

Soil Factors Affecting Survival of Microsclerotia of *Verticillium dahliae*

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ABSTRACT

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Survival of microsclerotia (MS) of *Verticillium dahliae* was determined over 60 mo in two soil types (Warsaw silt loam [WSL] and Chelsea loamy fine sand [CLFS]) at constant soil moistures of -0.001 , $-1/3$, and -15 bars matric water potential, fluctuating soil moisture (-0.001 bar to air dry) and constant temperatures of 4 C and 28 C. Survival was poorest in the WSL soil type, -0.001 bar, 28 C. In all other treatments there was an

initial propagule increase (10–80%) over a period of 3–28 mo followed by a gradual decline. Although differences varied, survival of MS was consistently higher in the CLFS soil type; however, soil moisture and temperature were not major limiting factors in survival except at the combined soil moisture extreme of -0.001 bar and the higher temperature.

Studies (1,4,9,12,13) have shown that *Verticillium dahliae* Kleb. (*V. albo-atrum* microsclerotial form) survives for long periods in soil. The primary survival structures are microsclerotia, while mycelia and conidia are short-lived in soil (4,12). Emmatty and Green (2) found that the microsclerotia are sensitive to fungistasis in soil and that germination does not occur except with a suitable carbon source. Root exudates are the most likely consistent nutrient source in soil and germination may be followed by colonization of both host and nonhost plant species (8). Thus, in the presence of crop and weed species, survival of *V. dahliae* may, at least in part, be due to root colonization.

Less is known of the effect of the physical factors of soil on survival of microsclerotia of *V. dahliae*. Nadakavukaren (10) found that survival of microsclerotia in vitro was lowest in flooded soil at temperatures >25 C. Carlstrom (1) infested three different soil types with microsclerotia from potato stems and followed the population under field conditions. Populations initially declined and then increased gradually for the remainder of the study. The increase was attributed to sporulation by the germinating microsclerotia. Also, soil type and geographic location influenced both survival and population fluctuation.

The present study determined the effects of two different soil types at constant and fluctuating soil moisture levels and two different temperatures on survival of microsclerotia of *V. dahliae* over a long time period.

MATERIALS AND METHODS

The two soils used were Warsaw silt loam ([WSL] clay—22% silt—58%, sand—20%, organic matter—2.6%, and pH 5.3) and Chelsea loamy fine sand ([CLFS] clay—2%, silt—10%, sand—88%, organic matter—1.4%, and pH 6.6). The WSL was cropped to corn, soybeans, or wheat for several years and the CLFS was from a fallow area. Neither soil contained an indigenous population of *V. dahliae*.

The drying boundary of the two soils was determined on the sintered glass plate (medium porosity) of a Haine's apparatus (7) and in a pressure membrane apparatus, using nitrogen as the compressing gas (11). The drying boundary of the soils, as determined by the Haine's apparatus for -0.01 and -0.1 bar and by the pressure membrane apparatus for -1.0 to -15 bars, shows the relationship between soil moisture and the corresponding matric water potential (6) (Fig. 1).

The propagules of *V. dahliae* used were primarily microsclerotia

(MS) recovered from naturally infected potato stems. The stems were collected from the field after crop senescence and the microsclerotia were recovered as follows. The stems were split lengthwise and soaked overnight in tap water. The central portion of the stem, consisting of collapsed pith cells, was stripped from the stem and the stem tissues were thoroughly agitated in a large volume of water to free the microsclerotia. These propagules were recovered by wet sieving on a series of US Standard sieves (W. S. Tyler Co., Cleveland, OH 44101) with openings of 250 μm (60-mesh), 149 μm (100-mesh), 74 μm (200-mesh), and 44 μm (325-mesh). The materials collected on the 100-, 200-, and 325-mesh sieves were further separated in a pestle-tube type tissue grinder followed by thorough washing on the same sieves. The MS on the 74- and 44 μm (200- and 325-mesh, respectively) sieves were combined and used to infest the two soils.

Microscopic examination of the final MS suspension indicated that some 30% of stem tissue fragments contained one to three or more MS per fragment.

