Growth Characteristics of Several Isolates of Verticillium albo-atrum and Verticillium nigrescens from Cotton

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ABSTRACT

Per cent germination of conidia, rate of sporulation, and the effect of C:N ratios on microsclerotial and chlamydospore formation was determined for cotton plant defoliating (T9) and nondefoliating (SS4) isolates of *Verticillium albo-atrum* and *V. nigrescens* (isolate 68). T9 conidia germinated more rapidly than conidia of SS4 at temp of 21, 24, 27, and 30 C. Conidia of SS4, however, failed to germinate at 33 C while those of T9 and 68 did germinate. Sporulation rates of T9 and 68 were twice that of SS4 after 80-hr incubation in vitro. Furthermore, T9 was capable of producing microsclerotia over a wider range of carbon:nitrogen ratios than isolate SS4. These data suggest that other factors as well as increased virulence may account for the increased prevalence of the defoliating strain T9 in California. Phytopathology 60:907-910.

Microsclerotial strains of *Verticillium albo-atrum* Reinke & Berth. are serious pathogens of cotton in the USA, particularly in the San Joaquin Valley of California and southeastern Missouri. Prior to 1960, isolations of *V. albo-atrum* from diseased cotton plants in California consistently yielded a pathotype that caused darkening of the vascular tissues, epinasty, stunting, and interveinal chlorosis of leaves, followed by necrosis upon reinoculation into cotton; severe defoliation was rare.

Since 1960, however, cotton fields in the San Joaquin Valley have had an unusually high incidence of severe wilt, often characterized by defoliation as well as the above symptoms. Greenhouse tests demonstrated that severe wilt is caused by a new distinct defoliating pathotype of *V. albo-atrum* (9). Field observations and data from numerous isolations from cotton in California indicate that strains of this pathotype are spreading and compete successfully with other strains of *V. albo-atrum*. This success may be partially due to the virulence advantage that the defoliating strains have over the other strains (9). Other factors, including superiority in growth and sporulation rates and survival capabilities, may also play important roles.

Inoculation of root balls of cotton with conidia in the greenhouse often causes a lethal response, depending on cotton variety, pathotype, and conidium concentration. A comparison of the sporulation rate between *V. albo-atrum* isolates might provide information useful in interpreting the reasons why one isolate may predominate over another. Recent evidence (7) indicates that the survival of conidia in soil is limited, yet conidia are capable of causing infection for at least 3 weeks. When conditions prevail that favor conidial production, they may become an extremely effective infection propagule. *Verticillium albo-atrum* can sporulate from inoculum fragments in the soil for as long as 2-7 weeks, suggesting that rate of spread through the soil may be increased by dispersion of conidia (11). The recently described (4) mechanism of conidium formation is similar for both *V. albo-atrum* and *V. nigrescens*.

This paper concerns two isolates of *V. albo-atrum* and one isolate of *V. nigrescens* pathogenic to cotton in California. It compares their conidial production, rate of germination, and the effect of the C:N ratio of the culture media on growth and microsclerotium or chlamydospore production.

MATERIALS AND METHODS.—Single spore isolates of *Verticillium albo-atrum* (isolates T9 and SS4) and *V. nigrescens* (isolate 68) were used in these studies. Isolates T9 and SS4 are black sclerotial forms in culture, and cause severe (defoliation) and intermediate (no defoliation) reactions, respectively, on susceptible cotton varieties upon inoculation. Isolate 68 is mildly pathogenic on cotton, causing mild leaf chlorosis. Conidial germination studies were made on distilled-water agar, and a minimum of 300 conidia were examined in each of three replications. The rate of conidium production was studied in potato-dextrose broth on a rotary shaker at 24 C. Each flask was seeded with 1.6×10^5 conidia/ml. Aliquots of the conidial suspension were removed at prescribed intervals, and the conidia were counted on a hemocytometer.

The effects of carbon:nitrogen (C:N) ratios on microsclerotial and chlamydospore production were determined by growing the isolates on a modified Czapek's agar at 24 C. The basic medium was $NaNO_3$, 3 g; KH_2PO_4, 1 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; KCl, 0.5 g; agar, 15 g; and distilled water to make 1,000 ml. The C:N ratios were adjusted by altering the sucrose content. Before transfer to the test medium, the organism was grown on a similar medium. Growth, chlamydospore, and microsclerotium production were observed periodically. A similar study was made with $(NH_4)_2SO_4$ substituted for $NaNO_3$ as the N source. The media were adjusted to pH 6.5.

