# The Inheritance of Factors in Cochliobolus sativus Conditioning Lesion Induction on Gramineous Hosts

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ABSTRACT

Sixty-three monoascosporic cultures were derived from crossing two isolates of *Cochliobolus sativus*, one pathogenic and one nonpathogenic to six gramineous species. Progeny cultures were tested for pathogenicity to the differentially reacting grass species, and seven different genes for lesion induction were identified. The assumption of pathogenic specificity conditioned by different genes seems valid because individual ascospores differed in pathogenicity to different testers, and recombinant phenotypes representing recombinant genotypes were observed on all paired combinations of the six tester grasses. Pathogenicity to a given species was simply inherited and dependent upon one or two genes. Phytopathology 61:1052-1054.

*Cochliobolus sativus* (Ito & Kurib.) Drechs. ex Dast., the incitant of spot blotch of barley, has been reported as pathogenic to a number of gramineous species (1). Isolates of *C. sativus* can be grouped in distinct pathogenicity types based upon reactions on differential gramineous species. This grouping suggests that a number of major genes condition pathogenicity to these grasses. A complex of pathogenicity genes has been shown to condition disease development in gramineous hosts in two related *Euhelminthosporium* species, *Cochliobolus carbonum* Nelson and *Cochliobolus heterostrophus* Drechs. (2, 3, 4). The observed qualitative differences in pathogenicity have usually been conditioned by one or two genes.

The objective of the study was to identify the number and possible relationships of genes conditioning pathogenicity of two isolates of *C. sativus* that differed in pathogenic reactions on six gramineous hosts. Sixty-three monoascosporic progeny cultures from a cross of the two isolates were tested for pathogenicity.

MATERIALS AND METHODS.—The two parental *C. sativus* isolates, CS7 and CS8, were isolated from *Hordeum vulgare* L. collected in Canada. Isolate CS7 induced a lesion reaction and CS8 induced no more than minute necrotic pits on each of the six gramineous hosts.

Gramineous hosts used as differentials included *Cynodon dactylon* (L.) Pers., *Dactylis glomerata* L., *Eleusine indica* (L.) Gaertn., *Panicum virgatum* L., *Phalaris stenoptera* Hack., and *Poa pratensis* L. Infection tests were conducted in an air-conditioned greenhouse maintained at ca. 24 C. Although there were differences among host species in numbers of plants per pot and in height and age of plants when inoculated, the plants of each species were comparable in each infection trial.

Sixty-three monoascospore progeny cultures were obtained from mating CS7 × CS8 in the following manner: 2-mm-diam mycelial plugs taken from actively growing cultures of each parent isolate were ground together in 1 ml sterile distilled water. The suspension was flooded over sterilized barley grains partially embedded in Sach's nutrient agar in petri dishes. Pairings were stored for 28 days in the dark at ca. 24 C. Single ascospores were isolated at random and maintained on tube slants of potato-dextrose agar (PDA).

Inoculum for infection tests consisted of a water suspension of spores and mycelial fragments obtained by scraping the surfaces of cultures grown for 7 to 14 days on PDA in petri dishes in the dark at 24 C. Inoculum was sprayed onto the test plants with a small hand-sprayer operated at a constant 10 psi. Inoculated plants were placed in a moisture chamber for 48 hr and placed in the air-conditioned greenhouse at 24 C. Disease reactions were evaluated 7 days after inoculation.

The parental isolates caused qualitatively distinct reactions on the leaves of test plants. The lesion reaction was characterized by the presence of well-defined, necrotic lesions with distinct limited borders. In the nonlesion reaction, no macroscopic evidence of disease development was apparent beyond the occasional observation of minute necrotic pits. The reactions expressed were essentially independent of inoculum concentration. The two reaction types were judged to be sufficiently stable and distinct to permit their use as genetic characters. Test species were selected that clearly showed differential, nonlesion reactions. All reactions were confirmed at least twice, and additional tests were performed when necessary to determine conclusively the reaction type of individual ascospore isolates.

RESULTS AND DISCUSSION.—Parent isolate CS7 induced a lesion reaction, and CS8 incited a nonlesion reaction on each tester. The ratios of nonlesion to lesion reactions on five hosts fit the 1:1 ratio expected if the parent isolates differed by one gene for pathogenicity on each of these hosts (Table 1). The ratio of progenies for nonlesion:lesion on *P. stenoptera* fit the 1:3 ratio expected if the isolates differed by two genes for pathogenicity on that host; and the lesion allele at either locus results in a lesion reaction.

