Vertical Distribution of Sclerotia of *Sclerotium cepivorum* and Host Root Systems Relative to White Rot of Onion and Garlic

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ABSTRACT

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Sclerotia of *Sclerotium cepivorum* placed 30 cm deep in beds in the field germinated and infected garlic bulbs. Mycelium grew upward on roots and hyphae radiated 1–2 cm into soil, frequently infecting nearby roots. Leaves wilted and plants died only when the pathogen grew onto the stem plate and leaf sheaths. Masses of sclerotia formed as leaf sheaths decayed. Few sclerotia formed in roots. Mycelium spread most rapidly from plant to plant 2–4 cm below the stem plates where roots grew laterally and root density was highest. Infections 1 cm or closer to the stem plate rarely resulted in disease of adjacent plants because downward movement of mycelium on roots was limited and few roots of neighboring plants grew laterally in this zone. The extent of plant-to-plant disease spread depended on timing and depth of infection. The greatest disease incidence resulted from inoculum placed at intermediate depths and from early infections.

Additional key words: Allium cepa, Allium sativum, disease development.

Sclerotia of *Sclerotium cepivorum* Berk. germinate in soil with a plug of mycelium erupting through the sclerotial rind (4). Roots and leaf sheaths are penetrated directly and the invading hyphae advance inter- and intracellularly through underground host tissues (1). As the stem plate, roots, and leaf sheaths are killed, leaves become yellow and flaccid; finally, the whole plant is killed.

In California, white rot appears anytime after emergence of garlic and progresses until harvest. The disease occurs in clusters of a few to 40 or more adjacent plants. Where and when in the soil profile infections occur to initiate such extensive plant loss is unknown. Scott (11) reported that the pathogen spread from inoculated onion bulbs to adjacent plants but did not spread when root systems were separated by nylon mesh even though mycelia from infected roots penetrated the mesh and encountered roots of neighboring plants. The extent of plant-to-plant spread depended on plant spacing. The most extensive spread (four adjacent plants) resulted when bulbs touched. Scott (10) also reported that *S. cepivorum* did not grow more than a few centimeters away from a colonized food source in unsterile soil and concluded that plant-to-plant spread of the fungus occurred only by root contact. Ryan and Kavanaugh (9) infested soil with sclerotia 2–4 cm deep at various distances from the planting row, but no disease developed. When inoculum was concentrated in the planting row or was distributed evenly over the bed, limited plant-to-plant spread occurred (one to four plants per infection site). In this study, the effect of sclerotia at different depths in the soil on disease development was investigated and related to growth and distribution of host roots.

MATERIALS AND METHODS

Inoculum production and preparation. Isolates used and preparation of inoculum were as described by Crowe et al (7). Sclerotia of isolate TL4-2, buried 4 mo at Tulelake, CA, and referred to as field-conditioned inoculum, were used to study germination and white rot disease development in controlled conditions.

Observation of infection. Ten sclerotia (field-conditioned inoculum) were spaced evenly in two rows of five sclerotia each, along 2 × 4 cm dampened strips of nylon mesh (3.5 grids per millimeter). Two nylon strips were placed lengthwise against opposite sides of the inner surface of 15 cm × 30 mm diameter glass tubes. The upper edge of each strip was 6 cm from the top of the tube. A rubber stopper with a hole covered by nylon mesh was inserted into the bottom of tubes, which were filled to within 1 cm of the top with 110 g of noninfested, unsterile Tulelake soil (unclassified soil, pH 6.8, 14% organic content). As tubes were filled with soil, one garlic clove (California Late), one corm of *Brodiaea peduncularis* (Salisb.) Greene, or four barley seeds (*Hordeum vulgare* L.) were placed in the top 1–3 cm of some tubes; other tubes contained no plants. Treatments were replicated three times per experiment. The soil was saturated by placing the tubes in separate beakers of distilled water. Tubes were inserted into holes in a wooden box so that only the soil surface at the top of each tube was exposed to light (8,000 lux) from both incandescent and fluorescent bulbs. Tubes were maintained at 15 C but were removed occasionally to observe pathogen and root growth at ×10–20 magnification with a stereomicroscope. Soil was rewatered when it appeared dry.

