

## Host Range and Survival in Soil of *Pyrenochaeta lycopersici*

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### ABSTRACT

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A semiselective medium (corky root medium) was developed for isolation, host range, and survival studies of *Pyrenochaeta lycopersici*, the causal agent of corky root of tomato. The host range of the fungus was tested by growing seedlings in infested vermiculite and by collecting plants growing in infested soils. Four new hosts (squash, *Datura stramonium*, spinach, and safflower) were identified in the seedling tests, and the last two were also found naturally infected. Other known hosts confirmed in the seedling test were pepper, eggplant, cucumber, and melon. Beet was a symptomless host. All hosts may occur in rotations in tomato production areas in California and therefore act as increase hosts for the fungus. An isolation technique was developed in which diseased tissue was blended and the fungus was isolated from the extract. The fungus was recovered from tomato roots buried for 6, 27, and 43 wk. Furthermore, *P. lycopersici* survived as microsclerotia in buried tissue, as was proven by removing single, viable microsclerotia from the extract of buried tissue.

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Corky root of tomato (*Lycopersicon esculentum* L.) is a common disease of field-grown processing and fresh-market tomatoes in California (3). The causal agent of corky root was known as the gray sterile fungus (GSF) until it sporulated and was identified as *Pyrenochaeta lycopersici* Schneider & Gerlach (13). *P. lycopersici* is a slow-growing organism and is difficult to isolate with consistency. Attempts to

isolate the fungus by conventional isolation techniques and media frequently fail because of the presence of saprophytic fungi (3,6; G. G. Grove and R. N. Campbell, *unpublished*). In addition to tomato, many genera and species of the Solanaceae are susceptible based on the occurrence of naturally infected plants or inoculation trials in the laboratory/glasshouse (3,7,8,12,14,15). The only known nonsolanaceous hosts are melon (*Cucumis melo* L.) (10) and cucumber (*C. sativus* L.) (15). Melon naturally infected with *P. lycopersici* has been found once in California (D. Gubler, *personal communication*). Other GSF that may have been *Pyrenochaeta* spp. have been isolated from *Fragaria* × *ananassa* Duch., *Pinus pinea* L., *Picea abies* L.,

*Solanum tuberosum* L., *S. sarachoides* Sendt., *Capsella bursa-pastoris* (L.) Medic., *Chenopodium album* L., and *Amaranthus retroflexus* L. (1,17).

Although *P. lycopersici* is soilborne, there is little information on its survival in soil. The pathogen forms microsclerotia in vitro or in the outer layers of infected roots (1,2,4,9,15,16). The assumption that microsclerotia are the perennating propagules is clear in most references. This assumption has been made because 1) the fungus forms microsclerotia (1,9,16) and 2) the fungus can be recovered from dried root pieces containing microsclerotia (4,9) or from dried soil presumed to contain microsclerotia (15).

The purposes of this study were: 1) to develop a selective medium and an improved method of isolating the fungus, 2) to test crop and weed species for susceptibility to *P. lycopersici* in order to assess their role in the maintenance of the fungus in the absence of tomatoes, 3) to demonstrate the survival of the fungus on infected tomato roots in the field, and 4) to identify the survival propagules.

### MATERIALS AND METHODS

**Selective medium.** A modification of the selective medium of Hockey and Jeves (6) was developed for host range, survival, and tissue isolation studies. The new semiselective medium, corky root medium (CRM), contained potato-dextrose agar (Difco) to which the

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following compounds were added after sterilization and before pouring: 100 µg/ml each of chloroneb and triadimefon (each as 50% wettable powders to give 50 µg a.i./ml) plus streptomycin sulfate, penicillin, tetracycline, chloramphenicol (each at 100 µg/ml), and 50 µg/ml rose bengal. The CRM should be stored in darkness at 2–10 C until use. The CRM was compared with Difco potato-dextrose agar acidified with 25% lactic acid to pH 4.5–4.7 (APDA) in some trials.

**Isolation and identification.** The standard tissue-planting method of isolating from diseased, field-collected tomato roots was followed. Tissue sections 1 cm long were taken from the centers of obvious corky root lesions with a scalpel, placed in a screen-covered jar with soap and tap water, washed for 5 min, surface-disinfested for 30 sec in 1% NaOCl, rinsed with sterile distilled water (SDW), plated on either APDA or CRM, and incubated for 7 days at 22 C. Four tissue sections were added to each plate.

