Relationship of Pathogenicity to Tobacco Leaves and Toxicity to Chicks of Isolates of *Alternaria longipes*

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One hundred thirty-seven single-conidium isolates of *Alternaria longipes* were tested for pathogenicity to tobacco leaves and for toxicity to 1-day-old chicks. Of 58 isolates pathogenic to tobacco, 43 (74.3%) had a significant effect on test chicks. Of 79 nonpathogenic isolates, 59 (74.7%) were nontoxic, 7 were toxic, and 13 were lethal. A relationship between pathogenicity and toxicity is suggested.

Toxicity of species of *Alternaria* to experimental animals and man has been established. Joffe (5) reported that cultures of *A. humicola* Oud. and *A. alternata* (Fries) Keissler (=*A. tenuis* Auct.) isolated from overwintered small grains that had been the source of several outbreaks of moldy grain toxicosis in man in the USSR during and shortly after World War II were toxic to experimental animals. Forgacs et al. (3) isolated an unidentified species of *Alternaria* from feed and litter that caused mycotoxicosis in chickens, and Forgacs and Carll (2) found that smoke aerosol generated from hay on which a species of *Alternaria* was grown caused pulmonary emphysema and other pathological changes in mice. Doupnik and Sobers (1) described an experimentally induced mycotoxicosis in ducks, turkeys caused by a metabolite produced by single-conidium cultures of *A. longipes* (Ell. & Ev.) Mason, the causal organism of brown spot of tobacco (Doupnik and Sobers, Bull. Ga. Acad. Sci. 26: 58, 1968; Phytopathology 58:1048–1049, 1968). Sobers (Phytopathology 58:731, 1968) and Sobers and Doupnik (6) indicated that a relationship apparently exists between conidium morphology and pathogenicity of isolates of *A. longipes* to tobacco leaves, and they (Sobers and Doupnik, Phytopathology 58:1068, 1968) suggested that pathogenic isolates of *A. longipes* were more likely to be lethal or toxic to chicks than were nonpathogenic isolates. Hamilton et al. (4) showed that 30 (78.9%) of 38 *Alternaria* isolates from uncured tobacco leaves infected with *A. alternata* (*A. tenuis* Nees) and 128 (60.4%) of 212 *Alternaria* isolates from cured tobacco leaves were toxic when cultures of these isolates were homogenized and injected into the peritoneal cavity of mice.

The primary purpose of this study was to determine the extent of the relationship of pathogenicity to tobacco leaves and toxicity to chicks of single-conidium isolates of *A. longipes*. Secondary objectives were to substantiate further the relationship between conidium morphology and pathogenicity, and to determine whether there were differences in the effects of the three conidial types of non-pathogenic isolates on chicks.

**MATERIALS AND METHODS**

** Cultures.** The 137 cultures of *A. longipes* used in pathogenicity and toxicity studies were derived from single-conidium isolations. Each isolation was made at random from a single, typical brown-spot lesion on leaves of one of 137 tobacco plants that included 17 varieties and four breeding lines of flue-cured tobacco, three cigar filler varieties, two cigar wrapper varieties, and one variety of burley tobacco. Cultures of all isolates were maintained at 4 to 8 C on slants of Difco potato-dextrose-agar (PDA), and were transferred at 4-month intervals.

Conidium morphology of each isolate was determined after 12 days of growth on V-8 juice-agar (V-8A). All designations of conidium type are based on descriptions presented in a previous study (6). Cultures to determine conidium morphology and for pathogenicity and toxicity tests were started at the same time to minimize variation.

** Pathogenicity tests.** Cultures used in preparing inoculum for pathogenicity tests were grown at 23 to 29 C on V-8A under continuous light provided by two 40-w F40D or F40D/3 Ken-Rad daylight fluores-
cent tubes suspended 30 cm above the surface of the cultures. When the cultures were 7 days old, they were scraped with a square-tipped spatula. Conidia from each plate were suspended in 5-ml portions of distilled water 5 days later. Suspensions containing 0.05 ml of Triton B-1956 (active ingredient, 77% modified phthalic glyceryl alkyd resin) for each 30 ml of inoculum were blended for 30 sec, filtered through a single thickness of cheesecloth, and adjusted to contain a maximum of 30,000 conidia per ml. A solution containing 0.05 ml of Triton B-1956 per 30 ml of distilled water was prepared for spraying on leaves of control plants. Each of the 137 isolates were tested for pathogenicity to ten 3- to 5-month-old Coker 187-Hicks tobacco plants. All test and control plants were maintained in a mist chamber for 48 hr after the inoculum was applied to the leaves, and then were placed in a greenhouse where temperatures varied from 21 to 34 C.

