In Situ Observations of *Phymatotrichum omnivorum* with a Borescope Mini-Rhizotron System

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ABSTRACT

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A new application of the borescope mini-rhizotron system is described. It was evaluated as a method for observing *Phymatotrichum omnivorum* in situ in field and greenhouse situations. At 31 mm from the objective being viewed, the borescope provides a 1-1 image and a field of view of 2 cm. Objects 50 μm and larger were easily resolved. Fungal strands of *P. omnivorum* were observed on cotton roots in the field 14 days before any aboveground symptoms appeared. This is the first reported indication made by nondestructive means of when the primary inoculum of *P. omnivorum* becomes active. The borescope was also used in a greenhouse study to determine how the presence or absence of a host affected sclerotial germination. In all treatments, sclerotia germinated regardless of the presence or absence of a host. The borescope mini-rhizotron is potentially a valuable tool for ecological and epidemiological studies of certain soilborne plant pathogens.

The study of soilborne pathogens has always been hampered by the difficulty in making in situ observations. Presently the most common methods for evaluating host-parasite interactions are destructive root sampling and observations of aboveground symptoms such as chlorosis and wilting. Actual in situ observations of soilborne pathogens are rarely reported by plant pathologists. The difficulty in making observations is one of the main reasons epidemiological and ecological studies of foliar pathogens are more advanced than studies of soilborne pathogens.

Over the years, agronomists have devised various methods of observing root distribution in situ; an excellent review of these has been published by Bohm (2). One apparatus, the mini-rhizotron, has gained a certain amount of popularity due to ease of installation and minimal disturbance of the soil-root environment (1,3,5,8). Upchurch and Ritchie (7) observed and recorded root densities by using a borescope and a video recording system in conjunction with mini-rhizotrons.

The purpose of our study was to evaluate application of their system as a potential method for observing *P. omnivorum* in situ. Advantages and disadvantages of this system and its application to the study of root pathogens are presented.

MATERIALS AND METHODS

The borescope (ACMI, 300 Stillwater Avenue, Stanford, CT 06902) consists of an eye piece, extension tube, and objective lens. Its use with mini-rhizotrons has been reported in detail (1,5,7). Our system was identical to the one used by Upchurch et al (7) for root density studies, except observations were recorded with a 35-mm camera instead of the video recording system.

Sixteen mini-rhizotrons were placed in a field with a known history of Phymatotrichum root rot. They were inserted at an angle of 30 degrees from vertical and the maximum depth of observation was 60 cm. Tubes were installed 4.5 m apart in a rectangular grid design. The mini-rhizotrons were placed directly in the rows after planting, but before plant emergence. Root observations were made weekly until the middle of July. Initial strand appearance, strand-root contact, and disease incidence were recorded.

Thirty-six mini-rhizotrons were also set up in 19-L containers in the greenhouse to observe host effects on sclerotial germination. Sclerotia were grown in 250 g of sterile soil in coffee cans. Sorghum seed, 50 g per container, was used as a carbon source and, after introduction of mycelial plugs of *P. omnivorum*, the containers were placed in an incubator at 28 C. Sclerotia began to form in the soil within 12 days and were mature after 8 wk.

After incubation the soil cores from these containers were treated in one of three ways:

(i) The intact soil core containing sclerotia was removed from the culture container without disturbing the soil or sclerotia. This was achieved by removing the bottom of the container and the intact core of soil and sclerotia.

(ii) The soil core was removed from the container and then coarsely ground, separating the sclerotia from the strands on which they were formed.

(iii) The soil core was taken from the culture container and the sclerotia were washed from the soil on a 500-μm sieve.

Following these treatments, mini-rhizotrons 5.1 cm in diameter and 38 cm in length were placed at an angle 30 degrees from vertical in 19-L pots which then were filled one-quarter of the way with nonsterilized Houston Black Clay (Udic Pellustert [fine montmorillonitic thermic]). The sclerotial preparations were placed on the upper surface of the Plexiglas tubes, soil was added to fill the container and cotton or sorghum was planted directly above the tube. Containers without a host were used as checks. After planting and inoculation, the soil in the containers was maintained at 28 C and a moisture level near field capacity. Observations were made every 2 days.

RESULTS

The borescope mini-rhizotron system proved to be an excellent method for observing *P. omnivorum* in the greenhouse and field. In the field, strands were observed coming into contact with cotton roots by 1 June, 2 wk before any aboveground plant symptoms appeared. Strands grew an average of 2.5–3.5 cm a day and the average strand depth at that time was 32 cm. This depth corresponds well with reported levels of sclerotial formation (6) suggesting that strands were observed soon after sclerotial germination. By 28 June, strands had contacted roots at 12 of the 16

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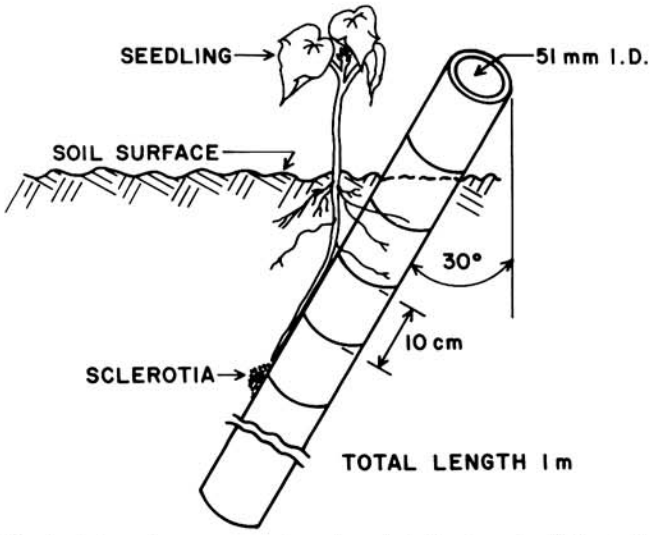