Microsclerotia were first incorporated into 5 kg of each soil by

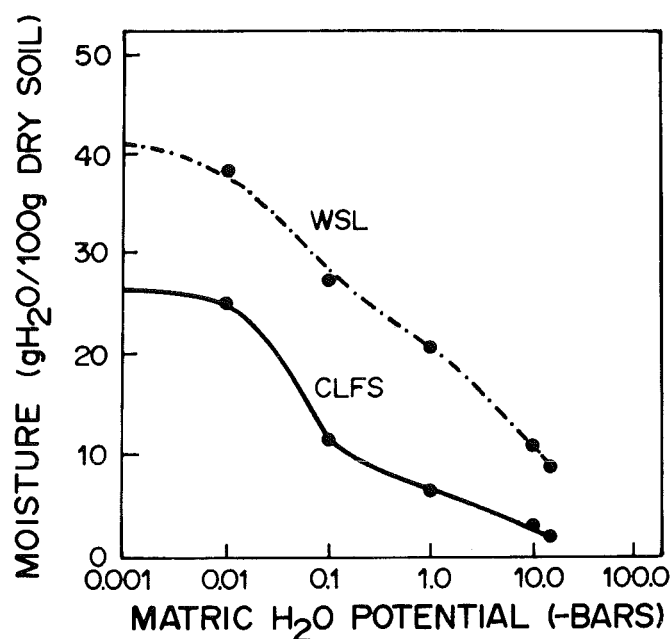


Fig. 1. Moisture characteristics (drying boundary curve) of Warsaw silt loam (WSL) and Chelsea loamy fine sand (CLFS) soils.

spraying the MS suspension on the soil to minimize clumping. These stock soils were mixed and sieved through an 841 μm (20-mesh) sieve for three consecutive days and then stored at a matric water potential of -15 bars for 2 wk at 22 C.

The selective medium described by Green and Papavizas (5) was used to assay the stock soils for viable MS per gram of soil. The final inoculum density in the soil was established by adding sufficient stock soil to noninfested soil for a final inoculum density of 3,000 MS/g soil at -15 bars moisture equivalent. The soil was mixed in a 15.1-L twin-shell blender (Patterson-Kelley Co., E. Stroudsburg, PA 18301) equipped with an intensifier.

The soil (2 kg) was placed in cylindrical plastic containers (15×20 cm), capped with lids and each replication was reassayed to determine the initial MS population. If the initial population varied $\pm 5\%$ from the calculated inoculum density of 3,000 MS/g, the results were adjusted in the calculations of surviving propagules over the course of this study.

The following soil moisture and soil temperature regimes were established: Soil moisture was adjusted to the soil matric water potential of -0.001 bar, $-1/3$ bar, and -15 bars in six replications of each soil type and the containers were closed with a cover that provided a small opening for air exchange. One treatment group was placed in an open container to permit soil moisture to fluctuate from -0.001 bar to become air-dry over each 30-day period. Three replications of each constant soil moisture treatment were placed at

a constant temperature of 4 C or 28 C. The fluctuating soil moisture treatment was held only at 28 C.

Survival of *V. dahliae* MS was determined as follows: After each 30-day period, soil samples were drawn from each replication of each treatment with a 1-cm-diameter soil probe. Two 5 g subsamples (moisture equivalent, -15 bars) were assayed on the selective medium (5). Soil moisture also was determined and adjusted as needed.

In the treatment in which the soil moisture fluctuated from -0.001 bar to air-dry, soil moisture was restored after sampling as follows: The soil was screened through a $0.84\text{-}\mu\text{m}$ (20-mesh) sieve and placed in a shallow container. Water was added with a sprayer with constant mixing to insure more uniform wetting. Final moisture adjustments were made in the storage containers.

Sampling was continued at 30-day intervals for 60 mo.