Effect of depth of infection on disease development in a controlled environment. Disease development from different depths of infection was observed on plants grown in upright wooden observation boxes with inside dimensions of 28.5 × 27 × 12.5 cm. One wall was window glass fitted into grooves at an angle in the 12.5 × 28.5 cm side wall so that the bottom of the glass was recessed 5.5 cm away from the front edge of the box to induce root growth against the glass. Additional grooves outside those holding the glass supported a removable sliding wall that excluded light. Observation boxes, containing 4,800 g of air-dried, noninfested Tulelake soil that had been passed through a 2-cm screen, were arranged in a randomized block design in a plant growth chamber at 15 C with 12 hr of light (8,000 lux). In one experiment, approximately 1,000 field-conditioned sclerotia were banded evenly against the plane of glass 2.5, 7.5, 12.5, or 17.5 cm deep in separate boxes as soil was added. A control of noninfested soil was included. Boxes and soil were soaked with water before 10 garlic cloves (California Late) were planted with stem plates located 1.5 cm deep against the glass. In a separate experiment, after garlic roots had grown 20 cm deep in all boxes, a soil core was removed from each box with a 0.8-mm diameter glass tube

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inserted from the top and rear of the box diagonally to a point near the center and in the plane of the glass 1.0, 6.0, 11.0, or 16.0 cm below the garlic stem plates. By reinserting the 0.8-mm tube with a 0.6-mm tube inside, 10 field-conditioned sclerotia were dropped through the inner tube to a point that was always near several garlic roots. Control boxes had tubes inserted to 7.5 cm deep, but no sclerotia were added. Each treatment was replicated three times in both experiments.

Effect of vertical distribution of inoculum on disease development in the field. Experiments on vertical distribution of inoculum and disease development in the field were done at Davis, CA, in Yolo fine sandy loam soil in beds 25.4 cm wide not infested with *S. cepivorum* (pH 7.4, <1% organic content). It was previously determined that 5-wk burial of inoculum produced in vitro was adequate to eliminate constitutive dormancy (7). For treatments in which inoculum was located at different depths below the stem plates, sclerotia were placed where desired 5 wk before planting. For treatments with sclerotia shallower than the garlic planting depth, sclerotia were buried in nylon bags 5 cm deep in adjacent beds for 5 wk before relocation to treatment sites at planting time. Garlic cloves (California Late) were planted 32.8 cloves per meter 4 cm deep in a single row per bed in mid-November 1976. Beds were irrigated after burial of sclerotia, at planting time, and every few weeks as needed. Weeds were removed without pulling (killed with a contact herbicide or clipped at the soil line) to avoid redistribution of sclerotia.

The effect of depth of infection on aboveground symptoms was determined by layering approximately 15,000 sclerotia evenly across 25.4 cm × 1.25 m sections of the bed 2.5, 5, 10, 15, 23, 30, or 45 cm deep. Noninfested bed sections of the same size were controls. Each treatment depth was replicated four times in a randomized complete block design.

The relationship of depth of infection to disease incidence and the extent of spread from a point of infection was determined by placing 10 sclerotia 2.5, 5, 10, and 20 cm deep in the center of beds. A noninfested control was included. Four sites per treatment depth were located in each of four randomized blocks.

Root density and distribution in the field. To determine if the number of sites at which disease appeared was related to root density, 10 2.5-cm diameter soil cores were taken from various locations across uninoculated beds and cut into 2.5-cm sections. Core sections from the same relative location in the bed were grouped together. This procedure was repeated four times. Samples were collected several times during the season. Immediately after sampling, roots were washed from soil onto stacked 0.850 and 0.425 mm screens and counted.

