

## Histopathology of Resistance in the Sorghum bicolor-Sphacelotheca reiliana Interaction

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### ABSTRACT

Hypodermic inoculation of five resistant sorghums with compatible monosporidial lines of *S. reiliana* resulted in three different host-parasite interactions. Early Hegari and F<sub>1</sub>'s having Early Hegari as the male parent were compatible with the pathogen for several days before becoming incompatible. The length of time before the host-parasite relationship became incompatible varied with incubation temp and the genotype of the female parent. The incompatible reaction was characterized by the disappearance of mycelium and the appearance of teliosporelike objects in the host.

Lahoma Sudangrass, White Kafir P.I. 48770, and kafir B65L-5427-3 had similar reactions to the monosporidial inoculum. In each entry, hyphal growth or sporidial germ tubes were present in the inoculation wound, but the pathogen did not grow into the host tissue. The reactions were not altered by different temp. Feterita Tx09 and F<sub>1</sub>'s involving this entry were characterized by the complete inhibition of sporidial development and fusion. This reaction was also stable over a range of temp.

A "squash" technique was developed to rapidly identify reaction type. *Phytopathology* 60:1365-1367.

Head smut became important to sorghum (*Sorghum bicolor* [L.] Moench) caused by *Sphacelotheca reiliana* (Kuehn) Clint. production in Texas when new susceptible hybrids were introduced in 1957 (3). Unlike the kernel smuts, head smut has not been successfully controlled by seed treatment. The use of resistant hybrids is the only present means of control. Fortunately, screening of available cultivars and breeding lines revealed many resistant entries (3). Every major sorghum group of economic importance (i.e. feterita, milo, hegari, and kafir) contained at least one resistant variety or line. The resistance from lines within the feterita group has been used and studied most extensively. Resistance is controlled by a single, dominant gene in Tx09 (D. T. Rosenow, *unpublished data*). Most varieties within the feterita group possess allelic genes for resistance. Inheritance of hegari resistance appears to be complex and controlled by genes nonallelic to that of the feteritas (D. T. Rosenow, *personal communication*). The inheritance of resistance within the other entries has not been determined.

At least two different host resistance mechanisms have been shown by field and greenhouse inoculation trials. Hybrids from a cytoplasmic-genic male-sterile kafir line were resistant in all field trials but fully susceptible when artificially inoculated at 3 weeks of age (R. A. Frederiksen, *unpublished data*). All other entries tested were resistant to both needle inoculation and field tests.

Studies to determine the mode of inheritance and the genetic homology in the various resistant sorghums have been initiated (D. T. Rosenow, *unpublished data*). Using the earlier data from the feteritas, hegari, and the male-sterile kafir, it could be predicted that the studies will show that the genes controlling the various sources of resistance are nonallelic. The question could then be raised as to the mechanisms for resistance conditioned by the various genes or gene complexes. Nonallelic genetic systems may control different resistance mechanisms.

The purpose of this study was to examine histopathologically the host-parasite interaction and demonstrate the existence of the different types of resistance expressed in various resistant host genotypes.

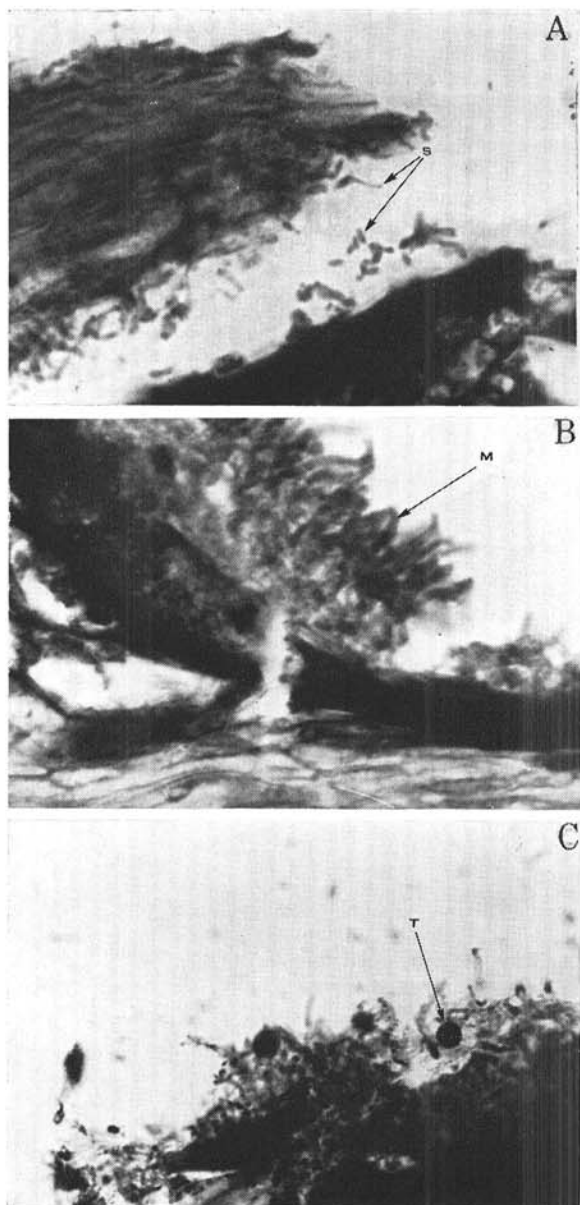
**MATERIALS AND METHODS.**—Varieties representing the sorghum groups chosen for this study and their reaction to the common race of head smut are shown as follows (R = resistant; S = susceptible). F<sub>1</sub> populations of resistant × susceptible varieties were included where possible. Combine Shallu SA394, S; Combine White Feterita Tx09, R; Early Hegari SA281, R; Lahoma FC32127, R; Martin A Tx398, S; Redlan A Tx378, S; Smut-Resistant Kafir B65L-5427-3, R (an experimental line); and White Kafir P.I. 48770, R.

