Existence of Chlamydospores of Alternaria porri f. sp. solani as Overwintering Propagules in Soil

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ABSTRACT

Dark brown, thick-walled, more or less round chlamydospores formed in the "curly type" mycelium of *Alternaria porri* f. sp. *solani* within infected tomato tissues as well as in agar cultures. These varied in diameter from 8 to 15 μ, and occurred in chains, in clusters, or singly. Similar round, thick-walled cells also formed in

normal conidia placed in natural soil. These propagules overwintered in soil, with or without host tissues, through soil and air temperatures ranging from −3.3 to 21.1 C and −31.1 to 27.7 C, respectively, for 7 months or more, and caused primary infection to the next crop. Phytopathology 61: 1347-1350.

Additional key word: fungistasis.

The author noted brown, thick-walled round cells in association with the mycelium and conidia of *Alternaria porri* f. sp. *solani* while examining tomato *(Lycopersicon esculentum)* seedlings with collar rot (15) symptoms. These thick-walled brown cells resembled chlamydospores recorded in other species of *Alternaria* (2, 5, 12, 14). Atkinson (2) remarked that the formation of chlamydospores in *A. raphani* was probably related to its prolonged survival (5 years) in dry soil cultures. In *A. porri* f. sp. *solani*, the cause of early blight of tomato, potato *(Solanum tuberosum)*, and other related crops, no evidence of any differentiated or resistant form of mycelium has been recorded (13, 15), although Angell (1) reported the occurrence of sclerotioid clusters of cells from which conidiophores developed in onion *(Allium cepa)* tissues. However, both conidia and mycelium of the early blight pathogen are known to be remarkably resistant to unfavorable conditions (13, 15). Rands (13) demonstrated the overwintering of conidia in infected potato leaves buried in soil, and believed that these conidia were the source of primary infection for the next crop. The possibility of overwintering of mycelium within host debris has also been suggested, but conclusive evidence was lacking (13).

This paper is on the existence and formation of chlamydospores in *A. porri* f. sp. *solani*, their ability to overwinter in soil, and their role in primary infection in the field.

MATERIALS AND METHODS.—A pathogenic culture of *A. porri* (Ellis) Cif. f. sp. *solani* (Ell. & Mart.) Neerg. was provided by Miss M. E. Elliott, Mycology Section, Plant Research Institute, CDA, Ottawa. Subcultures were made on potato-dextrose or peptone dextrose agar plates (6), on which the fungus developed only aerial and submerged mycelium. Heavy sporulation was induced by scraping off the aerial mycelium from the surface of the culture, then washing it in running water for 24 hr as described by Ludwig et al. (8). Conidia were washed off with sterile water, collected on filter papers, and stored at 5 C until required. Surface-sterilized (2% NaOCl) tomato *(Lycopersicon esculentum* Mill.)

seeds of the cultivar, John Baer, were used to produce disease-free seedlings. The soil mixture, for greenhouse and laboratory tests, was composed of garden loam, peat moss, and sand (2:1:1, v/v) and field tests were carried out in four clay-loam plots, each 16.5× 17.4 m, where tomatoes or potatoes had not been grown before. Each plot was separated from the others by at least 12.2 m, and accommodated 260 staked tomato plants grown in rows 1.4 m apart.

To determine the overwintering of the pathogen in the field as well as to obtain sufficient infected tomato tissues for greenhouse and laboratory tests, 520 fruiting tomato plants, in two of the plots designated "A" and "B", were spray-inoculated with a conidial suspension (4 x 10^4 cells/ml water) in July 1969. All plants became heavily infected in 7-10 days. At the end of the growing season, the plants of plot A were ploughed under, but those of plot B were allowed to remain aboveground throughout the winter months, November 1969 to April 1970. During this period of 181 days, the soil temperatures at 20 cm depth ranged from −3.3 to 10.0 C with 32 days at or below zero, and the air temperatures ranged from −31.1 to 27.7 C with 147 days at or below the freezing point. The depth of snow on the ground varied from 7.6 to 43.6 cm during January, February, and March 1970. The following spring (May 1970), overwintered diseased tomato tissues and associated soil samples were examined for viable structures of the pathogen. After necessary cultivation, tomatoes were planted in the two infested (A, B) and two noninfested plots. Careful observations were made to determine the onset of early blight and the number of infected plants in each plot.

