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EXISTENCE OF PHYTOPHTHORA CINNAMOMI AS CHLAMYDOSPORES AND OOSPORES IN ROOTS AND SOIL

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Persistence of the avocado root-rot fungus *Phytophthora cinnamami* Rands in soil for 6 years without a living host was reported by Zentmyer and Mircetich (8). The authors suggested that the fungus may survive in dead avocado roots as inactive mycelia, chlamydospores or oospores. Blackwell (1) considers chlamydospores of *Phytophthora* spp. to be resistant structures which allow these fungi to persist during adverse conditions, although she presented no experimental evidence to support this claim. According to Hawker (2), a fungus frequently survives adverse conditions temporarily by the production of thick-walled bodies, the simplest being mycelial chlamydospores. However, the literature contains little data on the exact conditions leading to morphogenesis of these structures.

Studies were initiated several years ago to elucidate the mechanism and conditions that permit the avocado root-rot fungus to survive in the soil without a living host. The purpose of this paper is to report information on the formation of resistant spores — chlamydospores and oospores — and their role in survival of the avocado root-rot fungus.

EXPERIMENTAL PROCEDURE AND RESULTS

Production of chlamydospores and oospores by P. cinnamomi. — Chlamydospores of this fungus have been recognized for several years (5) but little is known of the exact conditions leading to their morphogenesis. The effect of various factors on the formation of chlamydospores and oospores was investigated in the course of this work.

The influence of unidentified nutrients in V-8 juice broth (V8JB) and potato-dextrose broth (PDB) on formation of resistant structures by the fungus was studied. Production of chlamydospores was abundant (Fig. 1D) within 5 days in V8JB (100 ml cleared V-8 juice, 2 g, CaCo; and 900 ml, demineralized water). However, when the concentration of V-8 juice in the broth was increased, the formation of the spores was greatly delayed. Chlamydospores were not formed in PDB (effusion of 200g, potato; 20g dextrose and demineralized water to make 1 liter) cultures kept in laboratory for 1 year at 24 ± 3C. Although the isolate (SB216) used in these studies does not produce oospores in single culture on agar media, a few oospores formed in 45-day-old culture in V8JB.

Roots were inoculated in agar cultures, in nutrient solution, and in natural Bonsall and Vista soil series, to study the influence of avocado roots on the formation of chlamydospores by *P. cinnamomi,* Chlamydospores were observed in excised avocado roots 3 days after contact of the fungal mycelia with the roots on corn meal agar. No chlamydospores were formed on the agar outside of the roots. When avocado seedlings were inoculated in a complete nutrient solution as described by Zentmyer and Mircetich (7), the spores formed in the invaded roots within 5 days. These spores formed abundantly within 7 days in the roots of avocado seedlings grown in artificially infested Vista and Bonsall soil series. There were more spores in the roots infected in the Bonsall than in the Vista soil. The spores in the roots infected in the soil had a thicker wall than those formed in the agar culture. It is apparent that the thickness of the wall aids these spores to resist an unfavorable environment in natural soil.

When an avocado isolate (SB216) of the fungus was used singly to infect avocado roots on an agar medium, in the nutrient solution, or in the soil, oospores were not found in the roots during the limited time of observation. However, many oospores were detected when the roots were infected in petri dishes on potato-dextrose agar with two compatible cultures, the avocado isolate (SB216), and the macadamia isolate (H-1) of *P. cinnamomi.* Again, no oospores were observed on the medium outside of the roots.

Zentmyer and Mircetich (8) demonstrated that the avocado root rot fungus can make limited mycelial growth from a suitable food base in natural soil. Transformation of mycelium to resistant spores-oospores and chlamydospores, undoubtedly would greatly prolong the persistence of *P. cinnamomi* in soil in the absence of a living host or under conditions unfavorable to the fungal vegetative growth.

To study the production of chlamydospores and oospores by *P. cinnamomi* directly exposed to the environment of natural soil, fiberglass cloth, colonized in PDB or V8JB, was buried in soil. The fiberglass cloth from PDB was heavily colonized by mycelia only, while those from V8JB had mycelium bearing numerous chlamydospores.

After 15 days of incubation in natural soil at 24 C, many chlamydospores developed in mycelia on the fiberglass from PDB. Numerous oospores were formed on fiberglass pieces previously colonized in V8JB. The oopsores were simple, more or less filling the oogonium, golden brown in color; and very often they contained a large globule (Fig. 1C). No *Phytophthora* species other than *P. cinnamomi* were observed in material examined in this experiment.

