Effect of Soil pH and Volatile Stimulants from Remoistened Peanut Leaves on Germination of Sclerotia of *Sclerotinia minor*

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ABSTRACT

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Germination of *Sclerotinia minor* sclerotia embedded in field soil at pH 5.0–7.0 in 6-cm-diameter petri plates was studied in the presence of volatile stimulants from remoistened peanut leaves in enclosed desiccators. Germination of sclerotia invariably increased in the presence of remoistened peanut leaves. Optimum stimulation was produced by 0.25–0.50 g of dried peanut leaves; excessive peanut tissue (>1 g) tended to inhibit germination. Epidemiologic implications are discussed.

Additional key words: *Arachis hypogaea,* Sclerotinia blight

Sclerotinia blight of peanut (*Arachis hypogaea* L.), caused by *Sclerotinia minor* Jagger, was first described in the United States in 1971 in Virginia (8). The disease has since become widespread in certain peanut growing areas of Virginia, North Carolina, and Oklahoma (2,11). Factors that affect onset and development of disease are largely unexplored.

Sclerotia of *Sclerotinia* species germinate by three distinct methods, ie, carpogenic, eruptive mycelial, or hyphal germination (1,5,6). *S. minor* sclerotia germinate predominantly by the mycelial and hyphal methods.

Relatively little is known about factors affecting sclerotial germination of *Sclerotinia* species and its control mechanism. The purpose of this study was to determine the effect of soil pH and the presence of remoistened peanut leaves on germination of *S. minor* sclerotia.

MATERIALS AND METHODS

We used a culture of *S. minor* from peanut supplied by D. M. Porter, Tidewater Research Station, Holland, VA. The fungus was maintained on potato-dextrose agar (PDA) and transferred monthly. Sclerotia were produced on PDA at 27 C by transferring 4-mm-diameter agar disks of the fungus after 5 days to fresh PDA and incubating them an additional 9 days under a continuous fluorescent lamp. The sclerotia were collected, rinsed with distilled water, and dried. They were then embedded randomly in field soil (Norfolk sandy loam) at about 7% soil moisture to simulate natural survival conditions. After 8 days of incubation, sclerotia were recovered from soil using nested sieves (openings of 2.0, 0.85, and 0.36 mm). Sclerotia were air-dried and stored at room temperature before use.

All germination tests were conducted in field soil (Norfolk sandy loam, pH 5.7) that was adjusted to the desired pH (5.0, 5.5, 6.0, 6.5, and 7.0) using either calcium hydroxide solution or 0.8% sulfuric acid. After pH adjustment, the soil was incubated for 2 days, pH was rechecked, and adjustments were made if necessary.

Remoistened peanut leaves were used as a source of volatile stimulant for germination of the sclerotia. We evaluated the percentage of germination by using a desiccator system described by Beute and Rodriguez-Kabana (3) with slight modifications. In routine tests, 10 sclerotia were implanted on the soil surface in each 6-cm-diameter petri dish (15 g of soil per petri dish). Experiments with each pH were replicated twice, with a total of 10 soil plates for each desiccator (24 cm diam, 9.9 L). A 50-ml beaker containing barium peroxide (10 g/20 ml of distilled water) was placed at the bottom of the desiccator to absorb carbon dioxide and release oxygen. A 6-cm-diameter petri dish containing remoistened peanut leaves was put in the desiccator. Another 6-cm-diameter petri dish containing 5 ml of distilled water was put in the bottom of the desiccator to provide atmospheric moisture. As a control, a petri dish containing 0.5 ml of distilled water was substituted for peanut leaves. After 4 days of incubation at room temperature, we counted the number of germinating sclerotia with a dissecting microscope.

Soil samples were randomly collected from peanut fields at four locations in North Carolina and three locations in Virginia during September and October 1979. The samples, which were collected beneath the peanut canopy, included soil to a 3-in. depth.

RESULTS

Sclerotia that were isolated from field soil by sieving germinated by either the hyphal or mycelial method. However, most of the germination observed throughout this study was by the eruptive mycelial method. Without remoistened peanut leaves, germination was 13.3% on soil plates with a pH of 6.5. The percentage of germination increased in the presence of stimulants. Sclerotial germination was greatest when 0.25 and 0.50 g of remoistened peanut leaves served as a stimulant. The percentage of sclerotial germination declined when more than 0.50 g of peanut leaves was used (Fig. 1).

Soil pH also had a profound influence on sclerotial germination of *S. minor*. The percentage of sclerotial germination increased as pH increased from 5.0 (0.0%) to 6.5 (45.0%); it declined to 20.0% at pH 7.0 (Fig. 2). A soil pH of 6.0 and 6.5 was the most conducive for sclerotial germination.

There was a significant interaction (F test, $P = 0.05$) between soil pH and

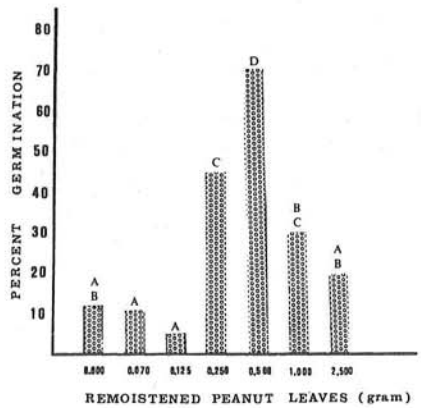

Fig. 1. Effect of quantity of remoistened peanut leaves on sclerotial germination of *Sclerotinia minor* on soil plates with a pH of 6.5. Values represent the average of three replicates with 20 sclerotia each. Data in bars with the same letters do not differ significantly ($P = 0.05$) as determined by Duncan's multiple range test.