RESULTS.—Per cent germination of conidia of *V. albo-atrum* isolates T9 and SS4 and *V. nigrescens* isolate 68 were compared at different temp.

After 10 hr, per cent germination was consistently higher for isolate 68 than for either isolates T9 or SS4. Germination for T9, however, was higher than for SS4 at 21, 24, 27 and 30 C (Fig. 1); although differences

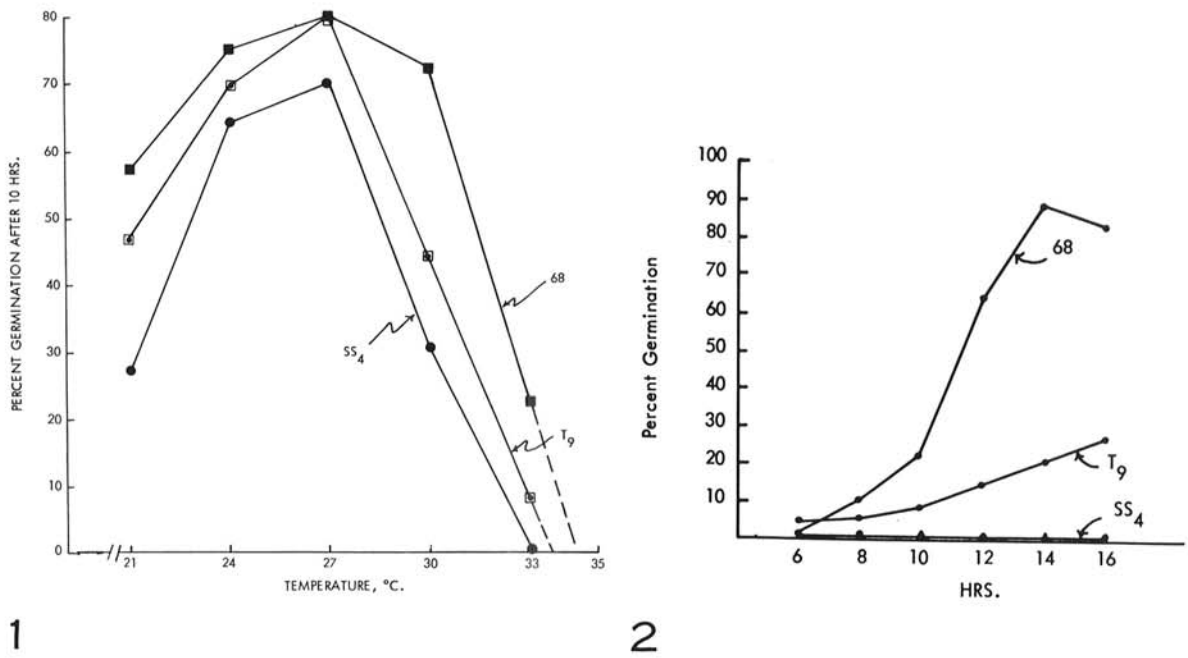

Fig. 1-2. **1)** Effect of temp on per cent germination of conidia of *Verticillium albo-atrum,* isolates T9 and SS4, and *V. nigrescens,* isolate 68, after 10 hr. **2)** Germination rate of conidia of *V. albo-atrum,* isolate T9, and *V. nigrescens,* isolate 68, at 33 C. Note that isolate SS4 of *V. albo-atrum* failed to germinate at this temp.

between SS4 and T9 after 12 and 14 hr were not significant. At 33 C, conidia of SS4 failed to germinate, whereas after 16 hr, conidia of isolates 68 and T9 germinated 83% and 26%, respectively (Fig. 2). There were no differences in the number of germ tubes produced by germinating conidia of these isolates.

The isolates T9, SS4, and 68 were compared for rate of conidium production in potato-dextrose broth. No significant rate differences occurred after 40 hr, at which time the conidia of the isolates had increased approximately 64- to 100-fold. By 42 hr, isolate 68 had increased 1,274-fold; T9, 860-fold; and SS4, 138-fold. By 80 hr, T9 and 68 had increased 4,200-fold, whereas SS4 had increased only 2,000-fold.