RESULTS

Observation of infection. Sclerotia were observed for 40 days in glass tubes in which garlic, *B. peduncularis*, or barley were grown and also in tubes without plants. Although roots appeared at different times and grew at different rates, they were present in all planted tubes about 2 wk after planting. Two to six of the 20 sclerotia germinated in tubes without garlic; 15–20 sclerotia germinated in tubes with garlic. Only hyphal-plug germination (4) occurred. Roots growing between the nylon and glass and just behind nylon strips were observed in relation to mycelial growth. After sclerotia germinated, mycelium grew for about 7–10 days and hyphae extended 1–2 cm from the sclerotial body. Infection cushions formed on glass, nylon, and roots of the three plant species tested, but only garlic roots appeared to be infected. After mycelial elongation from sclerotia stopped, infection of garlic roots did not occur, even though hyphae did not disintegrate until 10–15 days later. Mycelium growing from sclerotia through the nylon mesh infected garlic roots on the other side of the mesh. After infection, mycelium grew upward internally and externally on garlic roots and frequently formed infection cushions on the same root in advance of internal growth, although internal growth sometimes exceeded external growth. Mycelium also extended away (1–2 cm) from decaying garlic roots and infected nearby roots on either side of the nylon strips.

Effect of depth of infection on disease development in a controlled environment. In the experiment with inoculum banded at various depths in glass-walled boxes, garlic roots were evident 1.0 cm below the stem plates within 3 days after planting (week 0). After 1.5, 2.5, and 4.5 wk, roots had extended 6.0, 11.0, and 16.0 cm below the stem plates (Fig. 1). Inasmuch as sclerotia were concentrated approximately four sclerotia per millimeter in the band of placement, all visible roots grew within 1 mm of sclerotia as they intersected this band. Germination and hyphal growth were observed at ×10 with a hand lens. Sclerotia germinated and infection cushions appeared at all depths within 1–2 wk after roots penetrated the zones where sclerotia were present (Fig. 1). Nearly all visible roots penetrating the band of sclerotia became infected. Fewer infections occurred at greater depths because fewer roots reached these levels. After infection, the pathogen progressed up and down roots and grew internally and externally. The fungus was most active 1–3 mm behind the advancing external mycelium. This region generally collapsed and yellowed within a few days after infection. Further downward movement was limited because the pathogen did not grow into roots that collapsed below the area of infection. In areas of fungal activity, hyphae grew 1–2 cm through soil and often infected roots of the same or nearby plants. Hyphae did not continue to grow or infect other roots after the infected roots had decayed near the hyphal attachment. Spread of infection from root to root was most rapid when roots touched and was slower where distance between roots was greater. Horizontal spread of the fungus was obscured in this experiment because root systems of all plants became infected at various locations both from sclerotia and from mycelia of other infected roots. Infection of the bulb usually resulted from a single early-infected root on which the pathogen had grown rapidly upward ahead of the deeper, more extensive root decay. Time between planting and the first infections of stem plates increased with depth of sclerotial placement due to the increased time required for roots to penetrate to these depths and for the pathogen to grow up from the greater depths. First infections reached the base of plants 2.2, 6, 10.5, and 13.5 wk after planting from sclerotia banded at 1.0, 6.0, 11.0, and 16.0 cm below the plants (Fig. 1). Leaf wilting was evident within 1–2 days after the pathogen contacted the stem plate, penetrated leaf sheaths, and began to kill other roots. Plants rapidly died and masses of sclerotia

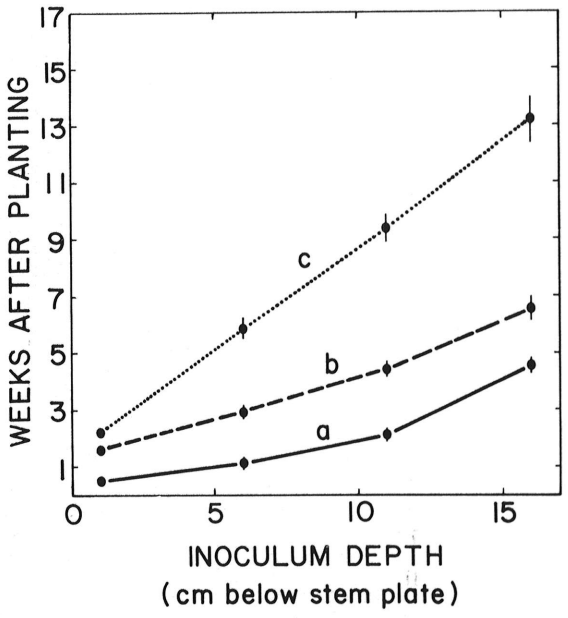

Fig. 1. Relationship between depth of inoculum and time (wk) for **a,** garlic roots to grow to depths at which sclerotia of *S. cepivorum* were banded; **b,** first root infections at infested depths; and **c,** stem plate infections from mycelium growing upward on infected roots at 15 C. Data points are averages of three replications and vertical bars represent standard deviations.

formed near the soil surface on the decaying leaf sheaths. A few sclerotia were formed in individual roots at various depths, especially when several infected roots were clustered together.