Fig. 1. Schematic representation of a mini-rhizotron. In field studies, natural inoculum is used; but in the greenhouse, laboratory-grown sclerotia serve as inoculum. Placement of the sclerotia directly on the tube surface allows observations of sclerotial germination and initial root-strand contact. Total length of the mini-rhizotrons varies, but all have orientation rings every 10 cm.

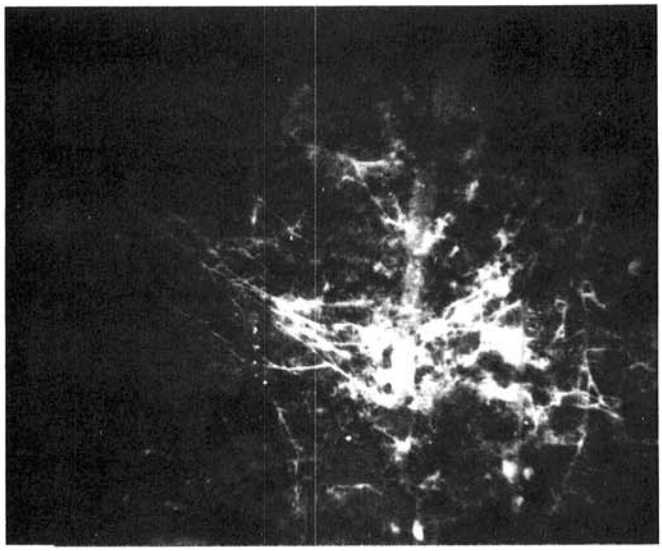

Fig. 2. Borescope mini-rhizotron view of young strands of *Phymatotrichum omnivorum* proliferating from an infected cotton root. Upon contact between the root and a fungal strand, a flush of new strand growth followed. Young strands could be differentiated from older ones by the lighter cream color of the former. ×4.5.

mini-rhizotrons, and the average depth of strand-root contact was 15 cm. No new strand growth was observed at deeper levels. Although there was root-fungus contact at 75% of the borescope tubes only 12% of the plants died in these plots, due to dry soil conditions.

Soil shrinkage was not a problem in the greenhouse where high soil moisture levels were maintained. This ensured a tight soil-to-mini-rhizotron interface and allowed clear accurate observations for the duration of the experiment. A schematic representation of a mini-rhizotron can be seen in Fig. 1. Sclerotia began to germinate in 3 days with all sclerotial preparations, hosts, and checks. Strand growth was occurring in all containers after 7 days. Young strands were a light cream color, but turned a dark caramel brown in 7–10 days. During the first few days of strand formation there was no detectable differences in quantity or rate of strand growth among any of the treatments. However, after 15 days young strands were detected only in containers with host plants (Fig. 2). It was impossible to determine visually whether old strands in the nonhost containers were still viable. Subsequent plantings of cotton in these containers, however, became infected without new strand formation from the sclerotia. Sclerotia of *P. omnivorum* are reportedly able to germinate more than once, but this was not observed.

DISCUSSION

The borescope mini-rhizotron observation system is potentially a useful tool for the study of plant pathogenic nematodes and certain soilborne fungal pathogens such as *Phymatotrichum, Armillaria, Gaeumannomyces,* and *Sclerotium*. All these fungi produce morphological structures which could be observed at the magnification reported. Increased magnification can be achieved photographically, but it was not required in this study. Individual hyphae of germinating sclerotia were easily resolved. The manufacturers of the borescope are making a new model which provides zoom capabilities. This should increase the effectiveness of the system.

Soil shrinkage was the most serious problem encountered with this system in the field. Houston Black Clay is a montmorillonitic soil and is subject to extreme shrinking and swelling. In the greenhouse, soil moisture levels were maintained near field capacity, which eliminated this problem. In the field, as the soil around the mini-rhizotrons dried the system became less useful. This problem will not be as serious in nonshrinking soils, or in years with adequate rainfall.

The borescope should be particularly useful for epidemiological studies. Normally, symptoms of Phymatotrichum root rot are not observed until 1–2 days before plant death. No nondestructive means of determining initial contact between pathogen and host have been available. With this system, however, we were able to observe initial contact 2 wk before any aboveground symptoms were observed.

The borescope mini-rhizotron system is an excellent tool for making in situ observations of roots or root-pathogen interactions. This system is applicable to the study of strand- or rhizomorph-producing fungi and also for pathogens that produce necrotic lesions on the root surface. In a recent review article (4), Huisman stated, "There is an urgent need to develop an extensive experimental data base in the area of root-pathogen interactions so as to permit the evaluation of current concepts and the development of new concepts." The borescope mini-rhizotron system of observing roots in situ should be a valuable tool in making these types of assessments.

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