Characteristics and Concentration of Propagules of Verticillium dahliae in Air-Dried Field Soils in Relation to the Prevalence of Verticillium Wilt in Cotton

J. E. DeVay, Linda L. Forrester, R. H. Garber, and E. J. Butterfield

Department of Plant Pathology, University of California, Davis 95616; and USDA Cotton Experiment Station, Shafter, California 93263.

We appreciate the financial support for this work by the California Planting Cotton Seed Distributors; the field assistance of Mr. O. D. McCutcheon, Farm Advisor, Kings County; the photographs by Mr. Jeff Hall; and the assistance of J. Hills and L. Leach with statistical analyses. We also thank the many cotton growers who helped with this study.

Accepted for publication 15 June 1973.

ABSTRACT

Viable microsclerotia of *Verticillium dahliae* were recovered from field soils that had been air-dried for at least 6 wk. These microsclerotia, ranging from 11 to 225 μ in widest dimension, consisted of clusters of hyaline cells (7-8 μ in diam) with thickened walls which were colorless to slightly pigmented. After 6 hr in a favorable environment, microsclerotia began to germinate, commonly producing up to 36 germ hyphae and several sporophores with verticillate branches and conidia. The correlation coefficient (r=-0.139) for the relationship between the concn of microsclerotia in air-dry soil samples from different fields and percentage of diseased plants (foliar symptoms) was not significantly different from zero; similarly, the relationship involving samples from a single field was again nonsignificant (r=0.175). These data indicate that the concn of microsclerotia in air-dry field soil is probably not the limiting factor in disease development.

Phytopathology 64:22-29

Additional key words: inoculum density, soil plating, epidemiology.

Verticillium wilt of cotton (*Gossypium hirsutum* L.) is a major problem and frequently a limiting factor in cotton production in some areas of the San Joaquin Valley. Efforts to control the disease have included cultural practices such as controlled irrigation and nitrogen applications, high plant populations, deep plowing, crop rotation, soil flooding, wilt-tolerant cotton varieties, fumigants, and systemic fungicides (6). Continuous culture of cotton, which is practiced by many growers, has increased the prevalence and severity of the disease and reduced the effectiveness of various control methods. At present, the best measures for reducing yield losses from Verticillium wilt are the use of wilt-tolerant varieties and crop rotations (13, 21, 37), but these may be relatively ineffective when soil propagules of *V. dahliae* are dense enough to cause near 100% prevalence of the disease (26). Moreover, under cool temperatures which favor wilt development (12, 16, 18), wilt-tolerant cotton varieties become highly susceptible to defoliating strains of *V. dahliae*. In fields where inoculum concns of *V. dahliae* cause near 100% prevalence of diseased plants, lowering the inoculum density may require soil flooding (29) or soil fumigation (5, 26, 39, 40) followed by grass crops such as barley (*Hordeum vulgare* L.), grain sorghum (*Sorghum vulgare* Pers.), or corn (*Zea mays* L.). Removal of cotton stalks, leaves, and upper roots has had little or no effect on incidence of Verticillium wilt (14). The effectiveness of cultural practices in controlling Verticillium wilt of cotton, as was emphasized by Ranney (28) and Turner et al. (37), has been well demonstrated on several large cotton acreages in the San Joaquin Valley (26, 37).

A major problem in studying inoculum concentration of *V. dahliae* in field soils, has been lack of a method for accurately estimating the number of infective units or propagules in relation to disease prevalence. In fact, the exact nature of the infective unit under field conditions is still unknown. Garber and Houston (15) convincingly demonstrated the infective nature of conidia and hyphal germ tubes of *V. dahliae*, and it is reasonable to speculate that both of these are actual infective units under natural conditions. However, the relative importance of conidia is still unknown (7, 17). Several published methods for estimation of soil propagules of *V. dahliae* (2, 11, 23, 25, 27) have been of limited use for our studies on air-dried field soils. The method of most use has been that described by Harrison and Livingston (19), in which 10-mg amounts of dry soil are distributed evenly with an Anderson Sampler through sieve plates onto agar medium in petri plates. To permit direct observations and determination of the concn of viable propagules of *V. dahliae* persisting in air-dried soil, we have modified their method to include Menzies' soil extract agar (27) and a layer of cellophane on the agar surface (2, 34).

The present study was done to describe the microscopic viable propagules of *V. dahliae* recovered from air-dried field soils, and to determine whether their frequency in soil was related to the prevalence of Verticillium wilt in cotton. A preliminary report of some of this work has been made (8).

In previous work we included microsclerotial strains of Verticillium under the specific name *V. albo-atrum*; we now use the name *V. dahliae* in view of the original descriptions of *V. albo-atrum* and *V. dahliae* and various cultural characters as interpreted by Isaac (22), Schnathorst (32), and Smith (35). It is apparent that these species are distinct, with *V. dahliae* forming microsclerotia, whereas *V. albo-atrum* forms one- and two-celled conidia and sporophores with black bases.

MATERIALS AND METHODS.—*Soil collections.*—Soil samples of about 1 liter each were collected about 10 to 20 cm below the soil surface at three or four locations between rows (10-ft sections) of 'Acala SJ-1' cotton ranging from 0 to 100% diseased plants. Sites were chosen on the basis of percentage diseased plants at the time of soil collection. In making soil collections, the surface soil was scraped away because the texture was

uneven and surface temperatures (>50 C) were high on hot days. Soil was collected from 51 sites in 13 fields just prior to defoliation in preparation for harvest of cotton. The soil was air-dried for 6 wk at 30-50% relative humidity to eliminate short-lived propagules such as conidia and mycelial fragments, and then mixed for 20 min in a stone mill. The stone cylinders used (2.0 cm × 2.0 cm in diam) and the time of rolling did not change the propagule count in test soils. Plant debris was well-decomposed in the soil that was collected, and no macroscopic pieces were included in the analyses.