Identification of gray to gray-green colonies thought to be *P. lycopersici* was done by the method of McGrath and Campbell (8): hyphal transfers from CRM were placed on water agar and incubated 3 days at 22 C in darkness followed by hyphal transfer to double-strength V-8 agar and incubation for 30 days at 18 C with an 18-hr photoperiod. Cultures were identified as *P. lycopersici* based on pycnidial, conidial, and conidiophore characteristics (13).

Another method was developed to recover the fungus by blending infected tissue and extracting the fungal propagules. Cortical tissue from lesion centers was removed, washed as described previously, and macerated in 10 ml of SDW with a mortar and pestle. The tissue slurry was then added to 200 ml of SDW and blended for 2 min at high speed in a Waring Blender. Tissue suspensions were then poured into nested sieves with pore sizes of 250 and 38 µm and washed for 5 min with tap water. Washed residue collected on each sieve was backwashed into a 200-ml beaker with 100 ml of 0.525% NaOCl and agitated for 30 sec with a stirring bar on a magnetic stirrer. The disinfestation treatment was terminated by pouring the disinfested root residue back on each respective sieve and washing with SDW for 2 min. Washed debris on each sieve was backwashed into flasks with 50 ml of SDW. One-milliliter samples were pipetted onto four plates of CRM and spread with a sterile glass rod. Plates were incubated 15 days at 22 C. The number of colonies of *P. lycopersici* was determined by visual inspection, and representative colonies were transferred, induced to sporulate, and verified as *P. lycopersici*.

**Survival studies.** Roots 5–10 mm in diameter were collected from severely

diseased tomato plants in a commercial field in the Salinas Valley and cut into 10-cm segments that had lesions on >90% of the surface. Each segment was sewn into a bag made of 10-µm mesh Nitex cloth (Tekto Inc., Elmsford, NY 10523) and buried in a field of Yolo loam soil at Davis, CA, on 25 October 1985. The site had been fallow for 10 yr and was kept free of plants during the study. Bags were buried in sets of eight with four replicate bags each at 15 and 30 cm deep. The center of each set was marked with a steel fence post. The four replicate bags were arranged at 90° to each other and buried at the specified depth 30 cm from the post to which they were attached with monofilament fishing line.

**Host range studies.** Three experiments were completed in controlled-environment chambers; an isolate that had been stored on soil tubes since 1980 was used in two experiments, and an isolate obtained from diseased tomatoes in August 1985 was used in the third trial. Conidial inoculum was produced as described by McGrath and Campbell (8). Conidia were harvested by flooding a plate with 20 ml of SDW and gently scraping the colonies with a sterile scalpel. Suspensions were poured through four layers of sterile cheesecloth. Conidia were counted in a hemacytometer and added to SDW to prepare inoculum so that 1 ml of inoculum added per 10 ml of vermiculite would yield the specified number of conidia per milliliter of vermiculite. Inoculum was atomized into vermiculite as it was tumbled in a rotating drum. About 25 seeds of potential hosts were sown in both infested and noninfested (prepared with equivalent amounts of SDW) vermiculite contained in 200-ml plastic cups with drainage holes. The cups were incubated 30 days in a controlled-environment chamber at 20 C with about 11,000 lux during a 16-hr photoperiod. There were four replicate infested pots for each host and an equal number of noninfested pots. The pots were arranged in a completely random design. Roots were washed free of vermiculite with running tap water, observed for lesions (with a dissecting microscope if necessary), sectioned, surface-disinfested for 30 sec in 0.525% NaOCl, and plated on CRM.

## RESULTS

**Isolation.** The CRM and APDA were compared for isolation of *P. lycopersici* by the standard method of plating tissue sections from root lesions. *P. lycopersici* was retrieved from 30 and 60% of the 40 tissue sections incubated on APDA and CRM, respectively.

The pathogen was recovered by the blending-extraction method from corky root lesions on tomatoes collected in 1985 and 1986. An average of 25 and 26 colonies of *P. lycopersici* grew on plates of CRM inoculated with samples from

the 38- and 250-µm sieves, respectively. Another isolation from roots from a different locality gave an average of 33 and 63 colonies on plates inoculated with samples from the 38- and 250-µm sieves, respectively. The colony numbers have no significance except to indicate that enough colonies were obtained to permit easy selection of well-isolated, representative colonies. Colonies of *P. lycopersici* appeared 4–7 days after plating on CRM; colonies were erumpent and light gray, gray-green, or dark gray. The colony diameter was about 5 mm at 7 days and 10 mm at 14 days. On the reverse, the colonies were typically black with red centers. Contamination by saprophytic fungi was negligible.

