely by differences in growth rate because there was no essential difference in the rate of growth of isolates of the three nonpathogenic types.

In the absence of further data, we suggest that these changes are genetic in nature, and will require further detailed study.

TABLE 1. Morphology of 137 single conidium isolates of *Alternaria longipes* on initial isolation and after 4 to 5 years in culture

	Number and percentage of isolates	
	Initial isolation	After 4 to 5 years
Pathogenic	58 (42.3%)	5 (3.6%)
Nonpathogenic[a]	79 (57.7%)	132 (96.4%)
Type I	55 (40.2%)	8 (5.8%)
Type II	16 (11.7%)	83 (60.7%)
Type III	8 (5.8%)	1 (0.7%)
Nonsporulating		40 (29.2%)[b]

[a] Type I, II, and III (6).

[b] Thirty-four of these isolates were type II before they became nonsporulating.

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