The shoot apices were inoculated, fixed, and prepared for examination according to the methods described previously for 1-week-old seedlings (4). Specimens were stained with thionin and orange G.

The R-1 test was planted in the greenhouse on 18 June 1968. Temp during the period of growth ranged from an average min of 22 C to an average max of 30 C. A second major test, R-2, was planted 12 September 1968. This was a temp-control study of the effects of three different temp. The treatments were: (i) constant 30 C and 12 hr of artificial light; (ii) constant 18 C and 12 hr of artificial light; and (iii) variable greenhouse temp with an average min of 18 C to an average max of 31 C.

Early Hegari was used to determine the effect of plant age at time of inoculation on the host-parasite interaction. Plants 1, 2, and 3 weeks old were inoculated on the same date, and specimens of each taken at 3 and 6 days after inoculation. Tissue preparation was the same as previously described (4).

**RESULTS.**—Lahoma Sudangrass, White Kafir P.I. 48770, kafir B65L-5427-3, the feteritas, and all hybrids developed from these cultivars showed a similar histopathological reaction. Three days after inoculation, host tissues were free of mycelium. The sporidia, which had been injected hypodermically, were ob-



**Fig. 1.** Incompatible host-parasite interaction of *Sphacelotheca reiliana* in *Sorghum bicolor*. **A)** Nonmated sporidia (s) in Tx09 3 days after hypodermic inoculation ( $\times 1600$ ). **B)** Mycelium (m) in Lahoma Sudangrass 3 days after hypodermic inoculation ( $\times 1600$ ). **C)** Teliosporelike objects in wound of Early Hegari 9 days after inoculation ( $\times 1600$ ).

served in the needle wound. In the feterita entries there was no evidence of sporidial fusion. Some sporidia had short germ tubes, but further development was restricted (Fig. 1-A). Shoot apices from seedlings 3 days after inoculation were placed in 0.1% acid fuchsin in lactophenol and autoclaved at 122 C for 15 min. Squash preparations of these coleoptiles confirmed the fact that sporidial fusion failed to occur in Tx09 while the susceptible check (Combine Shallu) had extensive mycelial development 3 days after inocu-

lation. Short mycelial strands were observed in the inoculation punctures of the two resistant kafir entries and Lahoma Sudangrass (Fig. 1-B). There was no difference in reaction between the resistant inbred lines and their respective hybrids.

Although mycelial development did not occur in the inoculated meristematic tissue of Lahoma and Redlan ms (male-sterile)  $\times$  Lahoma F<sub>1</sub>, mycelium was observed in the lower regions of the differentiated leaf tissue.

In the June planting (R-1), Early Hegari sampled 3 days after inoculation had the same amount of mycelial growth as was found in the susceptible control, Combine Shallu. Six days after inoculation, the mycelium in Early Hegari had disappeared. Concurrent with the disappearance of the mycelium was the appearance of teliosporelike structures within the inoculation wound (Fig. 1-C). Three days after inoculation, Redlan ms  $\times$  Early Hegari F<sub>1</sub> had no mycelium but did have the teliosporelike structures, whereas Martin ms  $\times$  Early Hegari F<sub>1</sub> had neither mycelium nor the "teliospores". Mycelium in Combine Shallu, at first intracellular with no haustoria, became intercellular with haustoria by the 6th day after inoculation.

The *S. reiliana* relationship with Early Hegari grown in the greenhouse and in 30-C tests was compatible at 3 and 6 days after inoculation. Mycelium was no longer present 9 days after inoculation, denoting incompatibility of the host-parasite relationship. At a constant 18 C, mycelium was noted 20 days after inoculation. However, this mycelium present in the subapical tissues failed to stain a bright blue as did the mycelium in the control plants. Brightly stained mycelium was observed in the leaf tissue of one Early Hegari specimen (grown at 18 C) 20 days after inoculation. Growth of the plants was retarded by a constant temp of 18 C.