**Host range.** Inoculated seedlings of tomato (cultivars Moboglan and VF-145 7879), spinach (*Spinacia oleracea* L. cv. Dixie Market), eggplant (*Solanum melongena* L. cvs. Pompano Pride and PSX 6674), pepper (*Capsicum annum* L. cvs. Yolo Wonder and Mild California), and squash (*Cucurbita pepo* L. cvs. Table Ace and Early Butternut) had necrotic taproots. Inoculated honeydew melon, *Datura stramonium* L., safflower (*Carthamus tinctorius* L.), and cucumber (cultivar Beit Alpha MR) showed slight to moderate discoloration of crowns, taproots, and lateral roots. All infected hosts were stunted compared with uninoculated controls that had no necrosis of the roots. *P. lycopersici* was reisolated from all infected hosts. No symptoms were apparent on roots of beet (*Beta vulgaris* L. cv. Ruby Queen), although *P. lycopersici* was reisolated from inoculated plants. No differences in severity of symptoms were observed when the two isolates were tested on the same hosts.

Symptomless, inoculated species from which the fungus was not isolated included: *Amaranthus hybridus* L., *A. retroflexus*, *Brassica nigra* (L.) Koch, *B. oleracea* var. *capitata* (L.) Alef. cv. Jackpot, *B. oleracea* L. var. *botrytis* L. cv. Snowball Y, *Capsella bursa-pastoris*, *Chenopodium album*, *Daucus carota* L. cv. Hi Color 9, *Echinochloa crusgalli* L., *Fragaria* × *Ananassa*, *Hordeum vulgare* L. emend. Bowden cv. Numar, *Ipomoea hederacea* (L.) Jacq., *I. incarnata* Choisy, *I. quamoclit* L., *I. setosa* Ker., *Lactuca sativa* L. cv. Climax, *Oryza sativa* L., *Phaseolus vulgaris* L. cv. Tendercrop, *P. aureus* Roxb. cv. Yuba mung bean, *Raphanus sativus* L. cv. Chinese White, *Solanum nigrum* L., squash cultivar Small Sugar pumpkin, and *Zea mays* L.

Plants were collected in random locations or in fields with histories of corky root to investigate the occurrence of the fungus in the field. Isolations onto CRM using standard tissue-planting techniques were made from roots, usually of five plants of each species. The plants that had no symptoms and from which the fungus was not isolated

included: *Amaranthus hybridus*, *A. retroflexus*, *Amsinckia* sp., *Brassica nigra*, *Chenopodium album*, *Convolvulus arvensis* L., *Dipsacus sylvestris* L., *Echinochloa crusgalli*, *Erodium mochatum* L'Her., *Helianthus annuus* L., *Martynia lutea* Lindl., *Physalis ixocarpa* Brot., *P. lanceifolia* Nees., *Plantago major* L., *Portulaca oleracea* L., *Prunella vulgaris* L., *Salsola kali* L. var. *tenuifolia* G. F. W. Mey., *Senecio vulgaris* L., *Solanum nigrum*, *S. rostratum* Dunal., and *Z. mays* L. A GSF was isolated from *D. stramonium* specimens with slight discoloration on the taproot, but it did not sporulate.

When the susceptible hosts were known, additional surveys were made to collect spinach and safflower from fields infested with *P. lycopersici* because these crops are grown in winter-early spring, when cool soils should favor infection. Spinach plants with root lesions were collected from one and three fields in Yolo and Monterey counties, respectively. Ten plants with root lesions were collected from each planting, and isolations from each plant were made on CRM medium. *P. lycopersici* was isolated from two specimens collected in Yolo County. Safflower specimens collected from a field in San Joaquin County had symptoms similar to those on tomatoes: a dark brown discoloration of taproot and lateral roots and a very slight cortical cracking. *P. lycopersici* was isolated from 10 of 10 specimens. *P. lycopersici* isolates from spinach and from safflower were pathogenic to tomato seedlings in controlled-environment pathogenicity tests.