Soil bioanalysis.—Amounts of air-dry soil varying from 5 to 500 mg were distributed evenly with an Anderson Sampler (1074 Ash Ave., Provo, Utah) through six sieve plates (Fig. 1) onto six plastic petri plates (15 × 100 mm), each containing 20 ml of soil extract agar (27) covered with sterile cellophane pressed to the agar surface. Since *Verticillium* spp. are highly cellulolytic (36), the cellophane increased the selectivity of the soil extract agar. The plates were incubated at 24-26 C, and colonies of *Verticillium* with microsclerotia were counted by the 10th day. When germination sequence was being followed in a propagule suspected of being *V. dahliae*, it was marked with a sterile piece of camel's hair to facilitate later identification. For most soil analyses, 10-mg amounts of dry soil were plated on a series of six petri plates. The soil particles were separated enough for identifying individual propagules and following their

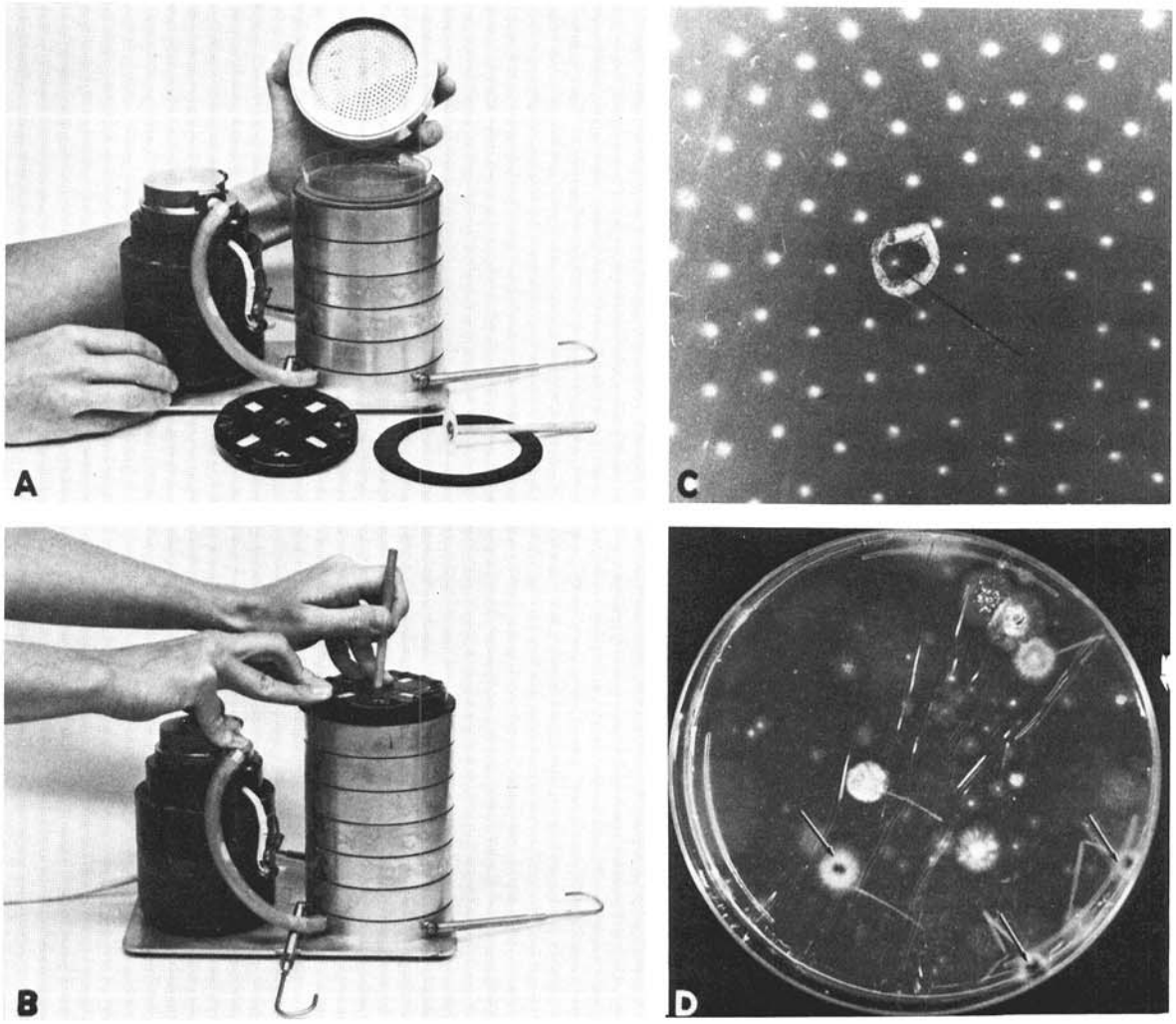

Fig. 1. Anderson Sampler used in soil bioanalyses. **A)** Vacuum pump which draws 0.028 m^3 (1 ft^3) of air/min over six sieve plates with pore sizes from top to bottom of 1181, 814, 711, 533, 343, and 254 μ. An open plastic petri plate is placed beneath each sieve plate. **B)** Soil is placed on a narrow plastic disk attached to the bottom of a dowel with a collar which, when pulled against an opening (9-mm diam) in a plastic petri plate cover placed over the top sieve, causes a partial vacuum to be drawn on the sieves and petri plates. When the dowel is pushed away from the opening, a sudden release of vacuum causes the soil to impact on these petri plates. **C)** Each sieve has 400 openings, which helps quantitative estimation of fungal colonies. Specific propagules are located with sterile camel's hair to help identify them for following a germination sequence. **D)** After 10 days, colonies of *Verticillium dahliae* (see arrows) are distinguished and counted on the basis of microsclerotia formation.

germination and the subsequent development of mycelium.

