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A New Method of Obtaining Systemic Infection of Soybeans by *Peronospora manshurica* (Naoum.) Syd.[1]

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INTRODUCTION

Downy mildew, caused by *Peronospora manshurica* (Naoum.) Syd. is a relatively common disease of soybeans. First symptoms appear as indefinite lighter green areas on the upper surface of the leaf. Later, these areas become more chlorotic and appear angular, being delimited at the edges by small veins. During periods of heavy dew, the fungus sporulates profusely from the under side of these chlorotic areas. Later in the season, these areas change to well-defined greyish brown lesions with chlorotic borders. Large numbers of oospores of the fungus may be found in the necrotic tissue of these lesions. Oospores in overwintered leaf debris or oospore encrusted seed are sources of initial infection, and conidia are responsible for secondary spread in the field.

Geeseman (1), Lehman (4, 5), and Grabe (2) have reported several physiological races of downy mildew in the United States. Grabe applied this physiologic specialization when he developed a laboratory technique for the identification of morphologically similar varieties of soybeans. (3).

MATERIALS AND METHODS

Leaves of Blackhawk soybeans infected with downy mildew were collected from plants in the field in October, 1957, and stored until February, 1958. Some of these leaves were stored in a loosely woven cloth sack exposed to normal weather conditions. Others were stored dry in the laboratory at room temperature or frozen at —10° C. These leaves were air dried and pulverized in a mortar before they were used as inoculum. This powder was deposited, by means of a small spatula, between the cotyledons of Hawkeye seeds from which the seed coats had been removed. Removal of the seed coats was facilitated by soaking the seed for 24 hours in water at 8° C. In another test, leaf material was deposited on the outer surface of soaked soybean seed before it was planted. The seed coat was left intact on one lot of seed, and removed prior to inoculation from another lot. For each test, 50 seeds were inoculated and planted at a

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depth of two inches in steamed soil. The tests were carried out in a greenhouse in which the temperature ranged from 18° to 22° C.

Experimental Results

The symptoms that appeared on seedlings arising from inoculated seed were identical to those that occurred on systemically infected seedlings from oospore encrusted seed. When the seedlings were about 2 weeks old, the presence of the disease was indicated by a clearly defined lighter green area which appeared first on the lamina near the petiole (Fig. 1-A). The infection quickly spread along the midvein and into the lamina until most of the leaf surface was infected (Fig. 1-B). About 7 to 10 days after the first visible evidence of infection, conidia were produced profusely on the under side of the infected area of the leaves when the plants were placed in a moist chamber for 12 hours. Individual isolates of the fungus were maintained by transferring conidia from infected plants to susceptible young plants every 10 to 12 days. Severely infected leaves of these plants gradually became chlorotic, and later, brown necrotic areas developed as the leaves shriveled and died (Fig. 1-C). Numerous oospores could be found in the necrotic areas of such leaves. They usually appeared clustered in the leaf tissue, bounded on each side by the small veins (Fig. 1-D).

Systemic infection (Table 1) occurred when leaf material used as inoculum was stored up to 5 months either indoors or outdoors. Exposure to freezing and thawing are not required by the oospores for germination, for 13.9% of the seedlings were infected when leaf material stored indoors was used as inoculum, and 13.5% infection occurred when leaf material stored outdoors was used. No infection resulted, however, when leaf material which had been frozen after collection was used for inoculum. The results also indicate that the location of inoculum in relation to the seed is important. Only one plant in 50 was infected when the inoculum was placed on the outside of the seed with the seed coat removed, whereas up to 13.9% infected seedlings resulted when inoculum was deposited between the cotyledons. No infection resulted when the leaf material was placed outside of seeds which did not have the seed coat removed.

Table 1

Percentage of Infected Soybean Seedlings Resulting from Inoculation with Leaf Material Containing Oospores of *P. manshurica* Stored Under Various Conditions

	Percentage infected seedlings when oospores were stored:		
	-10° C.	Indoors	Outdoors
Moistened seed covered with leaf material			
Seed coat intact	–	–	0
Seed coat removed	–	–	2
Leaf material deposited between cotyledons	0	13.9	13.5