RESULTS AND DISCUSSION

Survival of *V. dahliae* MS was poorest in WSL soil type at the matric water potential -0.001 bar and a constant temperature 28 C (Fig. 2A). The viable MS decreased from 3,000 MS/g soil to less than 400 in the first 90 days and no viable propagules were detected after 12 mo. More propagules survived in the CLFS soil type than in WSL in the same treatment until after 36 mo. In both soil types, survival was higher at 4 C, but there was a difference in survival

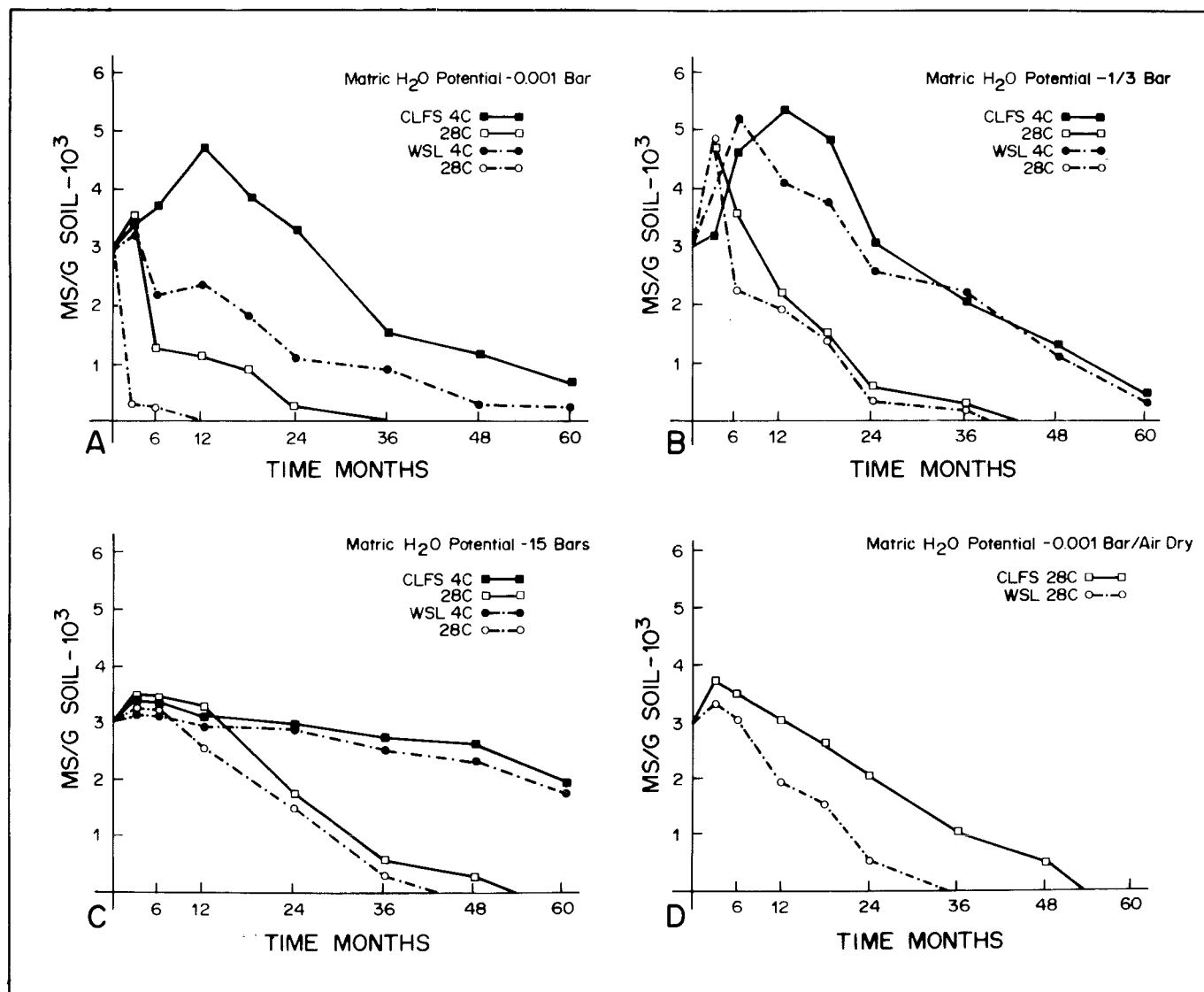


Fig. 2. Survival of microscerotia of *Verticillium dahliae* during 60 mo in Warsaw silt loam (WSL) and Chelsea loamy fine sand (CLFS) soil types at constant soil temperatures of 4 C and 28 C and at matric water potentials of: A, -0.001 bar; B, $-1/3$ bar; C, -15 bars; and D, -0.001 bar to air-dry (30-day cycle), 28 C.