TABLE 1. Segregations for lesion induction on six grass hosts by 63 ascospore progeny from a cross between *Cochliobolus sativus* isolates CS8 and CS7, respectively; nonpathogenic and pathogenic to the six grasses

| Differentially reacting grass hosts | No. progeny | | Expected ratios | | | $P$ values from $X^2$ test |
|---|---|---|---|---|---|---|
| | Nonlesion | Lesion | Nonlesion | | Lesion | |
| *Cynodon dactylon* | 27 | 36 | 1 | : | 1 | .30-.20 |
| *Dactylis glomerata* | 29 | 34 | 1 | : | 1 | .70-.50 |
| *Eleusine indica* | 28 | 35 | 1 | : | 1 | .50-.30 |
| *Panicum virgatum* | 27 | 36 | 1 | : | 1 | .30-.20 |
| *Phalaris stenoptera* | 17 | 46 | 1 | : | 3 | .80-.70 |
| *Poa pratensis* | 30 | 33 | 1 | : | 1 | .80-.70 |

The model genotype for factors conditioning nonlesion-lesion reactions on *C. dactylon* can be represented: $Cy_1$ (CS7) $\times$ $cy_1$ (CS8) yielding progeny with 0.5 bearing the lesion allele $Cy_1$ and 0.5 bearing the nonlesion allele $cy_1$. Similar models can be drawn for reactions on *D. glomerata, E. indica, P. virgatum,* and *P. pratensis*. Factors conditioning reactions on *P. stenoptera* can be represented: $Ph_1Ph_2$ (CS7) $\times$ $ph_1ph_2$ (CS8) yielding progeny of parental types $Ph_1Ph_2$, $ph_1ph_2$, and recombinant types $Ph_1ph_2$, $ph_1Ph_2$ in a 1:1:1:1 ratio. Because the lesion allele at either locus results in a lesion reaction, the progeny would be expected to segregate 3 lesions:1 nonlesion. The model genotype for the cross for pathogenicity to the six testers can be represented $Cy_1Da_1El_1Pa_1Ph_1Ph_2Po_1 \times cy_1da_1el_1pa_1ph_1ph_2po_1$.

The reactions of individual ascospore isolates on paired testers can be used to determine whether the gene(s) conditioning pathogenicity on one host is the same as the gene(s) conditioning pathogenicity on the other paired host. If the same gene controls pathogenicity to both testers, each ascospore culture would be identical to one of the parents in pathogenicity to the paired testers. The presence of recombinant types demonstrates that the genes are at different loci. The relative independence of two pathogenicity genes can be estimated from the ratio of frequencies of parental to nonparental types among progeny.

The numbers of ascospores in each of the two parent and recombinant phenotype classes of pathogenicity on the combinations of paired testers are recorded in Table 2. The probability values were obtained from the $X^2$ test for goodness of fit to a 1:1:1:1 ratio that would be expected if two independent genes condition pathogenicity to two testers, respectively.

The pathogenicity of 63 progeny on the paired testers, *C. dactylon* and *D. glomerata,* is representative of the results on all pairs of testers where pathogenicity to each is conditioned by one gene. Isolate CS7 induces a lesion reaction on the paired testers *Cynodon-Dactylis,* and its phenotype for pathogenicity to these two testers can be represented as L-L. CS8 does not induce a lesion on either host, and can be represented as NL-NL. If the same gene controls lesioning on the two testers, one-half the progeny would be expected to be L-L on both testers; one-half, NL-NL. The presence among progeny of phenotypes L-NL and NL-L, representing recombinant genotypes, show that two different genes condition lesion development on these two testers.