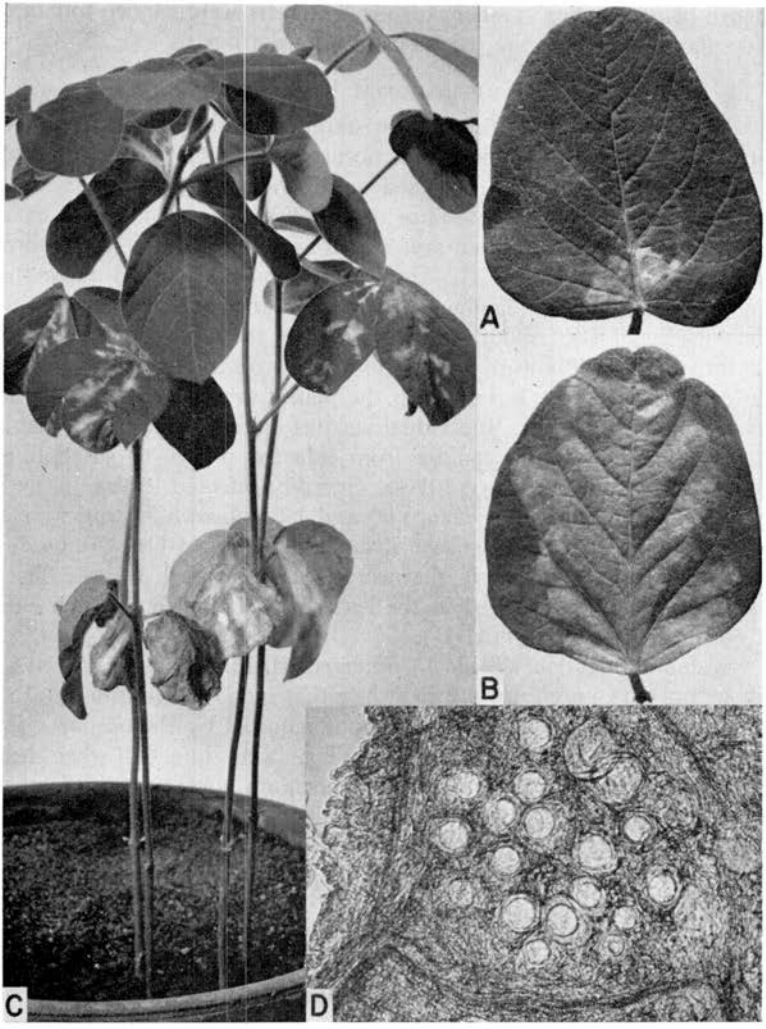

Figure 1. Symptoms of downy mildew on soybeans.
A. Early stage of systemic infection of a soybean leaf.
B. Advanced stage of systemic infection of a soybean leaf.
C. Chlorosis and necrosis resulting from conidial infection of greenhouse grown plants.
D. Oospores of *P. manshurica* as they appear in the necrotic tissue of infected soybean leaves. 110X.

Discussion

This method of placing inoculum between the cotyledons for obtaining systemic infection of soybean seedlings will have application in identification of physiological races of the downy mildew fungus, and possibly for the maintenance and preservation of races once they are identified. In the past, identification of physiologic races of

downy mildew has depended upon the location and use of oospore encrusted seed. A small percentage of systemically infected seedlings usually arises from planting such seed. This seed is difficult to find, however; and the presence of downy mildew in a field is no assurance that encrusted seed will be found there. Use of leaf material rather than encrusted seed as a source of oospores requires only that infected leaves be present in a field. These leaves can be collected, preserved, and used to obtain systemic infection of soybean seedlings.

Evidence obtained at this laboratory indicates that specific races of the downy mildew fungus obtained from oospore encrusted seed, can be reclaimed repeatedly from certain geographical areas in the United States. Presuming a given race can be reclaimed despite sexual reproduction, it may be possible to maintain races by preserving dried leaves obtained from plants infected with an identified race of the fungus. Maintenance of a specific race has been possible in the past only by the laborious process of transferring conidia from infected plants to susceptible young plants every 10 to 12 days. Conidia ordinarily do not remain viable long and all attempts to preserve them have been unsuccessful. Maintenance of identified races will be useful in the application of Grabe's method for distinguishing between morphologically similar varieties of soybeans. In addition, readily available sources of viable oospores will be useful for testing varieties and selections of soybeans for disease resistance.

Summary

Systemic infection in soybean seedlings was obtained with the fungus *Peronospora manshurica* by inoculating seed with infected soybean leaves containing oospores. Seed was inoculated by placing finely pulverized leaf material between cotyledons. It may be possible, therefore, to maintain races of the downy mildew fungus by preserving dried, infected soybean leaves which contain oospores.

Literature Cited

1. Geeseman, G. E. Physiologic races of *Peronospora manshurica* on soybeans. Agron. Jour. 42:257-258. 1950.
2. Grabe, D. F. Laboratory methods for identification of varieties of soybeans and oats. Unpublished Ph.D. thesis. On file at Iowa State College Library, Ames, Iowa. 1957.
3. Grabe, D. F. Identification of soybean varieties by laboratory techniques. Proc. Assoc. Off. Seed Anal. 47:105-115. 1957.
4. Lehman, S. G. Race 4 of the soybean downy mildew fungus. Phytopath. 43:460-461. 1953.
5. Lehman, S. G. Physiologic races of the downy mildew fungus on soybeans in North Carolina. Phytopath. 48:83-86. 1958.

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