To study the relative ability of the fungus to survive in soil, with and without host tissues, friably moist test soil was mixed with infected leaves or conidial suspensions in the following manner. Infected tomato leaves with 50-75% affected area were dried at room temperature and passed through a 40-mesh Wiley mill to obtain a fine leaf powder containing conidia and mycelium of the fungus. This leaf powder was mixed thoroughly with soil in

amounts of 1, 5, and 10% (w/w). Conidial suspensions were mixed with soil at rates of 1×10^2, 1×10^3, and 1×10^4 cells/g of soil. These two kinds of infested soil were held in 25.5-cm peat pots covered with aluminum foil. Pots without the pathogen were used as checks. Half the number of pots from each set were maintained indoors at 21.1 C, and the other half outdoors, from November 1969 to May 1970. Tomato seeds were sown in duplicate pots (100 seeds/pot) of each treatment, every 5 weeks for 7 months. The frozen pots from outdoors were allowed to thaw for 2-3 days in the greenhouse before seeding. Seedlings showing collar rot symptoms were indicative of the viability and pathogenicity of the organism held in soil for various periods of time.

To observe the fungus in soil, especially in terms of chlamydospore production, the organism was brought in contact with sterilized and nonsterilized soil held in petri plates in the following ways: (i) mycelial or sporulating agar cultures covered with soil; (ii) conidial suspension (4×10^5 cells/ml) placed directly on smooth soil surface as described by Lingappa & Lockwood (7); (iii) conidia embedded on agar slides buried in soil (11); (iv) conidia placed on filter papers covered with soil; (v) conidia placed between two Millipore filter papers (HAWP 04700, pore size 0.45 μ) buried in soil; and (vi) infected tomato leaflets, held between nylon mesh, covered with soil. The soil contained 23% moisture equivalent to 60% of its moisture-holding capacity, and the plates were incubated in a Copenhagen chamber at 27 C to prevent moisture loss (3). In addition to these procedures, the fungus was allowed to grow on agar plates until the medium became dry at room temperature (23 $\pm$ 2 C). The condition of the organism was noted on alternate days after making crushed tissue mounts or soil smears (9).

RESULTS AND DISCUSSION.—Chlamydospores (Fig. 1-6) developed in both mycelium and conidia of A. porri f. sp. solani, especially when the fungus was brought in contact with natural soil by methods described above. Chlamydospores in mycelium were observed in agar media (Fig. 1) as well as in infected tomato tissues (Fig. 2). In relatively old cultures, these were numerous and appeared to be normal components of the "curly type" mycelium (10), and were formed by rounding up of hyphal cells accompanied by thickening and darkening of cell walls. They varied in diameter from 8 to 15 μ and occurred in chains, in clusters, or singly. A chain or a cluster broke up, giving rise to individual chlamydospores which germinated readily in water (Fig. 3).

Chlamydospores developed inside normal conidia in the presence of natural but not sterilized soil (Fig. 4-6). Conidia, when placed on filter papers or embedded on agar slides, produced distinct chlamydospores 7-9 days after they were incubated in soil plates. In 3-4 weeks, 64-86% of the conidia contained 2-3 chlamydospores in each, and these were able to cause infection on tomato leaves.

The process of development of chlamydospores within the conidia of A. porri f. sp. solani resembled the formation of chlamydospores in Fusarium macro-conidia (11). Initially there was a contraction of the protoplasm of individual cells of the conidia followed by rounding up of the cell contents (Fig. 4), or the protoplasm of some cells moved into neighboring ones, making them denser and darker (Fig. 5). This was followed by thickening of the chlamydospore wall apart from the conidium wall (Fig. 6). The diameters of these chlamydospores ranged from 10 to 15 μ.