If the two genes that condition pathogenicity to *Cynodon* and *Dactylis* are independent, equal numbers of progeny would be expected in each of the parental and recombinant phenotype classes: L-L, NL-NL, L-NL, and NL-L. The preponderance of parental types (79%) suggests that the two genes are not independent. The distribution of progeny among the four phenotypic classes in each of the possible combinations of pairs among testers where lesion induction is monogenic show that five different genes condition the nonlesion reactions on the five different grass testers. The excess of parental phenotypes among the progeny suggests that the genes are not independent. The apparent linkage cannot be interpreted in terms of simple, linear

TABLE 2. Numbers of ascospores in four pathogenicity classes, based on the reactions of combination pairings of five tester species

| Paired testers | No. progeny with indicated pathogenicity on paired testers[a] | | | | Value of $P$ for 1:1:1:1 ratio |
|---|---|---|---|---|---|
| | Parent types | | Recombinant types | | |
| | L-L | NL-NL | L-NL | NL-L | |
| *Cynodon-Dactylis* | 28 | 21 | 8 | 6 | .005 |
| *Cynodon-Eleusine* | 28 | 20 | 8 | 7 | .005 |
| *Cynodon-Panicum* | 28 | 21 | 8 | 6 | .005 |
| *Cynodon-Poa* | 24 | 21 | 12 | 6 | .005 |
| *Dactylis-Eleusine* | 25 | 20 | 9 | 9 | .01-.005 |
| *Dactylis-Panicum* | 29 | 21 | 5 | 8 | .005 |
| *Dactylis-Poa* | 23 | 22 | 11 | 7 | .01-.005 |
| *Eleusine-Panicum* | 28 | 20 | 7 | 8 | .005 |
| *Eleusine-Poa* | 20 | 18 | 15 | 10 | .5-.250 |
| *Panicum-Poa* | 22 | 19 | 14 | 8 | .1-.05 |

[a] Pathogenicity on paired hosts hyphenated; L = lesion; NL = nonlesion.

genetic models, and this may be due to the relatively small number of progeny. Since each of seven pathogenicity genes obviously cannot be equidistant from each other, the tendency of the genes to remain associated may be due to some factor(s) other than linkage.

Two independent genes condition lesion induction on *Phalaris,* and the lesion allele at either locus is sufficient for lesion induction. The model genotype for factors conditioning lesion induction on *Phalaris* can be represented: $Ph_1Ph_2 \times ph_1ph_2$, yielding parental type ascospores and two recombinant types, $Ph_1ph_2$ (lesion) and $ph_1Ph_2$ (lesion). Whether these two genes are the same or different from the five previously discussed pathogenicity genes can be determined from the pathogenicity of progeny on *Phalaris* paired with the other five testers. The pathogenicity of progeny to the paired testers *Phalaris-Cynodon* is representative. A culture that does not induce lesions on *Phalaris* must be of the genotype $ph_1ph_2$. A lesion allele $Cy_1$ is essential to the induction of lesions on *Cynodon.* If $Cy_1$ were the same as $Ph_1$ or $Ph_2$, the recombinant phenotype that is nonpathogenic on *Phalaris* and pathogenic on *Cynodon* could not be recovered. However, three progeny that were nonpathogenic on *Phalaris* induced lesions on *Cynodon,* suggesting that $Ph_1$, $Ph_2$, and $Cy_1$ occur at different loci. The recovery of similar recombinant-type ascospores on the paired hosts, *Phalaris-Dactylis,* 3; *Phalaris-Eleusine,* 5; *Phalaris-Panicum,* 4; and *Phalaris-Poa,* 4 show that the two genes conditioning pathogenicity on *Phalaris* are different from the single genes shown to condition non-lesion-lesion reactions on these four testers.

The linkage between the two genes conditioning lesion induction on *Phalaris* and the single genes for lesion induction on the other testers cannot be precisely detected because the ascospore isolates that induce lesions on *Phalaris* could be $Ph_1Ph_2$, $Ph_1ph_2$, or $ph_1Ph_2$. Thus, the frequency of parent-type ascospore isolates that induce lesions on *Phalaris* cannot be determined from these data. The parent-type ascospores that are nonpathogenic on the two testers can be identified, and are in excess, in most instances, on paired testers. This suggests a degree of linkage between the single genes and one or both of the two conditioning reactions on *Phalaris.*

Seven different genes are identified in *C. sativus* that condition lesion induction on six gramineous species. Pathogenicity to a given species is simply inherited and dependent upon one or two genes. The detection of new pathogenic types among progeny of an interisolate cross provides a rationale for understanding pathogenic variability in nature. The number of possible recombinant types would be almost unlimited, as the seven identified genes for pathogenicity occur at different loci. Cross between isolates of the fungus differing in pathogenicity to grasses would result in a multiplicity of pathogenic types.

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