Sclerotia placed 1.0, 6.0, 11.0, and 16.0 cm below stem plates in the other experiment germinated and infections occurred at all depths 1–2 wk after inoculation. Roots of each plant grew down from the stem plate in a tight cluster for 1–2 cm before spreading in all directions and intermingling with roots of neighboring plants. Leaf symptoms appeared within the first week after infection on plants inoculated 1 cm below the stem plate. Roots were killed rapidly and collapsed below this level, but the pathogen rarely grew down to roots of neighboring plants. Increased depth of inoculum increased the time required for the fungus to reach the stem plate.

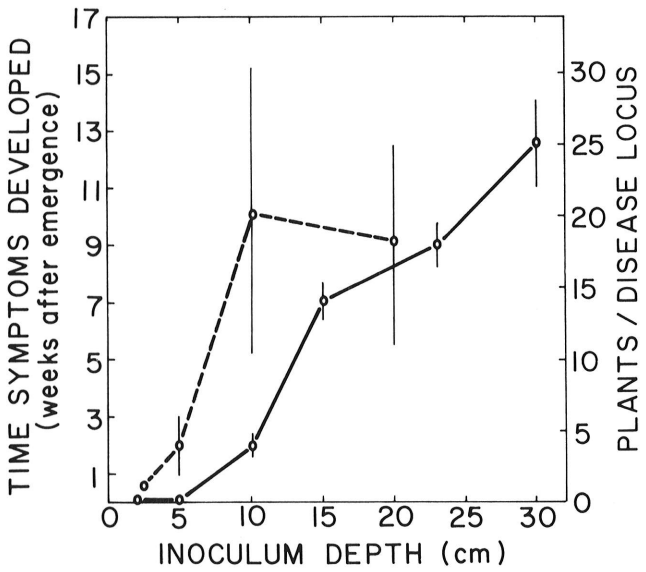

Fig. 2. Relationship between depth of placement of sclerotia of *S. cepivorum* and time of appearance of disease symptoms (————) on garlic in the field and the number of diseased garlic plants per disease locus at harvest (- - - - -). Data points are averages of four replications and vertical lines represent standard deviations.

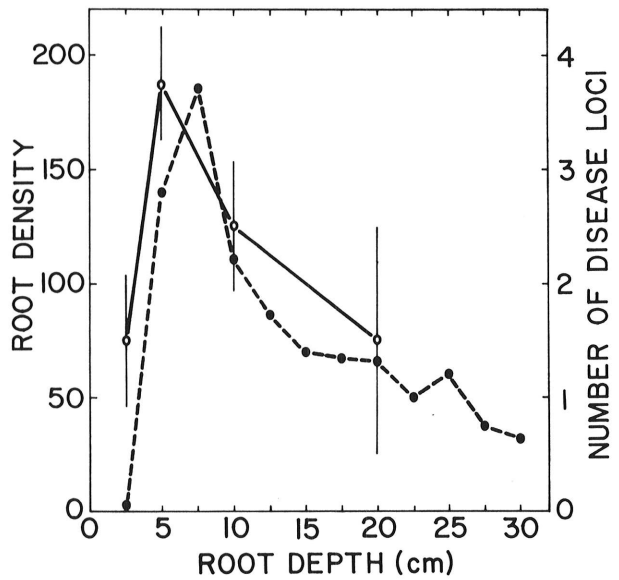

Fig. 3. Number of disease loci that developed from inoculum placed at different depths (————) in the field and the root density (number of roots in 19.67 cm^3 of soil) of garlic at various depths below the planted row (- - - - -). Four sites were infested per treatment depth per replication. Data points for the number of disease loci are averages of four replications and vertical bars represent standard deviations.