Microsclerotium formation by isolates SS4 and T9 was compared on media with different C:N ratios (Fig. 3, 4). These isolates behaved similarly, both producing more microsclerotia at higher C:N ratios than at the lower ratios. Major differences in microsclerotial production occurred at ratios of 3.8 or lower when N was supplied as $NaNO_3$. At a ratio of 3.8, both isolates responded to the increase in sucrose carbon, with isolate T9 producing greater numbers of microsclerotia (Fig. 3). In addition, T9 produced more microsclerotia at a C:N ratio of 1.7 than did SS4. Few microsclerotia were produced by either isolate at the ratio of 0.9 to 1.0. This greater capability of T9 for microsclerotium production continued through the higher C:N ratios. *Verticillium nigrescens* isolate 68 produced increasing numbers of chlamydospores as the C:N ratios were increased. Chlamydospore production was initiated at a C:N ratio of 3.8.

When the nitrogen source was $(NH_4)_2SO_4$, microsclerotium formation in isolate T9 and SS4 changed with increased amounts of sucrose (Fig. 4). Isolates T9 and SS4 initiated microsclerotium formation at a C:N ratio of 4.3 and increased their formation at higher C:N ratios. The density of the microsclerotia formed was greater for T9 than for SS4 at each C:N ratio tested.

The lowest C:N ratio at which *V. nigrescens* isolate 68 produced chlamydospores was 68 on media with $(NH_4)_2SO_4$, and 3.8 with $NaNO_3$.

Discussion.—The greater virulence of the defoliating strains (9) has been suggested as a possible reason for their greater competitive ability. This is indicated by the rapid spread of the defoliating strains into areas formerly inhabited by the nondefoliating ones, with accompanying increased inoculum levels. Their respective competitive saprophytic abilities (5) may also be involved.

The defoliating strain (T9) possesses a greater initial rate of germination than the nondefoliating strain (SS4) at several temp. This advantage is sometimes temporary, but could contribute to the colonization of the T9 strain in an ecological "microniche" ahead of the SS4 strain. Precedence has been established for the role of previous colonizers in excluding subsequent fungal invaders (1, 3, 12). These studies discuss colonization of organic substrates, but a similar phenomenon could exist for other microenvironments in the soil.

Conidia of the nondefoliating strain (SS4) do not germinate above 33 C, although 26% of the conidia of the defoliating strain germinated after 16 hr. This characteristic may provide the defoliating strain with an important competitive advantage, since temp exceeding