Since *P. cinnamomi* readily produced chlamydospores in fiberglass cloth colonized in PDB only after the cloth was buried in soil, it is apparent that some factor in soil influences morphogenesis of chlamydospores in the avocado root-rot fungus. Initially it was observed that production of chlamydospores in a 1% soil extract was inversely proportional to myceliai growth and sporangial formation. Zentmyer (6) reported that drying natural soil resulted in loss of the stimulatory effect on sporangia production by *P. cinnamomi.* In several experiments attempts were made to determine specific changes in soil microflora influenced by altering the level of soil moisture as well as the role of certain segments of the xnicrobial population on chlamydospore formation by *P. cinnamomi.*

Soil of the Bonsall and Vista series were collected from the field, screened, and placed

in 1 quart glass jars. The moisture level in the soils was adjusted to 30% and 60% moisture holding capacity and maintained throughout the experimental period. After the jars were incubated for 3 weeks at 24C, the 1% soil extract was prepared and tested for its stimulatory effect on chlamydospore production. *Phytophthora cinnamomi* produced chlamydospores abundantly in the soil extracts from soils with a 30% moisture-holding capacity level, while mycelial growth and sporangial formation was limited. The production of chlamydospores was limited in the extract prepared from the soils having a 60% moisture-holding capacity level, although an abundant vegetative growth and sporangial formation occurred in this soil extract. Bonsall soil series induced more abundant production of chlamydospores and sporangia, depending on the moisture content, than the Vista soil. The population of Actinornycetes was approximately twice as great in the soil with a 30% moisture holding capacity as in those with 60% moisture holding capacity.

A search for resistant structures in naturally infected tissue was made in dead feeder roots of avocado trees that either were in an advanced stage of the disease or had died. In all cases, specimens were collected from trees known to have been infected for at least 6 years. The roots from diseased trees were plated on pimaricin-vancomycin (PV) medium (3). After 18-hr, incubation those roots that were positive for *P. cinnamomi* were sectioned, stained and examined under the microspope for the presence of resistant structures.

Examination of these sections revealed the presence of both oospores (Fig. 1A) and chlamydospores (Fig. 1B), although the latter occurred in greater abundance. Chlamydospores were present only within cells of cortical tissue. Oospores, with amphigynous antheridia, were formed primarily in roots in an advanced stage of decay. These oospores were approximately the same size as those found in the colonized fiberglass material. The morphology of these oospores was identical to that described for *P. cinnamomi;* therefore, they are assumed to be *P. cinnamomi.* In support of this assumption, only *P. cinnamomi* was isolated from naturally infected avocado roots.

Role of chlamydospores, oospores and mycelia in survival of the avocado root rot fungus. — The relative importance of mycelia, chlamydospores and oospores in survival of *P. cinnamomi* in soil is uncertain. Several experiments were carried out under laboratory and greenhouse conditions to elucidate the role of these fungal stages in survival of the pathogen in soil.

Fiberglas cloth colonized by the fungus either in V8JB or PDB was buried in 8-dr glass vials containing Bonsall or Vista soil. Thus the fungus was directly exposed to a natural soil environment. Each vial received 10 fiberglass pieces (1x1 cm). The moisture level was adjusted in all vials at 21.6%. The vials were incubated in the laboratory 1 at 24 ± 3C. In half of the vials, the moisture level was maintained at 21.6% while the other half was allowed to dry out. To determine the fate of the fungal structures, the fiberglass pieces were removed every 15 days from 6 vials of each treatment and observed under the microscope. The persistence of the fungus in the soil was determined by plating the glass pieces on PV medium.