Pathogenicity tests.—Isolates of *V. dahliae* were subcultured from the assay plates directly onto potato-dextrose agar (PDA). After approximately 1 wk, conidia were suspended in water (10^7 conidia/ml), and stems of three SJ-1 cotton plants with first-lobed leaves were inoculated with a droplet (25-50 μl) of conidial suspension. In other tests, the root ball was sprayed with 10 ml of a conidial suspension (2×10^6 conidia/ml). Control plants were treated with distilled water. The plants were grown in the greenhouse in U. C. Mix (3) in 10.2-cm (4-inch) diam pots, and disease ratings were made 3 to 4 wk after inoculation.

Artificially infested soil.—The accuracy of the soil bioanalysis was determined on soil artificially infested with *V. dahliae*. The soil was prepared using microsclerotia from cultures grown on cellophane overlaid on PDA. The microsclerotia were scraped from the cellophane, dried, ground to a powder, then mixed with steam-sterilized sandy loam soil. Desired concns of microsclerotia were obtained by diluting the soil with sterile soil.

RESULTS.—*Soil bioanalysis and sample size.*—The relation between soil sample size and the number of viable propagules recovered was determined in two tests, one using field soil naturally infested with *V. dahliae* and a second using sterile soil artificially infested with microsclerotia. The results are shown in Figs. 2 and 3; each point represents an average of three analyses. The values on which the average values were calculated in Fig. 2 and 3 varied from 0-260 propagules/gram of soil. In the range from 5 to 500 mg, the 5-mg samples gave the highest counts of viable propagules/g soil. In the naturally infested soil, samples larger than 100 mg were difficult to analyze because of excessive microbial growth and inhibition of *Verticillium* spp. by other fungi, such as *Trichoderma* spp. With samples larger than 100 mg, the mass of soil on each plate interfered with identification of individual propagules and counts of individual colonies. The shape of the curve for naturally infested soil (Fig. 2) suggested a marked effect of soil fungistasis; the similar results with artificially infested sterile soil (Fig. 3), however, indicated that the physical mass of the soil sample also interfered with recovery. In addition, the

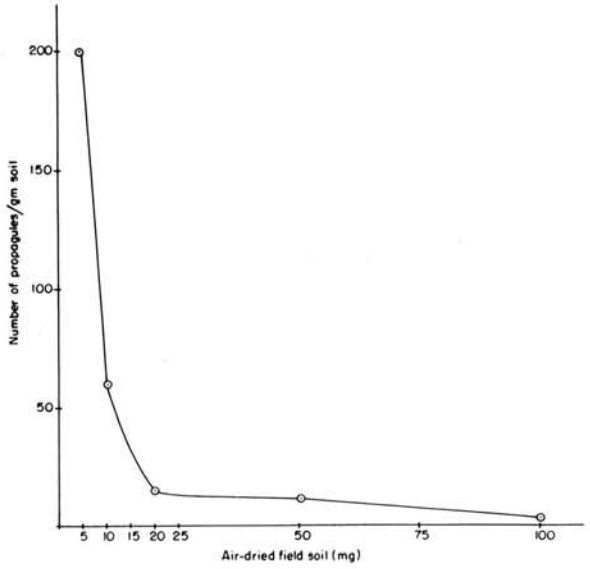

Fig. 2. Relationship of size of soil sample to the number of viable propagules of *Verticillium dahliae* recovered/g of air-dried natural field soil.

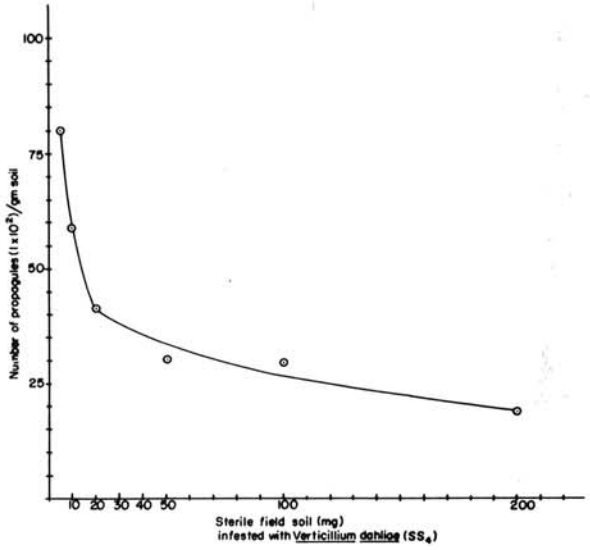

Fig. 3. Relationship of size of soil sample to the number of viable propagules recovered/g of sterile field soil infested with microsclerotia of *Verticillium dahliae*.

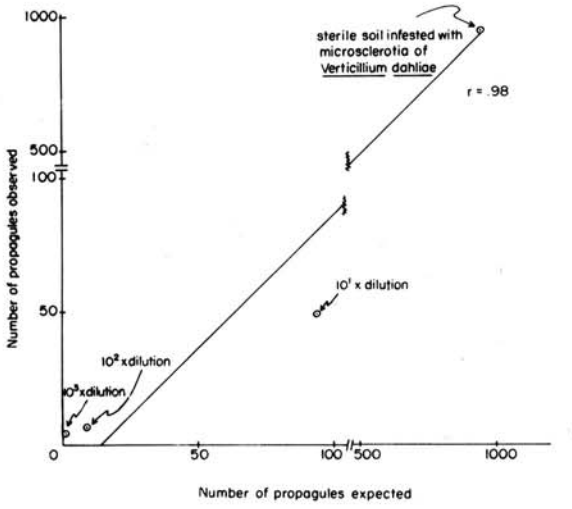

Fig. 4. Linear regression showing how the number of viable propagules expected in a sterile soil infested with microsclerotia of *Verticillium dahliae* is related to the number observed in bioanalyses of three ten-fold dilutions of the soil with sterile soil. The correlation coefficient is significant at the 1% level.

efficiency of soil plating decreased rapidly when soil samples were larger than 10 mg; the amount of soil impacted on cellophane from 10-mg samples was 97.5% of the total, whereas the amount recovered from 100-mg samples was only 63.6%. With large soil samples, appreciable amounts of soil were deposited on the sieves and on the filter leading to the vacuum line. For this reason, 10-mg samples were used in experiments on the