**Survival.** Infection of the roots selected for this study was confirmed by plating tissue from a random sample of 10 plants on CRM and recovering *P. lycopersici* from each plant. Sets of buried roots were recovered after 6, 27, and 43 wk and tested for *P. lycopersici* by both tissue-planting and extraction techniques. With the extraction technique, colonies of *P. lycopersici* appeared on plates inoculated with debris collected on both the 38- and 250- $\mu$ m sieves. The mean number (four replicate isolation plates for each of the four root samples) of colonies from the 38- $\mu$ m sieves was 42 and 23 for the samples buried for 6 wk at 15 and 30 cm deep, respectively; 110 and 95 for samples buried 27 wk at the same depths; and 33 and 21 for samples buried for 43 wk at the same depths. *P. lycopersici* also was isolated from debris collected on the 250- $\mu$ m sieves; however, the number of colonies was very variable because of the uneven distribution of inoculum from pipettes. *P. lycopersici* also was isolated from all sections of cortical and stelar tissue plated directly on CRM.

Additional plates were inoculated with extracts collected on the 38- $\mu$ m sieve from roots buried for 27 wk to

demonstrate that the microsclerotia were viable and responsible for the survival of the fungus. Twenty-four hours after transfer to CRM, the plates were observed microscopically for structures resembling germinating microsclerotia (1,9,16). These were transferred to separate plates of CRM with a sterile needle and incubated 7 days at 22 C. In the first trial, fungal colonies developed from 28 of 50 transfers; 23 of the colonies were *P. lycopersici* and the other five were *Fusarium* spp. In a second trial, *P. lycopersici* developed from 21 of 25 propagules; the other four failed to germinate. The mean dimensions of 25 suspected microsclerotia were 63.5 (50–75)  $\times$  44.8  $\mu$ m (31.3–50  $\mu$ m).

## DISCUSSION

The blending-extraction method of isolating *P. lycopersici* on CRM was superior to conventional isolation methods because competition from saprophytic organisms was minimized. Variation in colony numbers between the different samples processed with the improved method were probably due to variations in lesion depth or differences in thoroughness of tissue maceration. In the case of the soil burial trial, the roots buried for 27 and 43 wk were more friable and disintegrated more completely than those buried for 6 wk. It is also possible that the number of microsclerotia was increased in the 27-wk sample by saprophytic growth of the fungus.

Recovery of *P. lycopersici* from tomato roots buried for 43 wk confirms the observations that *P. lycopersici* survives in diseased root tissue (1,5,9,15). Survival of *P. lycopersici* for 43 wk ensures inoculum for successive tomato crops. Decaying, infested root tissue and/or survival propagules can also be moved via cultural practices (11), thus facilitating dispersal and/or distribution of the pathogen. Furthermore, we have demonstrated that the assumptions that the fungus survives as microsclerotia (1,5,9,15) are correct by isolating them directly from buried, diseased tissue. Some presumed microsclerotia did not produce colonies after isolation. Perhaps they were dormant, sensitive to the fungicide(s) contained in CRM (e.g., if they were produced by *Colletotrichum coccoides* [6]), or damaged during transfer. The dimensions of microsclerotia isolated in our trials (63.5  $\times$  44.8  $\mu$ m) were similar to the size ranges of 48–72  $\times$  35–55 (mean 59  $\times$  42) and 38–112  $\times$  9–45  $\mu$ m (mean 65.1  $\times$  32.7  $\mu$ m) for microsclerotia produced in vivo (9,16).

Our inoculation studies confirmed pepper (8,12), melon (10), cucumber (15), and eggplant (5,7) as hosts of *P. lycopersici*. New hosts identified in our trials were spinach, safflower, *Datura stramonium*, and squash, of which the first two also were found naturally infected. Although Termohlen (15)

reported cucumber was a symptomless carrier of a GSF (before it was recognized as *P. lycopersici*), it developed obvious symptoms in our experiments. Conversely, *D. stramonium* was susceptible in our trials but not in those of Termohlen (15). On the other hand, sugar beet was a symptomless host in our studies. *Solanum nigrum* was not susceptible to *P. lycopersici* in our studies, but it is one of many susceptible *Solanum* spp. (3,15). The discrepancies as to species susceptibility in different reports could be due to many factors: differences in the environment to which plants were exposed in the field or in the controlled-environment chamber, differences in pathogenicity among isolates, differences in host genotypes, or the effects of the age of seedlings tested. Thus, all of these species should be regarded as potential hosts. The natural occurrence of the fungus on spinach and safflower doubles the number of families in which hosts are known and which may play a role in maintenance or increase of inoculum.

Hosts of particular interest in California are melon, pepper, and safflower, which are summer crops, spinach, which is a winter crop, and sugar beet, which is a biennial crop. All of these crops are grown in rotation with tomatoes and could increase, or at least perpetuate, the corky root fungus.

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