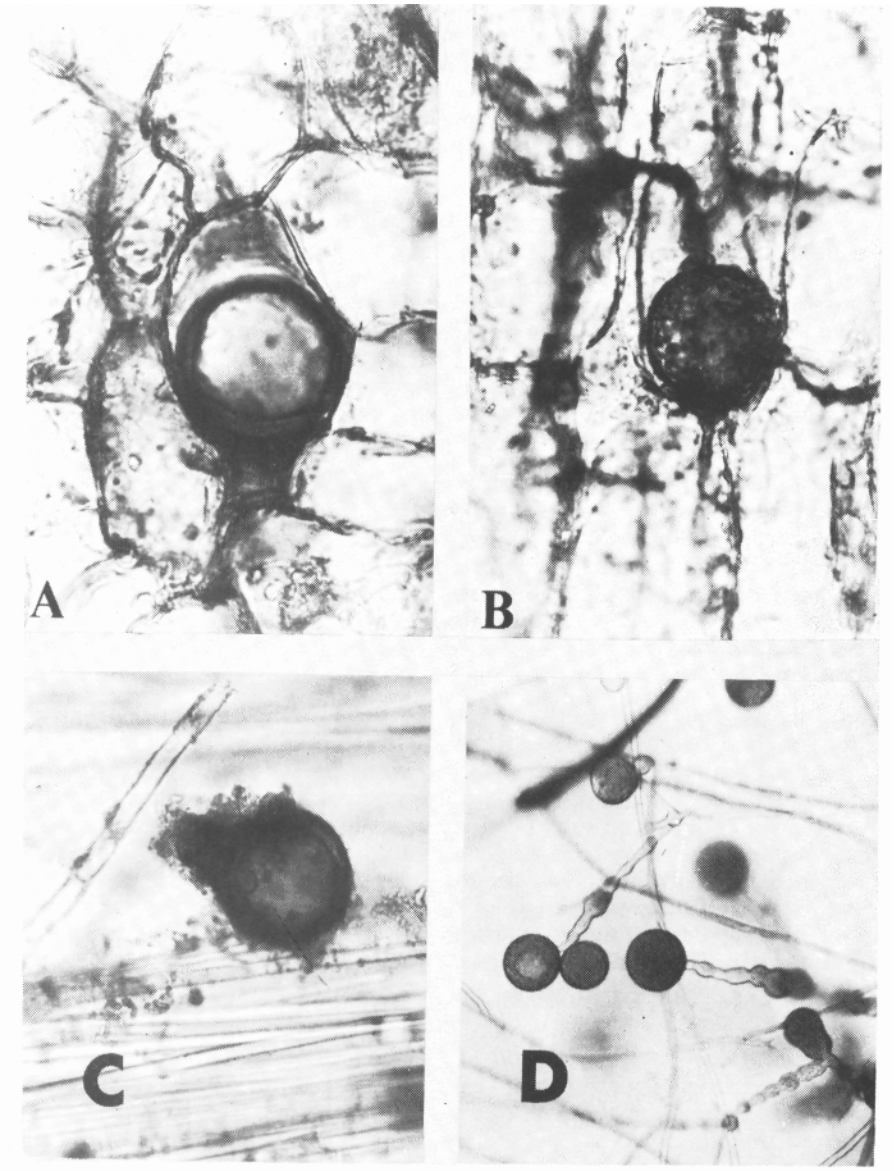

Figure 1. Oospores and chlamydospores of Phytophthora cinnamomi. A) Oospores in naturally infected avocado roots. B) Chlamydospores in naturally infected avocado roots. C) Oospores on fiberglass buried in soil for 8 months. D) Chlamydospores developed in V8JB, incubation 6 days at 24 ± 3C.

Direct observation of the fiberglass revealed that mycelia were completely lysed within the first month of incubation in the soil. *Phytophthora cinnamomi* was not recovered on PV medium beyond 1½ months of incubation in the soils which were allowed to dry out. The soil moisture in both the Bonsall and Vista soil dropped from 21.6% to approximately 2% within 1 month. When these dry soils were moistened and planted with *Persea indica* seedlings, a host of *P. cinnamomi,* the fungus could not be recovered in spite of the fact that numerous chlamydospores and oospores were present in the soil. Apparently chlamydospores and oospores are not capable of remaining viable under conditions of extreme drought.

The percentage recovery of *P. cinnamomi* was 100%, in soil with an adequate moisture level, within the first month. Thereafter the recovery gradually declined. The fungus survived 10 months in the Vista soil and it was isolated from 25% of the fiberglass pieces from the Bonsall soil at the end of the 12 month period.

Since the mycelia were completely lysed within the first month of incubation in the soil and since oospores did not germinate on the PV medium, the survival of the fungus beyond 1 month undoubtedly was due to the persistence of chlamydospores only. The colonies growing from the fiberglass on the medium were exclusively traced to chlamydospores.