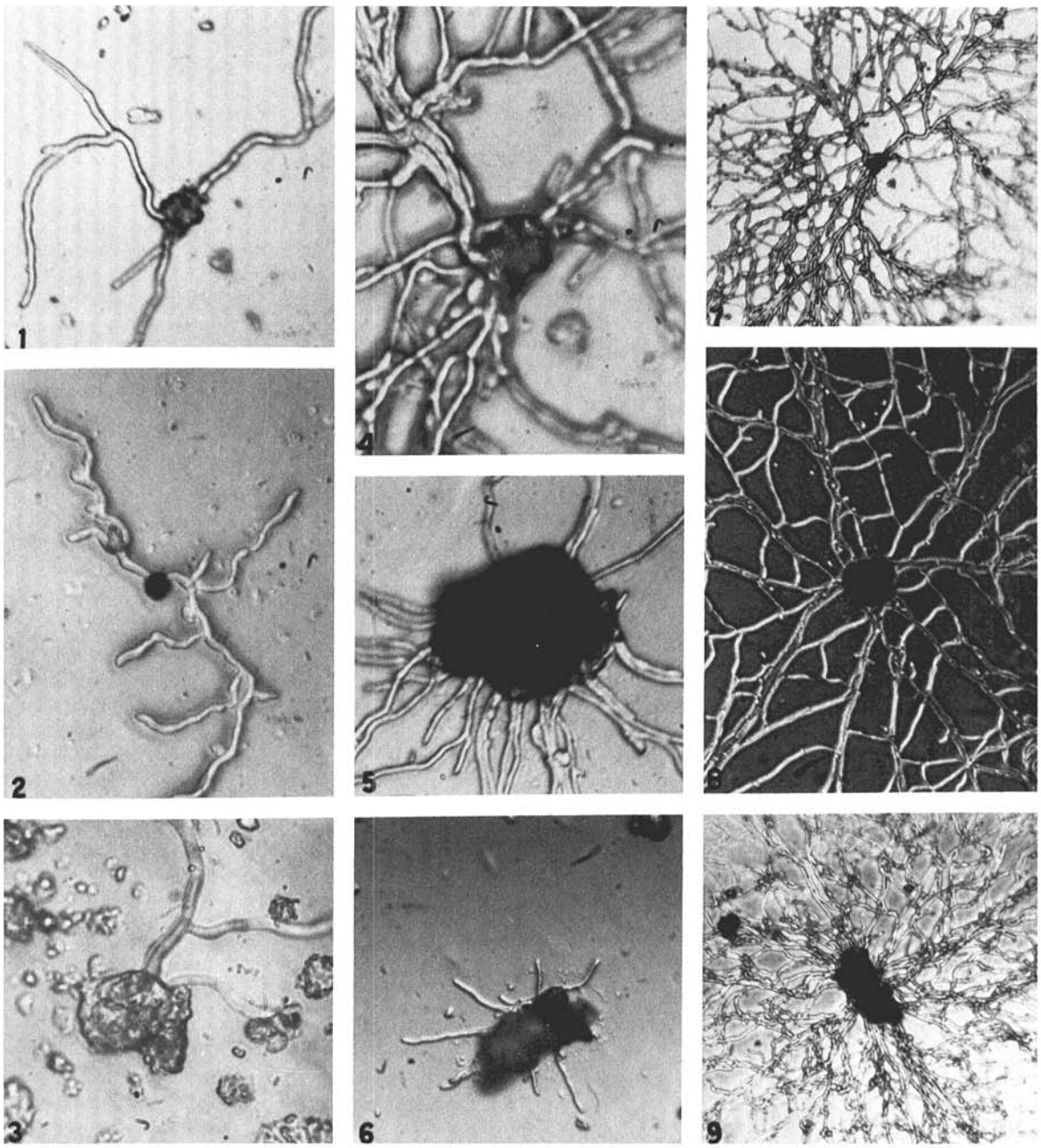

Fig. 5-(1-9). Microsclerotia of *Verticillium dahliae* recovered from field soil. Photos 1, 4, and 7 show a propagule (32 × 15 μ) at different magnifications and different stages of germination (**1**, after 41 hr; **4** and **7** after 65 hr); **2**, propagule (11 × 11 μ) 40 hr after germination; **3**, propagule (54 × 32 μ) 24 hr after germination; **5**, propagule (130 × 108 μ) 17 hr after germination; **6**, propagule (225 × 108 μ) 16 hr after germination; **8**, **9**, propagules (36 × 54 μ and 102 × 54 μ) 69 and 4 hr after germination, respectively. Germination usually begins after 6 hr in a favorable environment; multiple germ tubes are common. Microsclerotia in these photographs have hyaline cells but appear dark because of the focus or their thickness.

accuracy of the method, and for estimation of soil propagules in field soils.

Accuracy of the soil bioanalysis method.—Heat-sterilized soil infested artificially with SS4, a typical nondefoliating pathotype of *V. dahliae*, was diluted 10-fold three times, and 10 mg samples of the dry soil were analyzed. Recovery of the viable propagules at the different dilutions was nearly linear (Fig. 4), with a correlation coefficient of 0.98. Each point in Fig. 4 represents an average of three analyses.