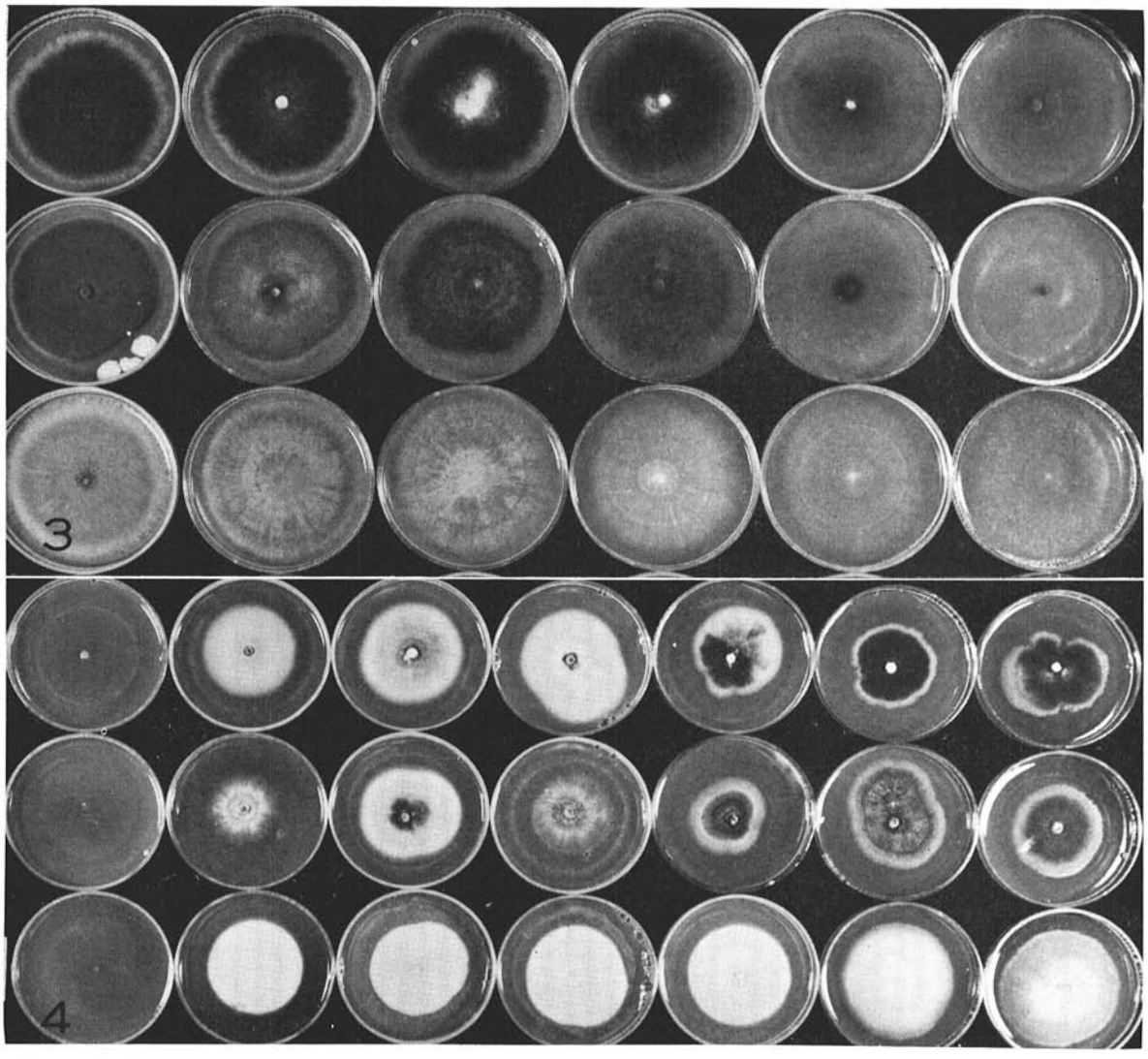

Fig. 3-4. **3)** Effect of carbon:nitrogen (C:N) ratios on microsclerotial and chlamydospore production of *Verticillium albo-atrum* and *V. nigrescens,* respectively, when $NaNO_3$ was used as the N source. From top to bottom, *V. albo-atrum* isolate T9, SS4; *V. nigrescens* isolate 68. From left to right,C:N ratios 120; 60; 30; 15; 7.5; and 3.8/1. **4)** Effect of C:N ratios on microsclerotial and chlamydospore production of *V. albo-atrum* and *V. nigrescens,* respectively, when $(NH_4)_2SO_4$ was used as the N source. From top to bottom, *V. albo-atrum* isolates T9, SS4; *V. nigrescens* isolate 68. From left to right, C:N ratios: No carbon or nitrogen, 4.3; 8.5; 17; 34; 68; 134/1.

30 C frequently occur in California during the growing season.

Garrett (6) interprets the influence of overwhelming "inoculum potential" as an important factor in the establishment and survival of pathogens in infested host tissues. Similarly, an organism capable of producing more propagules than its competitor seemingly possesses advantages in a soil environment even if saprophytic colonization of the host were not involved. Our data indicate that the sporulation rate of the defoliating strain is approximately twice that of the nondefoliating strain in liquid media substrates. This may also be true in the soil environment, permitting the defoliating strain to develop a superiority of numbers over the nondefoliating strain. The demonstrated ability of conidia to infect cotton plants in the soil environment and their survival for up to 3 weeks support the concept that increased numbers of conidia provide the competitive edge for the defoliating strain.

Microsclerotia have been linked with the basic role of survival of *V. albo-atrum* (10). Nutritional conditions favoring microsclerotium production would be important, especially if such factors resulted in one strain producing more microsclerotia than another strain. Our data indicate that the defoliating strain (T9) can produce more microsclerotia than the nondefoliating strain (SS4) over a wider range of C:N ratios, at least when sucrose is the carbon source used. The possibility exists that we may unintentionally have selected for nonmicrosclerotial forms when the C:N

ratios were lowered (2, 8). Since only mass transfers were used, however, the production of fewer microsclerotia in cultures growing at lower C:N ratios in a single culture generation is probably a valid observation, not the result of genetic selection for white mycelial types.

Greater microsclerotial capability suggests another probable reason for the increased prevalence of the defoliating strain of *V. albo-atrum* in California. Possibly microsclerotia do not serve a primary function as an infection propagule per se, but provide a base for initiating vegetative growth and eventual sporulation. This phenomenon, then, could account for the rapid spread of the pathogen through the soil (11).

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