Germination of chlamydospores was observed on agar medium as well as near the hypocotyl region of tomato seedlings, but no attempt was made to determine if host factors were related to it. However, A. porri f. sp. solani was sensitive to fungistasis, since its conidia did not germinate in nonsterile soil but did in sterilized soil, followed by extensive mycelial growth. In nonsterile soil, there was no new growth of mycelium. These results indicated that factors involved in fungistasis and chlamydospore production might be closely related, as in the case of Fusarium spp. (4, 7, 11).

The pathogen survived in potted soil, with or without host debris, as well as in the field for at least 7 months or more, and infected subsequently planted tomatoes. When tomato seeds were sown in soil infested with infected leaf tissues or conidia, the emerging seedlings became infected and showed collar rot symptoms in 3-4 weeks. The percentage of infected seedlings growing in soil that contained the pathogen, with or without host tissues, indoors or outdoors for 7 months, is given in Table 1. The organism survived equally well in the presence and absence of host tissues, but the effect of varying amounts of initial inoculum on the percentage of infected seedlings was not evident, probably because even the lowest level of inoculum was too high. However, it was clear that 100 conidia/g of soil or 1% infected leaf powder in soil resulted in infection of at least 20% of the seedlings used as a "catch crop" (indoors).

In preinfested field plots, the pathogen survived through soil and air temperatures ranging from −3.3 to 10 C and −31.1 to 27.7 C, respectively. Overwintered, infected tomato tissues (November 1969 to May 1970) and associated soil samples contained viable conidia, chlamydospores, and fragments of the

TABLE 1. The percentage of infected tomato seedlings in soil incubated with varying amounts of inoculum of Alternaria porri f. sp. solani for 7 months, indoors and outdoors, during winter

Proportion of initial inoculum introduced into soil	% Infected seedlings[a] in soil incubated	
	Indoors	Outdoors
1% Infected leaf powder (w/w)	24	8
5% Infected leaf powder (w/w)	23	9
10% Infected leaf powder (w/w)	27	11
1×10^2 conidia/g	20	14
1×10^3 conidia/g	23	15
1×10^4 conidia/g	30	20