Horizontal spread of the fungus onto adjacent roots was extensive for inoculations greater than 1 cm deep. The horizontal spread was most rapid in the zone of greatest root density and horizontal root extension 2–4 cm below the base of plants and was progressively slower at greater depths as root density decreased and roots grew predominantly vertically. All 10 plants became diseased in each box in which inoculum was placed 6.0, 11.0, and 16.0 cm below the stem plates. In contrast, one or two of 10 plants became diseased when inoculum was placed 1.0 cm below the stem plates. No white rot developed in the controls.

Effect of vertical distribution of inoculum on disease development in the field. Garlic emerged in mid-February (designated week 0) and symptoms were recorded one to three times per week until harvest in late June 1977 (week 17). When sclerotia were layered at various depths in the bed, emergence of garlic was uniform in all treatments. When sclerotia were placed 2.5 and 5 cm deep, many plants were infected immediately after emergence. Symptoms appeared progressively later with deeper inoculum placement (Fig. 2). Symptoms did not appear where sclerotia were placed 45 cm deep or in noninfested treatments.

The average number of sites at which aboveground symptoms eventually developed from placement of sclerotia at specific depths are presented in Fig. 3. Since several adjacent plants usually developed symptoms, these sites are referred to as disease loci. The average number of plants per disease locus at harvest was 1.2, 4.5, 20.4, and 17.7 for inoculum depths of 2.5, 5, 10, and 20 cm, respectively. (Fig. 2).

In an experiment to associate time of infection with disease development, garlic planted in beds free of sclerotia were inoculated at various times after emergence (week 0) by placing the bases of plants infected with white rot (taken from other experiments) next to roots 5 cm below the stem plate of plants (9 cm below the soil surface) 1, 6, and 11 wk after emergence. Uninoculated controls received the bases of healthy plants on week 1. Eight sites per treatment were located in each of four randomized blocks. White rot was evident 1–2 wk after inoculation at 68% of all sites of inoculation. For sites at which disease appeared, the average number of plants per disease locus at harvest (week 17) was 18.7, 8.1, and 5.1 for inoculations made 1, 6, and 11 wk after emergence, respectively (Fig. 4). No symptoms developed in the

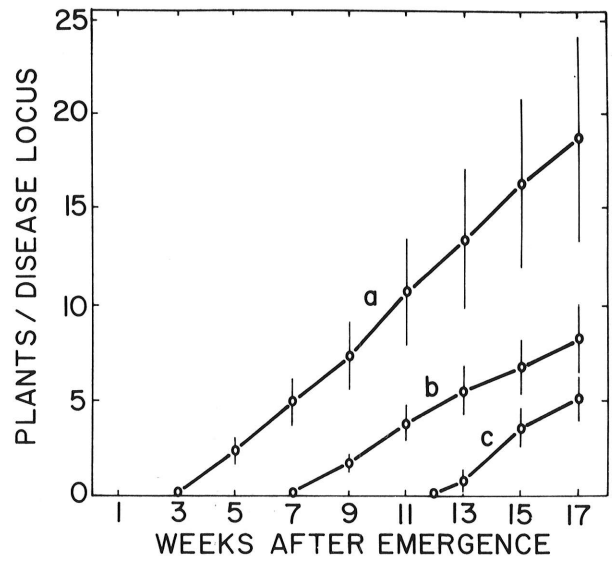

Fig. 4. Effect of time of inoculation on the number of garlic plants per disease locus. Roots below single plants at eight well-spaced locations were inoculated 5 cm below the stem plate in the field 2, 6, and 11 wk (**a**, **b**, and **c**, respectively) after emergence. Symptoms developed at 4–7 of these sites for each time of inoculation. Data points represent the averages and vertical bars represent the standard deviations of four replications adjusted for the number of disease loci in each treatment at various times during the season until harvest (week 17).

controls. The rate of increase in disease locus size was about the same for each inoculated treatment.

Root density and distribution in the field. Root density, the number of roots and side branches longer than 1 cm per 2.5-cm core section (19.67 cm^3) of soil, increased until week 14, but the relative proportions of roots at various depths remained the same. At week 14, two to four times as many roots were found 4–10 cm directly beneath plants than at depths greater than 15 cm (Fig. 3). Root densities 5–10 cm away from the planting row between 4 and 12.5 cm deep were about 40% less than directly in the planting row. At depths greater than 12.5 cm, root densities were similar across the bed. Few roots were recovered from the upper 2.5 cm at any location.

