

## Punch Inoculation for Measuring Resistance of Corn Leaf Tissue to *Helminthosporium maydis*

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The authors thank Barbara Wooding for her technical assistance.

### ABSTRACT

The punch-inoculation method was used to inoculate corn seedlings with *Helminthosporium maydis* Nisik. & Miyake. Spots are crushed on corn leaves with a punch just hard enough to injure the tissue without punching out a hole. A spore suspension is brushed on the crushed spot and the inoculated plants are incubated in plastic bags for 24 hr. This technique can be used to measure accurately the relative resistance of leaf tissue of various corn lines to southern corn leaf blight within 48 hr after inoculation. The method can also be used to measure the relative resistance of tissues from leaves of different ages.

Phytopathology 64:429-430

*Additional key words:* Southern corn leaf blight.

Various methods have been used to test the susceptibility of corn lines to *Helminthosporium maydis* Nisik. & Miyake (*Cochliobolus heterostrophus* Drechs.). Generally, the plants are either sprayed with a conidial suspension (3) or else treated with a solution of toxin obtained from cultures of the fungus (2, 3).

The method reported here makes use of punch inoculation, a technique devised by Misawa for investigating rice blast (4). He crushed spots on rice leaves and inoculated the spots with a spore suspension of *Pyricularia oryzae* Cav.

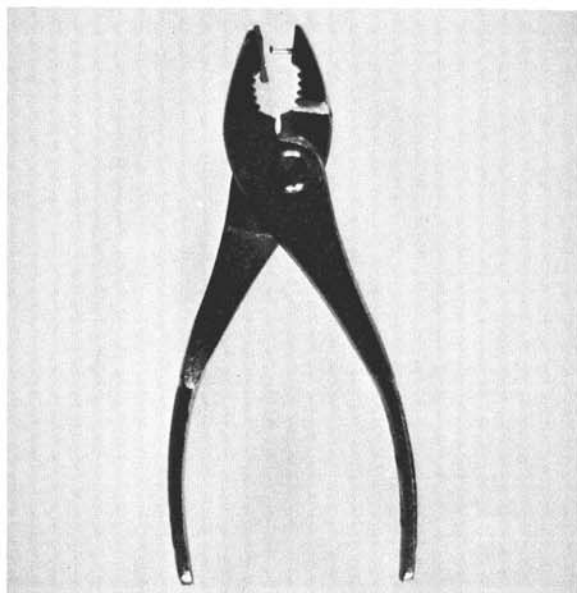


Fig. 1. Tool used for punch inoculation.

TABLE 1. Average lesion size (mm) 2 and 5 days after punch inoculation of lines of inbred SK2 corn with *H. maydis* (Race T). Fifth leaves were inoculated at five-leaf stage of growth. Each average represents five measurements on each of three plants<sup>z</sup>

Cytoplasm	Lesion diam (mm)	
	2 days	5 days
N	4.3 × 2.6 a	7.4 × 3.2 a
T	8.7 × 3.8 b	24.9 × 5.7 b
TRf	7.2 × 3.7 b	19.6 × 4.7 c

<sup>z</sup> Numbers in each column followed by different letters differ significantly at  $P = .05$  using Duncan's multiple range test.

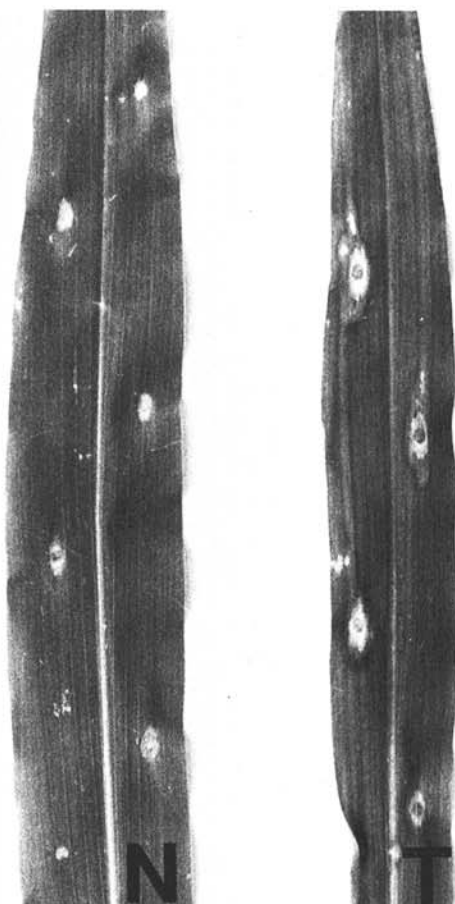


Fig. 2. Lesions on the fifth leaves of N (left) and T (right) cytoplasm SK2 corn 48 hr after punch inoculation of the leaves with *H. maydis* Race T at the five-leaf stage of growth.