The Martin ms  $\times$  Early Hegari F<sub>1</sub> plants grown at 30 C had mycelial development in the subapical tissues 3 and 6 days after inoculation. Nine days after inoculation, the mycelium within these tissues stained lightly and appeared almost transparent. In greenhouse-grown plants, the mycelium appeared darkly stained until the twelfth day after inoculation. Plants grown at the constant 18-C treatment had mycelium which was lightly stained 12 days after inoculation.

Mycelium in the Redlan ms  $\times$  Early Hegari F<sub>1</sub> plants grown at 30 C stained very lightly 9 days after inoculation but did not stain at 12 days. Mycelium within the tissues harvested from plants grown at 18 C stained dark blue until the 12th day after inoculation, when it stained only lightly or not at all.

Early Hegari plants inoculated at 3 weeks of age contained mycelium in the subapical tissues at both 3 and 6 days after inoculation. Plants 1 and 2 weeks old when inoculated had mycelial development 3 days after inoculation, but none at 6 days.

The feteritas and their F<sub>1</sub>'s exhibited the same reaction type at all 3 temp. Neither mycelium nor sporidial fusion was observed, the same reaction as described in the R-1 study.

The reactions of Lahoma Sudangrass, the Lahoma

F<sub>1</sub>, and White Kafir P.I. 48770 were also consistent over all the temp treatments of the R-2 test. The response was not different from that observed in the R-1 test.

Infection developed normally in both Redlan ms and Martin ms and did not differ from the reaction as described previously for Combine Shallu.

DISCUSSION.—Three different host-parasite interactions were identified among five resistant sorghums.

Candidates from the feterita class of sorghum, with resistance shown to be simply inherited, displayed a very rapid inhibition of pathogen development by inhibiting sporidial fusion. There was a pigmented zone, presumably caused by phenols, around the periphery of the inoculation wound. This discoloration was also noted in other resistant sources. Its possible role in resistance was questioned since the sporidia, after hypodermic infection, were not confined to the wound but distributed throughout the tissue through the vessels and intercellular spaces. The incompatibility of the host-parasite interaction was unchanged by various temp. This behavior of the resistance to head smut from Tx09 fits the concept of resistance controlled by a single, major gene. Single gene resistance is generally very stable over environmental influences, and is usually not altered in expression when placed in a different genetic background.

The Lahoma, White Kafir P.I. 48770, and the male-sterile kafir (B65L-5427-3) sources of resistance were similar in histopathological behavior to the reaction of the feterita-derived lines. Despite the similarities in reaction type, the two kafirs have recently been found to be resistant to a newly described race of head smut which is virulent to many feterita-derived lines (1). The observation of hyphae in differentiated leaf tissue but not the meristematic tissues of several specimens might indicate that the factor(s) controlling the resistance expression are effective only in meristems.

The male-sterile kafir, B65L-5427-3, possesses a specific type of resistance. This line, although resistant when inoculated at 1 week of age, was susceptible when inoculated at 3 weeks of age. Seedling resistance of this type would be quite effective under natural field conditions, since infection usually takes place in the early stages of seedling development (2).

Reactions observed in the entries with resistance from Early Hegari differ from those observed in the

other incompatible host parasite interactions. In general, plants examined 3 to 6 days after inoculation appeared susceptible. The time of incompatibility-expression varied with the tests, the temp, and the male-sterile parent used in the F<sub>1</sub>'s.

The exact identity of the teliosporelike objects seen in certain hegari sections is not known. They were within the size range of teliospores, but no spore-wall echinulations could be distinguished. The criterion used for determination of transition from compatible to incompatible host-parasite interaction was the stain reaction of the mycelium. Some of the hegari entries had no visible mycelium, while others had mycelium which stained very lightly or not at all. Since the susceptible controls contained well-stained mycelium, and since thionin is specific for the protoplasm of the mycelium, this nonstaining reaction was interpreted as indicative of dead mycelium.

Although plant growth was greatly reduced by a constant temp of 18 C, there was no evidence that the pathogen was adversely affected.

Plant age at time of inoculation is a factor in the longevity of the compatible relationship of Early Hegari and *S. reiliana*. Plants 1 and 2 weeks old at inoculation time contained no mycelium 6 days later. Plants inoculated at 3 weeks of age contained mycelium 6 days after inoculation. These results indicate a slower mobilization or action of the resistance factors in the older plants.

In general, Early Hegari exhibited a host-parasite relationship which varied with the influence of different environments and genetic backgrounds. This variable behavior is interpreted as being indicative of a character of complex inheritance.

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