In another series of greenhouse experiments, an attempt was made to evaluate the effect of soil type and soil moisture on persistence of *P*. *cinnamomi* in the infected tissues. Avocado roots, uniformly infected in nutrient solution, were cut into 2-cm pieces, incorporated into natural Bonsall and Vista soil and placed in 2-gal containers. The containers were placed in the greenhouse, and saturated with water. Each soil series consisted of 8 replications, half of which were allowed to dry out and the other half were watered every day as needed throughout the experimental period. The viability of the fungus in the roots was determined by planting 30 root pieces from each replicate on PV medium at bimonthly periods. The roots were also sectioned and examined microscopically for the presence of mycelia, chlamydospores and oospores at the time of plantings.

The fungus survived better in the moist Bonsall than in Vista soil. The percentage of positive isolations of the fungus from the Bonsall soil did not decline significantly during the experimental period of 14 months. However the recovery of the fungus from the Vista soil declined rapidly within the first 2 months and remained relatively constant throughout the experiment. Chlamydospores and mycelia were observed in the roots when they were sectioned and examined every 2 months. Oospores were not observed in these roots. Since the origin of mycelia in a large number of the roots was traced to the chlamydospores it is apparent that these spores were functional.

When the soil was permitted to dry, the fungus could not be recovered after 2 months, at which time the soil moisture was 2%. When these soils were planted with *P*. *indica* seedlings and kept for 4 months at optimum conditions for infection and disease development, the seedlings remained healthy. The fungus failed to survive under these conditions.

GENERAL DISCUSSION

Several factors are involved in the morphogenesis of chlamydospores and oospores of *P*. *cinnamomi.* The formation of chlamydospores and oospores under aseptic conditions in V8JB and in avocado roots hut not in PDB suggests that formation of these fungal structures is influenced by a specific nutritional condition. Chlamydospores and oospores were formed readily by mycelia directly exposed to a natural soil environment or in infected avocado roots in soil. Also it is apparent therefore that competition between the soil microorganisms for available nutrients and metabolic products of the soil biophase, particularly Actinomycetes, plays an important role in the formation of

these structures by *P*. *cinnamomi* in its natural habitat. Thus formation of resistant structures is an effective mechanism developed by the avocado root-rot fungus for survival during adverse conditions in the soil.

Zentmyer (4) demonstrated that *P*. *cinnamomi* is homothallic and that formation of oospores by this fungus under controlled conditions can be induced by substance exuding from avocado roots. During this investigation, chlamydospores and oospores were found for the first time in naturally infected roots of avocado. The fact that this fungus forms chlamydospores and oospores in soil and naturally infected roots, accounts for the ability of the pathogen to persist for long periods in soil without a living host.

Mycelia, when directly exposed to the soil environment, are susceptible to lysis. However the fungal mycelium was able to form chlamydospores and oospores in soil before it was completely lysed within 1 month. Therefore, the importance of this fungal stage in survival of the pathogen lies not in its persistence in the soil but in its ability to form resistant structures. Chlamydospores were shown to be adapted for prolonged survival in soil. These spores persisted for 10 months in Vista and over 12 months in Bonsall soil when directly exposed to the soil environment. Thus it is apparent that these spores may persist in the soil for a long period of time after the tissues which harbored them are completely disintegrated.

The debris of dead plants infected during the active parasitic or saprophtic phase by a soil-borne pathogen may provide a place more favorable for survival of the pathogen than the soil matrix. It was shown in this investigation that infected avocado roots provided a more favorable site for *P*. *cinnamomi* than the soil matrix, since recovery of the fungus was higher from the infected roots than from the colonized fiberglass buried in soil. The pathogen was capable of maintaining its population at a higher level in Bonsall than in Vista soil. A good correlation in this respect was observed in both cases: in the dead avocado roots and the colonized fiberglass. The greater capacity of the fungus to survive in Bonsall soil than in Vista soil series may partially account at least for the greater incidence and severity of the avocado root-rot disease occurring in the field in the former soil.

The pathogen failed to survive beyond 2 months in the soils which were allowed to dry out to approximately 2% moisture. Therefore oospores and chlamydospores are not important factors in survival of the fungus in soil under prolonged adverse conditions of drought. They are of significance only in soils of higher moisture levels.

Elucidation of the mechanisms of survival and behavior of the avocado root-rot fungus in the soil should contribute to a better understanding of the role of different stages of the fungus in pathogenesis, as well as aid in developing more effective control measures for avocado root rot.

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