

## SHORT PAPERS

### SOUTHERN CORN LEAF BLIGHT: GENETIC CONTROL OF PATHOGENICITY AND TOXIN PRODUCTION IN RACE T AND RACE O OF *COCHLIOBOLUS HETEROSTROPHUS*\*

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TWO physiologic races, T and O, of *Helminthosporium maydis* Nisikado and Miyake (perfect stage; *Cochliobolus heterostrophus* Drechsler) cause southern corn leaf blight (SMITH, HOOKER and LIM 1970). The two races are morphologically similar but race T is specifically pathogenic to corn containing [cms-T] (Texas male-sterile) cytoplasm while race O is not. Race T produces a pathotoxin in culture that is highly toxic only to susceptible [cms-T] corn (HOOKER *et al.* 1970), whereas race O produces only a small amount of a toxin non-specific to cytoplasms. The fungus is heterothallic and the perfect stage can be obtained by matings of compatible isolates (NELSON 1957). A study of intra-specific crosses among three wild-type isolates of *C. heterostrophus* from various geographical areas showed that toxin production amount is under genetic control (SMEDEGARD-PETERSON and NELSON 1969).

The unexpected invasion of the race T into most of the corn growing areas of the U.S. in 1970 caused significant losses in yield and reduced seed supplies for the 1971 crop. Race O which has been prevalent for many years throughout the corn growing area in the southern part of the U.S. has rarely caused serious losses. An understanding of the inheritance of pathogenicity and toxin production in race T and O of *H. maydis*, as given in this paper, should contribute to studies of epidemiology, host resistance, and pathogenicity.

Monoconidial isolates for both race T and O were obtained from infected corn leaves collected in Illinois. For ascospore production, senescent corn leaves, 4 × 2 cm, were autoclaved and placed in 90 × 15 mm plastic dishes containing 20 ml of sterile SACH'S nutrient agar. Inocula of race T and race O of different compatibility mating types were placed on the agar on opposite sides of the leaf section. The matings were incubated at 24°C for 25–30 days in the dark. Two or three mature perithecia, produced on the corn leaf tissue, were collected randomly from each of 25 matings and bulked in 1 ml of water. The perithecia were agitated to release ascospore from the asci and individual ascospores were transferred to potato-dextrose agar plates. A total of 82 single ascospore cultures were obtained.

Pathogenicity of parental and ascospore isolates was determined on susceptible (P-A-G SX 29 [cms-T]) and resistant (P-A-G SX 29) corn seedlings in the

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greenhouse. The two hybrids are genetically similar except for the [cms-T] cytoplasm of the susceptible hybrid. For inoculation, conidial suspension, adjusted to 10,000–15,000/ml, were sprayed onto corn seedlings in the fifth to sixth leaf stage and incubated for 16 hr in a moist chamber. Seedling reactions were scored 7 to 12 days after inoculation. Forty-five ascospore isolates exhibited the host-specific pathogenicity to plants with [cms-T] cytoplasm like the parental isolate of race T. Thirty-seven isolates did not show this host-specific pathogenicity; lesions on both resistant and susceptible plants were similar to the lesions incited by race O or incited on resistant plants by race T isolates. The segregation fits a 1:1 ratio ( $\chi^2 = 0.78$ ;  $P = 0.30-0.50$ ).

It was evident that the degree of pathogenicity based on lesion size differed both within the race T type isolates and within the race O type isolates. A few ascospore isolates which exhibited specific pathogenicity to the [cms-T] corn seedlings produced larger and a few produced smaller lesions on the susceptible plants than did the parental isolates of race T. Similar variations in the size of lesions on both resistant and susceptible plants were observed among the ascospore isolates which showed no specific pathogenicity to seedlings of the two cytoplasm types. This suggests that degrees of pathogenicity within each race may be quantitatively controlled by other genes.