Toxicity tests. Rations for toxicity tests were prepared as follows. Fernbach flasks containing six parts (500 g) of cracked corn and five parts (417 ml) of distilled water were autoclaved for 1 hr each on consecutive days. Discs (10 mm) of each isolate from 12-day-old cultures grown on V-8A were placed in flasks and incubated at room temperature (23 to 29 C) under continuous fluorescent light for 14 days. The flasks were shaken each day to prevent mycelial matting. At the end of the growth period, the contents of each flask were removed, dried at 50 C for 15 to 18 hr, ground, and mixed with a 36% protein supplement, 6:4 (v/v). Approximately 1,500 g of the corn-fungus mixture was required for ad libitum feeding to groups of ten 1-day-old Babcock B-300 cockerels for 14 days. Control chicks of the same age and number received sterile corn that was similarly treated and mixed. Weights were recorded for each group of chicks at the start of the test and for those that survived the test at the end of 7 and 14 days. All birds were sacrificed, and a gross examination was made at the end of the test period. Those that died during the test were examined as soon after death as possible.

Isolates referred to as lethal in this study are those that caused death of 50% or more of the chicks in the group to which they were fed. Toxic isolates are those that caused death of less than 50% of the birds to which they were fed, those that suppressed weight gain by 20% or more of the control birds, or those that had both effects. This level of weight suppression was selected as significant because all but one of the lethal isolates caused weight suppression of 20% or more of the control.

RESULTS AND DISCUSSION

Pathogenicity tests. Fifty-eight (42.3%) of the 137 single-conidium isolates of A. longipes were pathogenic to leaves of 3- to 5-month-old Coker 187-Hicks tobacco plants. Lesions were apparent 2 to 4 days after conidial suspensions were applied to the leaves, and typical brown-spot lesions were evident 3 weeks after inoculation.

Conidium morphology. All pathogenic isolates were distinguished morphologically from nonpathogenic isolates. Conidia produced after 12 days of growth on V-8A exhibited long beaks, whereas beaks of nonpathogenic isolates were 70 to 80% shorter.

Conidia of the 79 nonpathogenic isolates were classified as being one of three types based on length, width, and shape. Fifty-five isolates exhibited type I conidia (long, narrow, and cylindrical), 16 produced type II conidia (short, narrow, and cylindrical), and eight had type III conidia (short, wide, and obclavate). A comparison of the four types of conidia produced by A. longipes is given in Table 1.

These results substantiate those of a previous study (6) and indicate a relationship between conidium morphology and pathogenicity.

Toxicity tests. A relationship between pathogenicity and toxicity is suggested by the following results. Forty-three (74.2%) of 58 pathogenic isolates had a significant effect on 1-day-old chicks; 34 (58.7%) isolates were lethal, 9 (15.5%) were toxic, and 15 (25.7%) had no effect on test birds. By comparison, only 20 (25.3%) of the 79 nonpathogenic isolates had a significant effect on test chicks. Of the 79 nonpathogenic isolates, 55 were type I; these included 9 lethal isolates, 7 that were toxic, and 39 that had no effect. None of the type II isolates was lethal or toxic. Four of eight type III isolates were lethal, and four had no effect (Table 2).

Comparing the average per cent kill for all pathogenic isolates with that of all nonpathogenic isolates shows a 51.2% average kill as compared with an 18.7% kill for nonpathogenic isolates. The average per cent weight of control of surviving birds was -20.5% for pathogenic isolates and -1.1% for nonpathogenic isolates.

The effect of nonpathogenic isolates on chicks based on conidium type shows the average kill for type I isolates as 18.6%, 0.6% for type II isolates, and 56.3% for type III. The average effect on per cent weight of control was -1.7% for type I isolates, +1.4% for type II, and -5.2% for type III. These data show that the average kill levels for all pathogenic isolates fall in the lethal and toxic group designations, and that the average nonpathogenic isolate falls into the no-effect group. On an individual basis, all of the nonpathogenic types fall into the no-effect group, with the exception of type III isolates which show a 56.3% kill. The reason for the high average kill in this group is not known, but may reflect the small number of isolates of this type.

The performance of individual isolates on test birds with respect to per cent kill and per
Pathogenic
Nonpathogenic

and valid.

birds revealed the

died before the

proventriculous described.

Isolates that killed 100% of the test birds (nine pathogenic, seven type I, and two type III) are not shown because most died before the 7-day weights were made. The few that survived through the 7th day were so badly dehydrated because of their inability to get water that such weights were deemed invalid.

Gross examination of dead and sacrificed birds revealed changes in the ventriculous and proventriculous similar to those previously described (1).

Because of the high percentage of pathogenic isolates of *A. longipes* that are toxic to chicks, the significance of lesions due to *A. longipes* on marketed tobacco leaves should be reassessed.

**LITERATURE CITED**

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