Characteristics of soil propagules.—Figure 5 shows the kinds of viable propagules of *V. dahliae* recovered from soils planted to cotton. They were microsclerotia ranging from 11 μ (Fig. 5-2) to 225 μ (Fig. 5-6)in greatest dimension and consisting of culsters of mainly thick-walled hyaline to lightly pigmented cells, 7-8 μ in diam. Some appear dark in Fig. 5 mainly because of the focus of the microscope, the density of the cell clusters, or the thickness of the lightly pigmented cell walls. When soil was plated onto cellophane, most cells of the microsclerotia began to germinate after 6 hr; propagules producing up to 36 germ tubes were common (Fig. 5), which indicates absence of the self-inhibition of cell aggregates that characterizes certain other fungi. Figure 5-1, 4, 7 shows stages in the germination of a propagule from soil at 41 hr and 65 hr. Figure 5-4 is the same as Fig. 5-7 but at greater magnification. Sporophores and conidia were commonly produced by the propagules in addition to germ hyphae. Microsclerotia such as in Fig. 5 have been described in tissues of cotton plants by Ashworth et al. (2), Brinkerhoff (4), Evans et al. (10), and Schnathorst (31). We have seen such microsclerotia in cotton stalks also, especially in plant material buried in soil for several months; when isolated from plants and placed in 3% KOH, cells of the young microsclerotia are separable and appear hyaline to slightly pigmented and thick-walled (Fig. 6A, B); if placed on an agar medium they germinate (Fig. 6C). Cell clusters of young microsclerotia from cultures of *V. dahliae* on agar media tend to resemble the viable microsclerotia from soil or diseased plants (Fig. 6); however, the black melanized microsclerotia typical of older cultures were uncommon in our soil and plant samples.

When germinating microsclerotia from field soils were marked with camel's hair, less than 10% thus identified gave rise to colonies of *V. dahliae*. Many of these cell clusters from soil gave rise to *V. nubilum* Pethyb., which produced gray colonies and many chlamydospores when transferred to PDA. Since these isolates in vitro did not produce microsclerotia like those of *V. dahliae*, they were apparently not *V. tricorpus* Isaac.

Data presented in Figs. 2, 3, 4, 5, 6C, 7, and 8 refer only to propagules which produced colonies of *V. dahliae* and which were positively identified on the basis of microsclerotia formation on agar medium. We found that it was impossible to distinguish between aggregates of chlamydospores of *V. nubilum* and microsclerotia of *V. dahliae* in soil samples because of their similarity in structure; only by the germination of these propagules and the production of microsclerotia or chlamydospores could the species be discerned. Evans et al. (10) also isolated microsclerotia from soil preparations and viewed them through the compound microscope. Of 25 units cultured, 17 yielded *Verticillium* spp.; they did not, however, state which species of *Verticillium* were recovered. Isaac et al. (23) reported that soils from potato fields contained relatively few propagules of *V. dahliae* compared with other species of *Verticillium* they recovered.

Among the fungi regularly isolated in high numbers from field soils by this plating method were *Cephalosporium* spp., *Stachybotrys* sp., *V. nubilum, V. lateritium* Berk., and *Trichoderma* sp.

Pathogenicity of recovered isolates of Verticillium species.—Pathogenicity tests of 40 isolates of *V. dahliae* recovered at random from soil platings indicated that 33 of the isolates were nondefoliating pathotypes, three were defoliating pathotypes and four were nonpathogenic. Of isolates from randomly picked leaves of diseased cotton plants in 10 fields with a high percentage of nondefoliating isolates from soil, 2% were nondefoliating whereas 98% were defoliating pathotypes. It appears that the propagules recovered from soil do not reflect the pathotypes most prevalent in diseased plants. A possible reason is that the nondefoliating pathotype of *V. dahliae* requires at least 100 times as many infective propagules per unit volume of soil as does the defoliating pathotype to cause a severe disease reaction in cotton cultivar 'Acala 4-42' (32). The virulence advantage of the defoliating pathotype (T-9) over the nondefoliating pathotype (SS-4) may be due to its tolerance of the high temperature (33 C), that prevents growth of the nondefoliating pathotype, and a rate of conidia production which is twice that of the nondefoliating pathotype (41).

When conidial suspensions (10^7 conidia/ml) of eight isolates of *V. nubilum*, four isolates of *Stachybotrys* sp.,

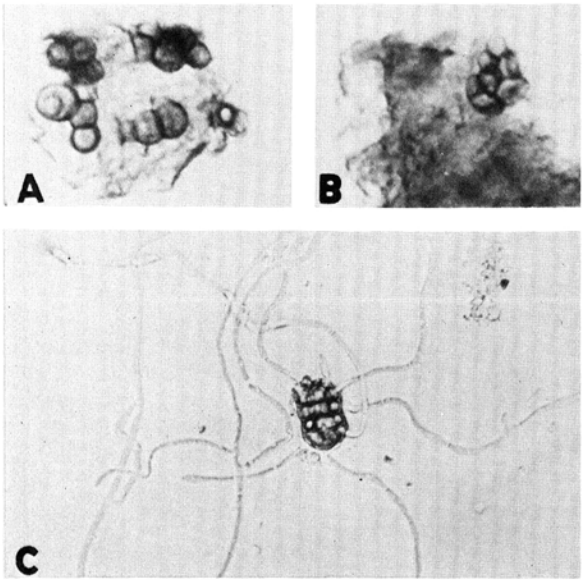

Fig. 6. Microsclerotia of *Verticillium dahliae* recovered from pieces of cotton stalks which overwintered in soil. **A)** Small clusters of hyaline microsclerotial cells closely resembling branching chlamydospores of *V. nubilum* (see Ref. 20, Fig. 5). **B)** Typical microsclerotium with hyaline cells. **C)** Microsclerotium 27 hr after germination with hyaline cells and slightly pigmented cell walls.

or 25 isolates of *Cephalosporium* spp. were injected into stems of cotton plants, none were pathogenic.

Relationship of concn of microsclerotia of V. dahliae in soil to percentage diseased plants.—Figure 7 shows how the number of microsclerotia of *V. dahliae*/ g of soil is related to the percentage of diseased (foliar symptoms) plants at the soil collection sites. Each value represents an average of three analyses. When results from 30 different sites in 13 fields were analyzed, the correlation coefficient (r=–0.139) was not significantly different from zero, indicating that the count of viable microsclerotia in air dry soil is not a reliable indicator for determining the potential of Verticillium wilt in cotton fields. When 21 soil samples from within a single field were compared, the correlation coefficient (r=0.175) was again nonsignificant (Fig. 8). In the comparisons of samples from a single field, 12 soil collections had 0 microsclerotia but the collection sites for these samples varied from 5 to 58% diseased plants; whereas, one site with 160 microsclerotia/ of soil had no diseased plants. These data indicate that microsclerotia are not distributed uniformly in soil and that they are not necessarily the factor determining the prevalencew of diseased plants. Isaac et al. (23) also found that distribution of viable propagules of *V. dahliae* was rarely uniform in soil samples.