The parental and ascospore isolates were assayed for the host-specificity and the amount of pathotoxin produced. All isolates were grown in 125 ml Erlenmeyer flasks containing 50 ml of FRIES modified medium supplemented with 1% yeast extract and adjusted to pH 4.0 with 5% HCl. Each flask was seeded with a sporulating culture grown for 8–10 days on potato-dextrose agar. Seeded flasks were incubated at 24°C for 18–22 days; then culture solutions were filtered to remove fungus growth. All culture filtrates were diluted with deionized distilled water in a 1:10 ratio (v:v). Previous studies had shown that more concentrated culture filtrates often did not give a differential reaction with corn seedlings due to the salt concentration in the medium.

For the seedling bioassay, seeds of susceptible and resistant corn, soaked in distilled water for 8 hr at 24°C, were placed embryo side down on moist paper in 150 × 20 mm Petri dishes and allowed to germinate for 24 to 48 hr. Germinated seeds with 5 mm of primary root length were selected for use in the bioassay. Twenty ml aliquots of the diluted culture filtrate from each fungal isolate were placed in 90 × 15 mm plates. Five germinated seeds of susceptible and of resistant corn were placed embryo side down in the filtrate and allowed to grow for 48 hr at 24°C in the dark. The length of the primary root of each seedling was then measured.

Thirty-eight ascospore isolates and the parental race T produced a host-specific toxin which markedly inhibited root elongation of susceptible corn compared with the root elongation of resistant corn. Using this criterion, 44 ascospore isolates and the parental race O did not produce the host-specific pathotoxin. Root elongation of both susceptible and resistant corn was equally inhibited in these non-specific pathotoxin filtrates. The inhibition was much less than that of susceptible but greater than that of resistant corn in race T pathotoxin. This segregation fits a 1:1 ratio ( $\chi^2 = 0.44$ ;  $P = 0.50-0.70$ ).

The amount of pathotoxin produced by all isolates was determined on the basis of percentage inhibition of root elongation in a pathotoxin filtrate compared with root length in a sterile culture solution. Roots of susceptible and resistant seedlings were 29.1 and 30.2 cm (mean of 5 seedlings) long, respectively, after 48 hr incubation in the sterile culture solution used as a check. The percentage of inhibition in root elongation of susceptible corn ranged from a low of 62.0% to a high of 98.7% for the 38 pathotoxin filtrates showing host specificity. The pathotoxin filtrate of the parental isolate of race T inhibited root elongation by 88.1%. With 44 of the non-specific toxin filtrates, the percentage inhibition of root elongation of resistant corn ranged from 53.3% to 86.3%. Similar results were obtained with susceptible corn. The toxin filtrate obtained from the parental isolate of race O inhibited root elongation by 61.5%. "End-point dilution" assays of the toxin filtrates showed that host-specific toxin preparations ranged from 1:80 to 1:180 (parental race T was 1:160). The non-host-specific toxin preparations ranged from 1:15 to 1:40 (parental race O was 1:20). These results suggest that the amount of toxin produced by race T and race O isolates may be quantitatively inherited.

All ascospore isolates which produced the host-specific pathotoxin exhibited the host-specific pathogenicity to the susceptible and resistant plants. Seven of the 44 ascospore isolates, however, which were classified as not producing the host-specific pathotoxin in the root bioassay test exhibited host specificity in their pathogenicity to susceptible and resistant plants. This discrepancy in classification may be attributed to limitations in the pathotoxin assay utilizing seedling roots. These isolates may have produced too low a concentration of the host-specific pathotoxin to be detected or the salt concentration in the filtrates may have interfered with the specific reaction of seedling roots to the pathotoxin.

#### SUMMARY

Segregations among ascospore progeny obtained from the cross of Illinois isolates of race T with race O showed that both the selective pathogenicity and the specific pathotoxin production of race T to plants with [cms-T] cytoplasm for male sterility are monogenic in inheritance. The severity of pathogenicity expressed and the amount of pathotoxin produced may be polygenic in inheritance. The two characters are highly associated.

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