The horizontal spread of individual roots was determined by removing soil from around plants. Within 7 wk after emergence, roots in the upper 6 cm frequently had grown horizontally along the bed for about 30 cm before growing downward.

DISCUSSION

Scott (11) suggested that the white rot pathogen spreads from plant to plant only at points of root contact, but he did not observe spread between plants when healthy onion roots were separated from infected onion by only a thin nylon mesh that was penetrated by hyphae of the pathogen. In our observations, hyphae from germinating sclerotia and infected roots grew through soil, penetrated nylon mesh, and infected healthy garlic roots. No infection occurred on barley or *Brodiaea*, although the latter is a close relative of *Allium*. This confirms reports that only *Allium* spp. are susceptible (3). Scott (10) also reported that mycelium grew a few centimeters from a colonized nutrient base, and our observations confirm this.

Sclerotia survive 4 yr in soil at various depths with little loss in viability (3) and during this period become well distributed vertically in the soil profile from farming operations. In this study, sclerotia of *S. cepivorum* germinated, mycelia infected garlic roots, and the pathogen decayed bulbs from as deep as 30 cm. Symptoms were not evident, however, until the pathogen had reached and partially rotted the stem plate or leaf sheaths. At infections 2–4 cm or more below the stem plate, extensive plant-to-plant spread occurred because the pathogen spread among roots of the same and neighboring plants as the mycelium grew along roots, radiated from active sites of colonization, and infected other roots up to 1–2 cm away. Infections spread among plants most rapidly at points of root contact. Distances between roots were least in the zone of highest root density 2–4 cm below stem plates, and in this zone roots frequently extended horizontally to provide a direct path for mycelial growth. Infections from sclerotia on roots within 1 cm below the stem plate or directly on the stem plate or leaf sheath rarely resulted in spread of the pathogen from plant to plant, because the roots below died rapidly before the pathogen grew extensively downward, and few roots from neighboring plants grew horizontally in this zone. Other organisms may have colonized these regions and precluded colonization by *S. cepivorum*.

After inoculating single bulbs, Scott (11) observed disease spread among four or less adjacent plants when bulbs were touching. The reasons for this are not clear since mycelium would have a direct path from plant to plant. Possibly there was insufficient time for more extensive spread. He observed even less spread when bulbs were slightly spaced, probably because plants were inoculated on the bulb and mycelium failed to grow down into the root zone. Ryan and Kavanaugh (9) also did not observe disease spread involving more than four plants when inoculum was placed at points directly in the planted row 2–4 cm below the soil surface (probably 1–3 cm below the base of the onion plants). That failure of more extensive spread may have been because of shallow depth of infection or delayed germination of sclerotia (4) that were incorporated directly into the soil from in vitro cultures. Delayed germination was not a problem in our study because sclerotia were placed in soil several weeks before planting. Ryan and Kavanaugh (9) also observed no disease when sclerotia were placed 2–4 cm below the soil surface and several centimeters from the planted row. Our root distribution studies indicated that very few garlic roots were in the top 2.5 cm of bed. Onion roots also were sparse in the upper 2–3 cm of soil, especially if mechanically cultivated, even though seed is planted about 1 cm deep.

Experiments in controlled conditions and in the field indicated that the extent of plant-to-plant spread depends on the time and depth of infection, root density, and root distribution. The number of root infections probably is influenced by both inoculum density and root density. Root density varied along bed width and with depth. Root density and distribution probably would be changed only slightly by increased plant spacing that would still allow economic production of onions and garlic. Ali et al (2) noted only a slight decrease in disease incidence with increased plant spacing.

The high (50%) incidence of disease described previously (7) from uniform inoculum densities of 0.001–0.004 sclerotia per gram of soil was due to the high percentage of sclerotial germination in the presence of *Allium* spp. (4–8) and extensive spread of the fungus onto the roots of adjacent plants. At high uniform inoculum densities >0.01 sclerotia per gram of soil, most plants probably become infected directly from sclerotia.

Plant-to-plant spread of the pathogen may increase the nutritional base on which inoculum is produced; the rapid increase in inoculum density from very low inoculum density levels (7) may be associated with this pattern of disease development.

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