DISCUSSION.—Many articles on the microsclerotial form of *Verticillium* have dealt with the taxonomy (22, 32, 35), nature, distribution and longevity of inoculum of this fungus in soil (12, 25, 38), the relative merits of different types of propagules as inoculum (17),

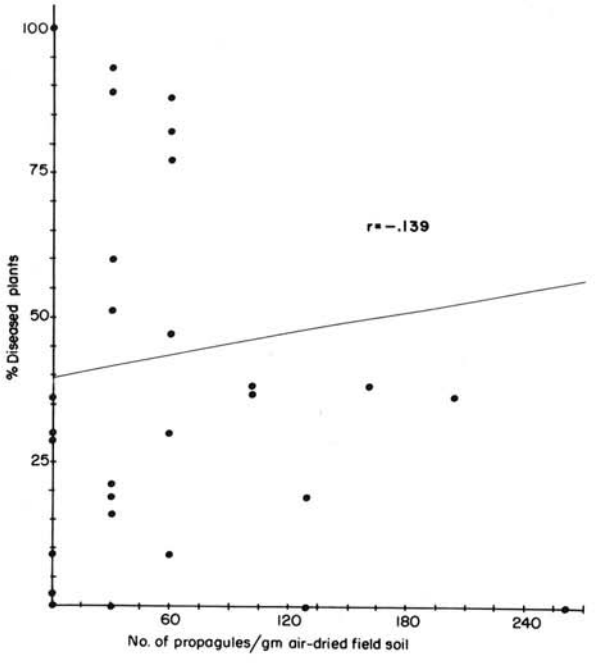

Fig. 7. Linear regression showing how the number of microsclerotia of *Verticillium dahliae* that germinated/g of air-dried natural field soil is related to the percentage diseased plants (foliar symptoms) from 30 different sites in 13 fields. The correlation coefficient is not significantly different from zero.

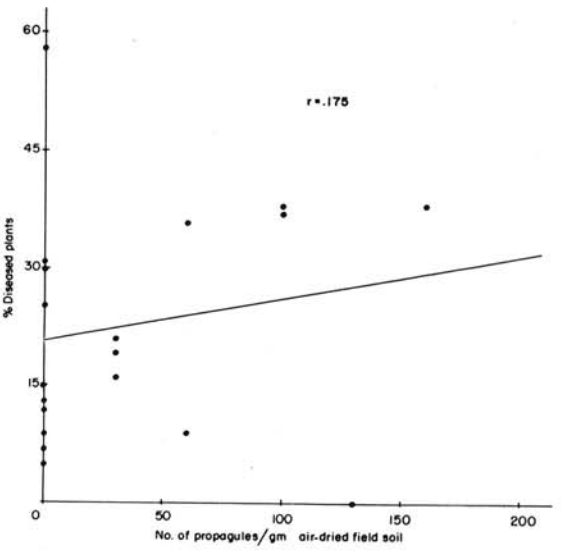

Fig. 8. Linear regression showing how the number of microsclerotia of *Verticillium dahliae* that germinated/g of air-dried natural field soil is related to the percentage diseased plants (foliar symptoms) from 21 different sites within a single field. The correlation coefficient is not significantly different from zero.

methods for estimating the concn of inoculum (2, 11, 17, 19, 23, 27), and descriptions of the size (1) and germinability of differently pigmented cells of microsclerotia (5, 12, 24, 30). Still, the exact nature of the infectious propagule(s) of this fungus under natural conditions remains unknown; the controversy is still not settled over the role of microsclerotia as the most important soil propagule (23); and no general agreement has been reached on the specific name. The succession of methods for estimation of soil propagules, each indicating the inadequacy of previous ones, further attests to the diversity of views on the biology of this highly important plant pathogen.

This paper focuses on the nature and concn of microscopic soil propagules which persist in air-dry soil. As presumed by other workers(2, 11, 12, 17, 24, 27),these propagules are microsclerotia; they consist mainly of hyaline to lightly pigmented cells, 7-8 μ in diam. These propagules are not uniform in size but vary from 11 to 225 μ in widest dimension. Under favorable temperature and moisture, most cells germinate, thus showing no self-inhibition; also produced are sporophores with verticillate branches and conidia. It might be predicted that what we have observed in soil platings also occurs in field soil. Until direct observation is made, however, there is still reasonable doubt. Farley et al. (12) reported repeated germination of microsclerotia in soil but have extrapolated in vitro data obtained from microsclerotia produced on agar medium to a field situation. The high degree of melanization characteristic of most microsclerotial cells produced in culture was not characteristic of viable microsclerotia recovered from field soils that had hyaline to lightly pigmented cells. Therefore, germination data for microsclerotia produced in vitro may not be applicable to field conditions.

The method of soil bioanalysis used here was adapted from that of Harrison and Livingston (19); using their soil samples we were able to confirm the concn of microsclerotia tthey found in soil (approximately 150) from potato fields with a history of severe wilt. The use of cellophane-covered agar (2, 34) and smaller soil samples allowed us to study the propagules in detail and make a time-course study of their germination. The dry-soil plating method used was highly sensitive in that each 10 mg sample of soil was distributed through 2,400 sieve openings over six petri plates which facilitated the quantitative assay of high numbers of microsclerotia per gram of soil. Microbial antagonists and mycostasis observed by others using loam soils (23) was largely overcome with this method of sampling. Methods involving soil-dilution assays, such as those used by Green (17) and Jordan (25), yielded higher numbers of propagules/g soil but their assays included both conidia and microsclerotia. Figure 2 and 3 show that the size of the soil sample which is plated affects the concn of propagules of *V. dahliae* recovered.