The punch used to crush spots on the corn leaves was made by forcing a headless, flat-tipped nail partially into a hole on the inner surface of one jaw of a pair of pliers and gluing a rectangle of thin hardboard to the inside surface of the opposite jaw (Fig. 1). The spot to be inoculated was crushed with the punch just enough to injure the tissue but not hard enough to punch out a hole.

TABLE 2. Average lesion size (mm) 4 days after punch inoculation of N and T lines of three different corn inbreds with *H. maydis* (Race T). Fifth leaves were inoculated at 5-leaf stage of growth. Each average represents five measurements on each of three plants<sup>z</sup>

Inbred	Lesion diam (mm)	
	N-cytoplasm	T-cytoplasm
OH43	6.0 × 3.1 a	15.9 × 4.4 b
WF9	9.1 × 3.6 a	18.3 × 4.9 bc
SK2	9.3 × 3.4 a	19.6 × 4.3 c

<sup>z</sup> Numbers followed by different letters differ significantly at  $P = .05$  using Duncan's multiple range test.

TABLE 3. Average lesion size (mm) on leaves of different ages 2 and 8 days after punch inoculation of N and T lines of inbred SK2 corn with *H. maydis* (Race T). Plants inoculated at six-leaf stage. Each average represents five measurements on each of five plants

Days after inoculation	Cytoplasm	Leaf		
		6th	5th	4th
2	Na	3.7 × 2.5	3.6 × 2.1	3.9 × 2.1
	T	7.1 × 3.1	6.5 × 3.1	7.1 × 3.8
8	Na,b	7.8 × 3.4	8.8 × 3.8	11.4 × 3.6
	T	31.0 × 5.3	31.4 × 5.1	dead

<sup>a</sup> N-cytoplasm significantly different from T-cytoplasm on both dates ( $P = .01$ ).

<sup>b</sup> The three leaves of N-cytoplasm at day 8 differ significantly from each other ( $P = .05$ ).

A spore suspension of *H. maydis* Race T, containing about 0.2-0.5% of carboxymethyl cellulose, was applied to the injured spot with a camel's-hair brush.

Texas male sterile (T), restored Texas male sterile (Tff), and normal (N) cytoplasm strains of the corn inbred lines (WF9, OH43, and SK2, respectively) were punch-inoculated at the 5th or 6th leaf stage of growth, and were kept in a closed plastic bag for the first 24 hr after inoculation.

Table 1 and Fig. 2 show that the difference in lesion size between T and N corn was very obvious within 2 days after inoculation. At that time, the lesions on both T and

TRf, the restored T, were significantly larger than the lesions on N corn, but were not significantly different from each other. Five days after inoculation the differences were even greater, and now the lesions on the T corn were also significantly larger than those on the Tff corn.

The lesions on the N lines of the inbreds SK2, OH43, and Wf9 (Table 2) did not differ from each other in size, but were all significantly smaller than the lesions on their T-cytoplasm counterparts. On T-cytoplasm, the lesions on SK2 were significantly larger than those on OH43, but the lesions on Wf9 could now be differentiated in size from those on the other two inbreds.

When *H. maydis* Race O was punch inoculated, we could detect no significant differences in size between the lesions that developed on T and N corn.

The punch-inoculation method works equally well with mycelial fragments instead of spores.

In rice blast, resistance to the pathogen increases with leaf age (1). Our results with southern corn leaf blight (Table 3) indicate that, if anything, the reverse is true. The effect of leaf age on lesion development would not be detected on either N or T cytoplasm SK2 within 48 hr after inoculation. However, differences did appear as the lesions continued to expand during the next 6 days. The lesions became significantly larger with leaf age on the N corn. On the T corn, the lower leaves were dead by the 8th day.

The precision of the punch inoculation method makes it useful for studying small differences in susceptibility of corn strains and corn tissue to *H. maydis*, and to other fungi.

#### LITERATURE CITED

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