between the soil types. Also, in these and other treatments there was an initial increase in viable MS during the first 3–28 mo. This occurred in all treatments except WSL at -0.001 bar and 28 C (Fig. 2A–D). The increase varied from 200 (WSL at -15 bars and 4 C) to more than 2,400 MS (CLFS at $-1/3$ bar and 4 C) above the original 3,000 MS/g soil.

With the soil matrix water potential $-1/3$ bar, survival characteristics of MS of *V. dahliae* was similar in the two soils at the same temperatures. No viable MS were found after 39 mo in WSL and 42 mo in CLFS at 28 C (Fig. 2B). At 4 C, viable MS were found (WSL, 380 MS/g; CLFS, 570 MS/g) after 60 mo. In this treatment the increase in propagules during the first 3–16 mo also was greater than in all other treatments.

Survival characteristics also were similar in the two soil types at the same temperatures when the soil matrix water potential was -15 bars (Fig. 2C). No viable MS were found after 42 mo in WSL and after 52 mo in CLFS. Survival was highest in both soils at this moisture regime at the soil temperature 4 C; however, the increase in viable propagules during the first 12 mo was less than in any other treatment.

When the soil temperature was constant (28 C) but the soil moisture fluctuated from -0.001 bar to air-dry over a 30-day period (Fig. 2D) the survival characteristic of *V. dahliae* MS in the two soils was similar to the treatment of constant -15 bars, 28 C. There was a slight increase in viable propagules during the first 3–6 mo, followed by a decline until no viable MS were detected after 34 mo (WSL) and 51 mo (CLFS).

Although differences varied depending upon the treatment, survival of *V. dahliae* MS was consistently higher in the CLFS than in the WSL soil type. The greatest differences in MS survival in the two soil types occurred at -0.001 bar matric water potential at both 28 C and 4 C. This may have been due, in part, to the difficulty in maintaining a constant -0.001 bar soil moisture. However, the tendency sometimes was for the lighter soil (CLFS) to have free water on the surface so that “flooding” alone was apparently not a factor in MS survival. Carlstrom (1) also found that survival characteristics of MS differed in different soil types both in the field and under laboratory conditions.

The explanation for the increase in viable MS during the first 3–28 mo in all treatments except WSL at -0.001 bar and 28 C is not clear. Both Farley et al (3) and Carlstrom (1) reported similar increases and attributed the increase to sporulation (conidia) by germinating MS. Farley et al (3) illustrated spore production in soil that was first dried and then wet either with 0.1% sucrose solution or water. Emmatty and Green (2) found no sporulation by germinating microsclerotia in sucrose-amended soil, but they did detect the development of viable “secondary” microsclerotia. In the present study, intermittent wetting-drying cycle did not alter either the temporary increase in viable propagules observed in other treatments in the first 6–28 mo or the effects of soil types on survival.

When microsclerotia were recovered from infected plant tissues

it was virtually impossible to completely separate these propagules from fragments of plant material. As indicated, microscopic examination of the final MS suspension showed some 30% of the tissue fragments contained one-to-three or more MS per fragment. On the assay plate, growth from these fragments appeared as a single colony. From the data, the magnitude of the increase in viable propagules (10–80%) suggested that MS from tissue fragments were released by microbial degradation of the fragments over time. Also, the maximum population occurred within 3–6 mo in both soil types in all treatments at 28 C, but did not occur until after 12 mo or longer at 4 C. This also suggests release of MS from tissue fragments rather than sporulation.

Soil type appears to have some influence on survival of MS of *V. dahliae*, however, these results suggest that soil moisture and temperature are not major limiting factors in the survival of these propagules except at the moisture extreme of soil saturation (-0.001 bar) combined with the higher temperature.

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