The minimum number of microsclerotia of *V. dahliae*/g of dry soil that is required to cause a prevalence of 100% disease in greenhouse tests with cotton is considered to be about 10^4 on the basis of soil inoculation (33). For potatoes, more than 40 propagules/g of soil are required at planting time (23) to induce disease. We found that air-dried soils from different fields showed an extremely low correlation (r=–0.139) between the concn of microsclerotia of *V. dahliae* and percentage of diseased plants (Fig. 7). When soil samples from a single field were analyzed (Fig. 8) the correlation (r=0.175) also was nonsignificant. These data indicate that the microsclerotial concns in air-dry samples of field soils do not reflect the percentage diseased plants growing in those soils.

The soil bioanalyses in the present study omitted conidia and mycelium, both free in the soil and in macroscopic pieces of plant material in soil; the latter has been considered to be the most potent source of inoculum (7). In addition, disease development also appears to be influenced by factors such as crop history, nitrogen fertilization, soil moisture, and aeration, plant populations, and changes in the population of soil microorganisms (9, 13, 21, 37).

The production of multiple germ tubes and conidia by microsclerotia has a definite bearing on inoculum potential and may be an important variable. We were able to make limited use of our soil bioanalyses for changes in microsclerotia due to soil treatments with a field, but it is apparent that microsclerotia are not the limiting factors in disease development.

There is an apparent contradiction between our results and conclusions and those of Ashworth et al. (1, 2) concerning the concn of microsclerotia in soil and the prevalence of Verticillium wilt in cotton. Differences in recovery of viable propagules in the two methods reflect mainly the differences in soil preparation and in methods of soil plating. Ashworth et al. (2) stated that "our principal difficulty was in assaying soils having 20 or more microsclerotia/g"; whereas with the present method, soil samples having several thousand propagules/g were easily analyzed. The minimum number of propagules/g soil that could be counted using the present method was 30. However, since previous work (33) has shown that for cotton (Acala 4-42) more than 100 microsclerotia/g soil are required to cause disease symptoms, the minimum number of microsclerotia seen with the present soil assay method was well below the number required to cause wilt symptoms in Acala cotton. Another point of difference between the two studies is that we made no attempt to survey percent infection of cotton plants which was the parameter used by Ashworth et al. (2) since infection of cotton roots and stems by *V. dahliae* can be widespread without the appearance of foliar disease symptoms. The present study focused on the nature and concn of microscopic propagules of *V. dahliae* which persist in air-dry soil in relation to the percentage of cotton plants growing in that soil which show foliar-disease symptoms.

LITERATURE CITED

1. ASHWORTH, L. J., JR., O. D. MC CUTCHEON, and A. G. GEORGE. 1972. Verticillium albo-atrum: the quantitative relationship between inoculum density and infection of cotton. Phytopathology 62:901-903.
2. ASHWORTH, L. J., JR., J. E. WATERS, A. G. GEORGE, and O. D. MC CUTCHEON. 1972. Assessment of microsclerotia of Verticillium albo-atrum in field soils. Phytopathology 62:715-719.
3. BAKER, K. F. 1957. The U.C. system for producing healthy container-grown plants. Calif. Agric. Exp. Stn. Manual 23. 332 p.
4. BRINKERHOFF, L. A. 1969. The influence of temperature, aeration, and soil microflora on microsclerotial development of Verticillium albo-atrum in abscised cotton leaves. Phytopathology 59:805-808.
5. BROWN, M. F., and T. D. WYLLIE. 1970. Ultrastructure of microsclerotia of Verticillium albo-atrum. Phytopathology 60:538-542.
6. COTTON RESEARCH TASK GROUP. 1969. California Cotton Research. Division of Agricultural Sciences, Univ. of Calif., Berkeley. 57 p.
7. DE VAY, J. E. 1970. Physiology of Verticillium wilt: host-pathogen interaction. p. 43-53. Cotton disease research in the San Joaquin Valley, California. Research report for 1968-1969. Div. of Agric. Sci., Univ. of Calif., Berkeley, 251 p.
8. DE VAY, J. E., and L. L. FORRESTER. 1972. Nature and concentration of propagules of Verticillium dahliae in field soils relative to the prevalence of Verticillium wilt in cotton plants. Phytopathology 62:754 (Abstr.).
9. DE VAY, J. E., O. D. MC CUTCHEON, A. V. RAVENSCROFT, H. LEMBRIGHT, R. H. GARBER, D. JOHNSON, M. HOOVER, and J. QUICK. 1970. Fumigation and other chemical soil treatments for control of verticillium wilt and potassium deficiency symptoms in cotton. p. 218-226. Cotton Disease Research in the San Joaquin Valley, California. Research Report for 1968-1969. Div. of Agric. Sci., Univ. of Calif., Berkeley. 251 p.
10. EVANS, G, W. C. SNYDER, and S. WILHELM. 1966. Inoculum increase of the Verticillium wilt fungus in cotton. Phytopathology 56:590-594.
11. EVANS, G., S. WILHELM, and W. C. SNYDER. 1967. Quantitative studies by plate counts of propagules of the Verticillium wilt fungus in cotton field soils. Phytopathology 57:1250-1255.
12. FARLEY, J. D., S. WILHELM, and W. C. SNYDER. 1971. Repeated germination and sporulation of microsclerotia of Verticillium albo-atrum in soil. Phytopathology 61:260-264.
13. GARBER, R. H. 1971. Effect of continuous cotton culture