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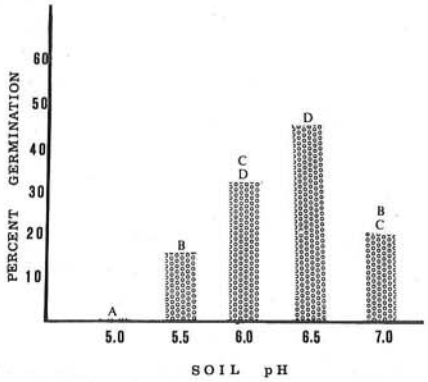

Fig. 2. Effect of soil pH on sclerotial germination of *Sclerotinia minor* in the presence of 0.25 g of remoistened peanut leaves. Values represent the average of three replicates with 20 sclerotia. Data in bars with the same letters do not differ significantly ($P = 0.05$) as determined by Duncan's multiple range test.

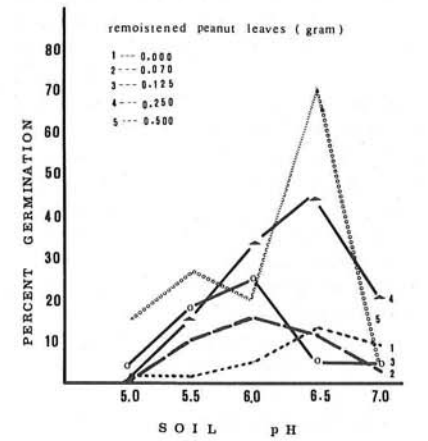

Fig. 3. Effect of soil pH and quantity of remoistened peanut leaves on sclerotial germination of *Sclerotinia minor*.

stimulation of germination of *S. minor* sclerotia (Fig. 3). The peak of sclerotial germination shifted from pH 6.0 to 6.5 as the amount of stimulant increased from 0.07 to 0.50 g of peanut leaves (Fig. 3). The pattern of germination indicated that sclerotia were less sensitive to the stimulant at low pH and that sensitivity increased as the soil pH increased from 5.0 to 6.5.

Soil pH of the three Virginia peanut fields (fields 1, 2, and 3) were 6.6, 6.3, and 6.3, respectively. Soil pH of the four peanut fields in North Carolina (fields 4, 5, 6, and 7) were 5.8, 5.6, 5.6, and 5.5, respectively.

DISCUSSION

Adams and Tate (1) described the process of mycelial germination with *S. sclerotiorum* and demonstrated that sclerotia have a dormancy period of 8–10 wk. More recently, Beute and Rodriguez-Kabana (3) demonstrated that enzymatically released methanol from peanut tissues can induce sclerotial germination of *S. rolfsii*, the causal agent of stem rot of peanut. The role of peanut tissue or other physical factors of soil on sclerotial germination of *S. minor* has not been studied. We need to know the factors that lead to epidemics so that effective disease control measures can be developed for this destructive disease of peanut.

The process of mycelial germination observed in this study is identical to that described by Adams and Tate (1) for sclerotia of *S. sclerotiorum*. Although carpogenic germination of *Sclerotinia* species has been investigated, there is surprisingly little information on factors that affect the ability of sclerotia to germinate myceliogenically (7,9,10). Mycelial germination is required for the infection of many crops (4).

This study suggests that volatile stimulants evolved from remoistened peanut leaves are important stimulants of mycelial germination of *S. minor*. Recently, Beute and Rodriguez-Kabana (3) demonstrated that enzymatically released methanol from remoistened, undecomposed plant tissues is a major stimulant of germination of *S. rolfsii* sclerotia. Although the volatile stimulant responsible for stimulating mycelial germination of sclerotia of *S. minor* evolved from peanut leaves has not been identified, preliminary results indicate that germination of sclerotia of *S. minor* was not stimulated by methanol (F. C. Hau, *unpublished*).

To our knowledge, this is the first report on the effects of soil pH on sclerotial germination of *S. minor*. Several important epidemiologic phenomena appear to be involved in the interaction between germination stimulant and soil pH. In these tests, sclerotia were less sensitive to the stimulant at low soil pH, and sensitivity increased as soil pH increased from 5.0 to 6.5. Our results suggest that germination of sclerotia is favored by high soil pH (6.0–6.5) and the presence of plant debris within the canopy. Mycelia that emerge from the sclerotia at pH 6.0–6.5 in the presence of remoistened peanut leaves may be capable of infecting the host plant directly without any exogenous food base.

Soil pH data collected from various fields in North Carolina and Virginia showed a strong correlation between pH levels above 6.0 and incidence of Sclerotinia blight of peanut as observed by the investigators. A high incidence of the disease was observed in Virginia. Fields sampled by the investigators in Virginia also consistently had soil pH above 6.0. In North Carolina, peanut fields normally have a pH below 6.0, and disease severity was considerably lower than that found in Virginia except in those peanut fields (eg, field 4) where inoculum had built up to high levels.

ACKNOWLEDGMENT

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