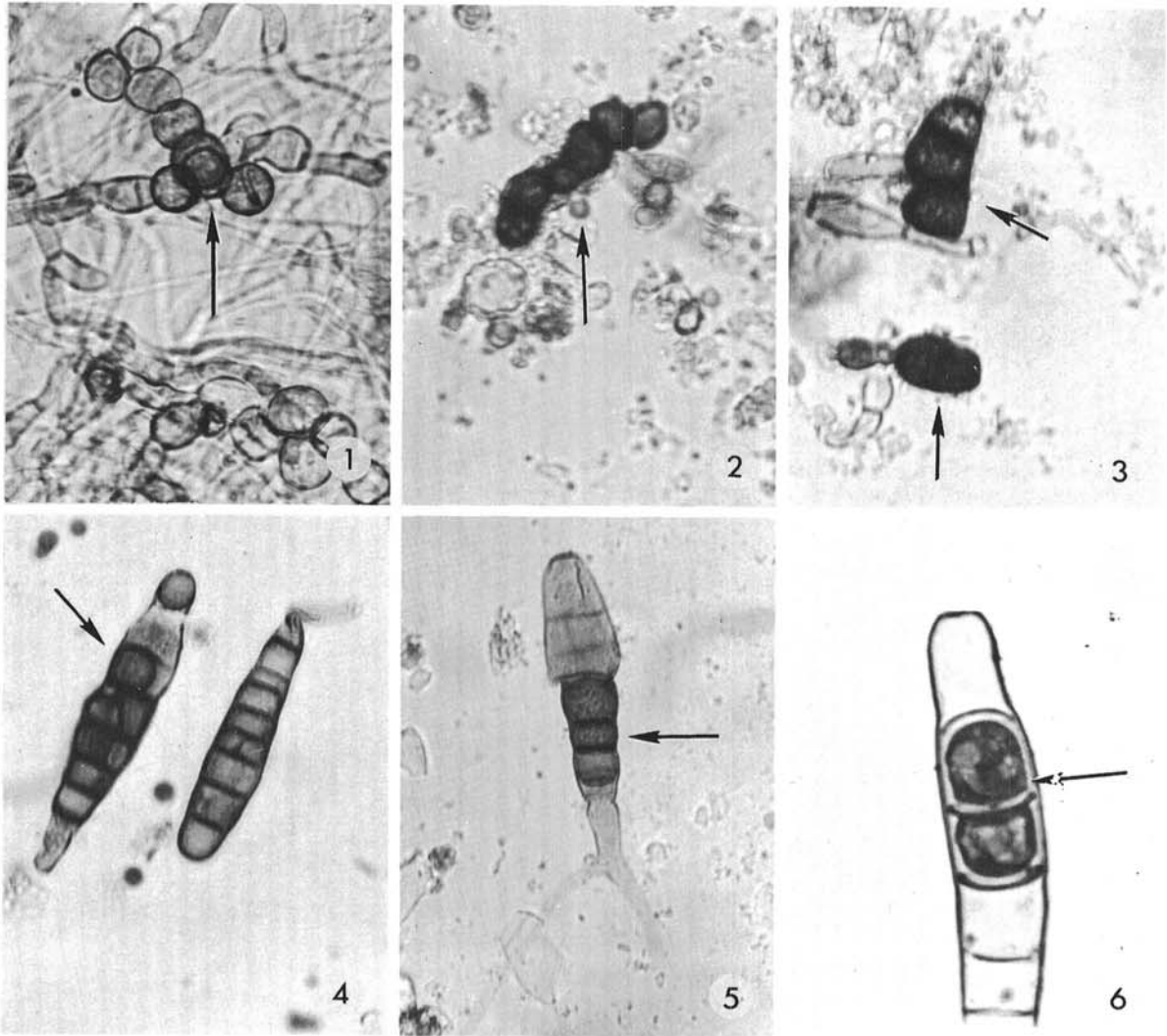[a] Percentage based on 200 seedlings/treatment.

Fig. 1-6) Chlamydospores in mycelia and conidia of *Alternaria porri* f. sp. *solani*: **1)** Mycelial chlamydospores in agar cultures; **2)** chlamydospores in chain from infected tomato tissues; **3)** germinated chlamydospores; **4)** rounding up of cells in the left conidium as compared to a normal one on the right; **5)** protoplasm moved in three cells of a conidium forming chlamydospores; and **6)** thick wall, indicated by arrow, and dense protoplasm of two chlamydospores as observed under oil immersion (×1,000).

mycelium of the fungus. When tomatoes were replanted in the two infested (A, B) and two noninfested plots, all the 520 plants in plots A and B became infected early in the season. Only 5% of the plants in the noninfested plots showed evidence of infection, which was apparently due to the movement of overwintered diseased tissue during cultivation. Careful observation from the beginning of the growing season indicated that primary infection started invariably from the bottom leaves near the ground, confirming Rands' (13) earlier report. It should be noted that the organism survived equally well in plots where diseased plants were ploughed under before winter (plot A) or were left aboveground until the following spring (plot B). This indicated that ploughing under a diseased tomato crop, which is usually practiced, would barely reduce the amount of primary inoculum in the field.

Crop rotation (13, 15) as a means of reducing the primary inoculum has been suggested, but the longevity of chlamydospores of *A. porri* f. sp. *solani* over a number of years in natural soil and the methods for their eradication need further study. In the light of the present work, it would be advisable to remove the diseased plants after harvest, and practice sanitary procedures in order to reduce the number of infective propagules in soil.

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