on severity of Verticillium wilt. p. 23. *In* Proc. Beltwide Cotton Production Research Conf., Atlanta, Ga.; National Cotton Council, Memphis, Tenn. 84 p.
14. GARBER, R. H., and L. CARTER. 1970. Relationship of crop residue management to Verticillium wilt of cotton. p. 25. *In* Proc. Beltwide Cotton Production Research Conf., Houston, Texas; National Cotton Council, Memphis, Tenn. 93 p.
15. GARBER, R. H., and B. R. HOUSTON. 1966. Penetration and development of Verticillium albo-atrum in the cotton plant. Phytopathology 56:1121-1126.
16. GARBER, R. H., and J. T. PRESLEY. 1971. Relation of air temperature to development of Verticillium wilt on cotton in the field. Phytopathology 61:204-207.
17. GREEN, R. J., JR. 1969. Survival and inoculum potential of conidia and microsclerotia of Verticillium albo-atrum in soil. Phytopathology 59:874-876.
18. HALISKY, P. M., R. H. GARBER, and W. C. SCHNATHORST. 1959. Influence of soil temperature on Verticillium hydromycosis of cotton in California. Plant Dis. Reptr. 43:584-587.
19. HARRISON, H. D., and C. H. LIVINGSTON. 1966. A method for isolating Verticillium from field soil. Plant Dis. Reptr. 50:897-899.
20. HOES, J. A. 1971. Development of chlamydospores in Verticillium nigrescens and V. nubilum. Can. J. Bot. 49:1863-1866.
21. HOOVER, M. 1970. Some effects of cultural practices on cotton in disease situations. p. 184-191. *In* Cotton disease research in the San Joaquin Valley, Calif. Research Report for 1968-1969. Div. of Agric. Sci., Univ. of Calif., Berkeley. 251 p.
22. ISAAC, I. 1967. Speciation in Verticillium. Annu. Rev. Plant Pathol. 5:201-222.
23. ISAAC, I., P. FLETCHER, and J. A. C. HARRISON. 1971. Quantitative isolation of Verticillium spp. from soil and moribund potato hauls. Ann. Appl. Biol. 67:177-183.
24. ISAAC, I., and Q. MAC GARVIE. 1966. Dormancy and germination of resting structures of Verticillium spp. Trans. Br. Myc. Soc. 49:669-678.
25. JORDAN, V. W. L. 1971. Estimation of the distribution of Verticillium populations in infected strawberry plants and soil. Plant Pathol. 20:21-24.
26. LEMBRIGHT, H. W., and J. E. DE VAY. 1970. Chemical soil treatment and disease control. p. 208-217. Cotton disease research in the San Joaquin Valley, Calif. Research report for 1968-1969. Div. of Agric. Sci., Univ. of Calif., Berkeley. 251 p.
27. MENZIES, J. D., and G. E. GRIEBEL. 1967. Survival and saprophytic growth of Verticillium dahliae in uncropped
28. RANNEY, C. D. 1973. Cultural control. Pages 98-104. *in* Verticillium wilt of cotton. ARS-S-19. Poceedings of a workshop conference held at the National Cotton Pathology Research Laboratory, College Station, Texas, 30 Aug - 1 Sept 1971. Published by U.S. Dep. of Agric., Agric. Res. Serv., Publication Div., Beltsville, Md. 398 p.
29. REED, F. 1963. La culture du coton en Iran. Bull. Ec. Nat. Sup. Agron. 5:199-218.
30. SCHNATHORST, W. C. 1965. Origin of new growth in dormant microsclerotial masses of Verticillium albo-atrum. Mycologia 57:343-351.
31. SCHNATHORST, W. C. 1969. Origin of new growth of Verticillium albo-atrum in dried infected plant tissue. p. 30. *In* Proc. Beltwide Cotton Production Research Conf. New Orleans, La..; National Cotton Council, Memphis, Tenn. 134 p.
32. SCHNATHORST, W. C. 1973. Nomenclature and physiology of Verticillium spp. with emphasis on the V. albo-atrum vs. V. dahliae controversy. Pages 1-19. *in* Verticillium wilt of cotton. ARS-S-19. Proceedings of a workshop conference held at the National Cotton Pathology Research Laboratory, College Station, Texas, 30 Aug - 1 Sept 1971. Published by U.S. Dep. Agric., Agric. Res. Serv., Publication Div., Beltsville, Md. 398 p.
33. SCHNATHORST, W. C., and D. E. MATHRE. 1966. Host range and differentiation of a severe form of Verticillium albo-atrum in cotton. Phytopathology 56:1155-1161.
34. SCHREIBER, L. R., and R. J. GREEN, JR. 1962. Comparative survival of mycelium, conidia, and microsclerotia of Verticillium albo-atrum in mineral soil. Phytopathology 52:288-289.
35. SMITH, H. C. 1965. The morphology of Verticillium albo-atrum, V. dahliae, and V. tricorpus. N. Z. J. Agr. Res. 8:450-478.
36. TALBOYS, P. W. 1958. Degradation of cellulose by Verticillium albo-atrum. Trans. Br. Myc. Soc. 41:242-248.
37. TURNER, J. H., E. G. SMITH, R. H. GARBER, W. A. WILLIAMS, and H. YAMADA. 1972. Influence of certain rotations upon cotton production in the San Joaquin Valley, Calif. Agron. J. 64:543-546.
38. WILHELM, S. 1955. Longevity of the Verticillium wilt fungus in the laboratory and in the field. Phytopathology 45:180-181.
39. WILHELM, S. 1966. Chemical treatments and inoculum potential of soil. Annu. Rev. Phytopathol. 4:53-78.
40. WILHELM, S., J. E. SAGEN, and W. PENDERY. 1965. Control of Verticillium wilt of cotton by soil fumigation. p. 47-54. *In* Cotton Disease Research in the San Joaquin Valley, California. Research Reports for 1963 and 1964. Div. of Agric. Sci., Univ. of Calif., Berkeley. 78 p.
41. WYLLIE, T. D., and J. E. DE VAY. 1970. Growth characteristics of several isolates of Verticillium albo-atrum and Verticillium nigrescens from cotton. Phytopathology 60:907-910.