Environmental Effects on the Development and Dissemination of *Cladosporium carpophilum* on Peach

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ABSTRACT

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In the laboratory, mycelial growth of *Cladosporium carpophilum* was best at 20–30 C, and conidial germination was best at 98–100% relative humidity (RH). Some conidia germinated on glass coverslips without free water, but conidia germinated much more readily on water agar. Sporulation on twig lesions occurred at 80–100% RH, but was most abundant at 98–100%. Some peach scab lesions appeared on new twig growth about the same time as on fruit and they became numerous on twigs later in the season. The fungus did not sporulate abundantly in lesions until after overwintering. At 100% RH, *C. carpophilum* produced conidia in lesions for 20–30 hr, after which conidia were released. Moistening infected twigs before exposure to 100% RH did not increase production of conidia. Conidia were present in the atmosphere of a peach orchard and in rainwater runoff from infected twigs. In the spring, conidia were most abundant 2–6 wk after the calyx-split stage of development. High numbers of conidia were not always associated with rainfall. Conidia of *C. carpophilum* were most numerous early in May.

Additional key words: spore sampling.

Peach scab, which is caused by the fungus *Cladosporium carpophilum* Von Thümen, occurs in most peach-producing areas of the world where environmental conditions favor its development. Peaches grown in arid regions of the western USA are not affected. The market value of the fruit is reduced by the appearance of small, oval, olive-to-black spots. In severe cases, fruit may crack and become infected with *Monilinia fructicola* Wint. Honey (1).

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In his monograph of *C. carpophilum,* Keitt (3) reported that the fungus overwintered primarily as mycelium in twig lesions. Koch (4) showed that the mycelium continues to grow throughout the winter in twig lesions, forming secondary lesions that appear the next growing season. Conidia produced on twig lesions in the spring are carried by rainwater runoff to young, growing fruit (3). Keitt (3) found 20–27 C to be optimal for germination and growth.

Except for the results of fungicide trials, little has been published about *C. carpophilum* since Keitt's monograph. With the current interest in pest management, more information on the biology and epidemiology of the fungus is needed to provide for more efficient use of fungicides.

The objectives of this research were to examine environmental factors that affect germination, growth, sporulation, and conidia dispersal of *C. carpophilum.*

MATERIALS AND METHODS

Temperature effects. Cultures of *C. carpophilum* used to study temperature and relative humidity (RH) were isolated on potato-dextrose agar in 1977 and 1978 from infected 1-yr-old peach twigs.

Four-millimeter-diameter disks of mycelium were removed with a cork borer from the margins of 3-day-old colonies, transferred to the center of plates of acidified PDA (one drop of 88% lactic acid per 30 ml of medium), and incubated at temperatures ranging 10–35 ± 1 C in growth chambers. The diameters of four colonies per temperature treatment were measured after 4 days, and the entire series was repeated twice in 1977 and twice in 1978.

We studied the effects of temperature on conidial germination by using conidial suspensions prepared from 3- or 5-day-old cultures of the fungus. Concentrations of conidia were measured with the aid of a hemacytometer and adjusted to 625 conidia per milliliter. Two milliliters of the suspension were poured over the surface of water agar (20 ml Difco Bacto-agar per liter of water) in 100 ×10-mm plastic petri dishes. Inoculated plates were incubated at constant temperatures ranging in 5 C intervals from 10 to 35 C. Percent germination on four plates at each temperature was determined after 24 hr. The entire series was repeated twice both in 1977 and in 1978. Conidia were counted as germinated if the length of the germ tube was equal to or more than the diameter of the conidium.

Relative humidity (RH) effects. We prepared humidity chambers by dissolving appropriate amounts of granular (NaCl, $CaCl_2$, or granulated sucrose in 250 ml of distilled water to regulate the desired RH in sealed containers as described by Scott (6). The solutions were poured into 500-ml flasks, and the flasks were sealed with rubber stoppers. The chambers were stored at a constant temperature of 25 C to prevent condensation. Conidia from one isolate were removed with a camel's hair brush and dusted on dry glass coverslips (approximately two conidia per square millimeter), and the coverslips were placed inside humidity chambers regulated at 94, 96, 98, 99, and 100% RH. Percent germination was determined from counts of conidia from four chambers at each RH treatment after 1, 2, 3, 4, 7, and 10 days.

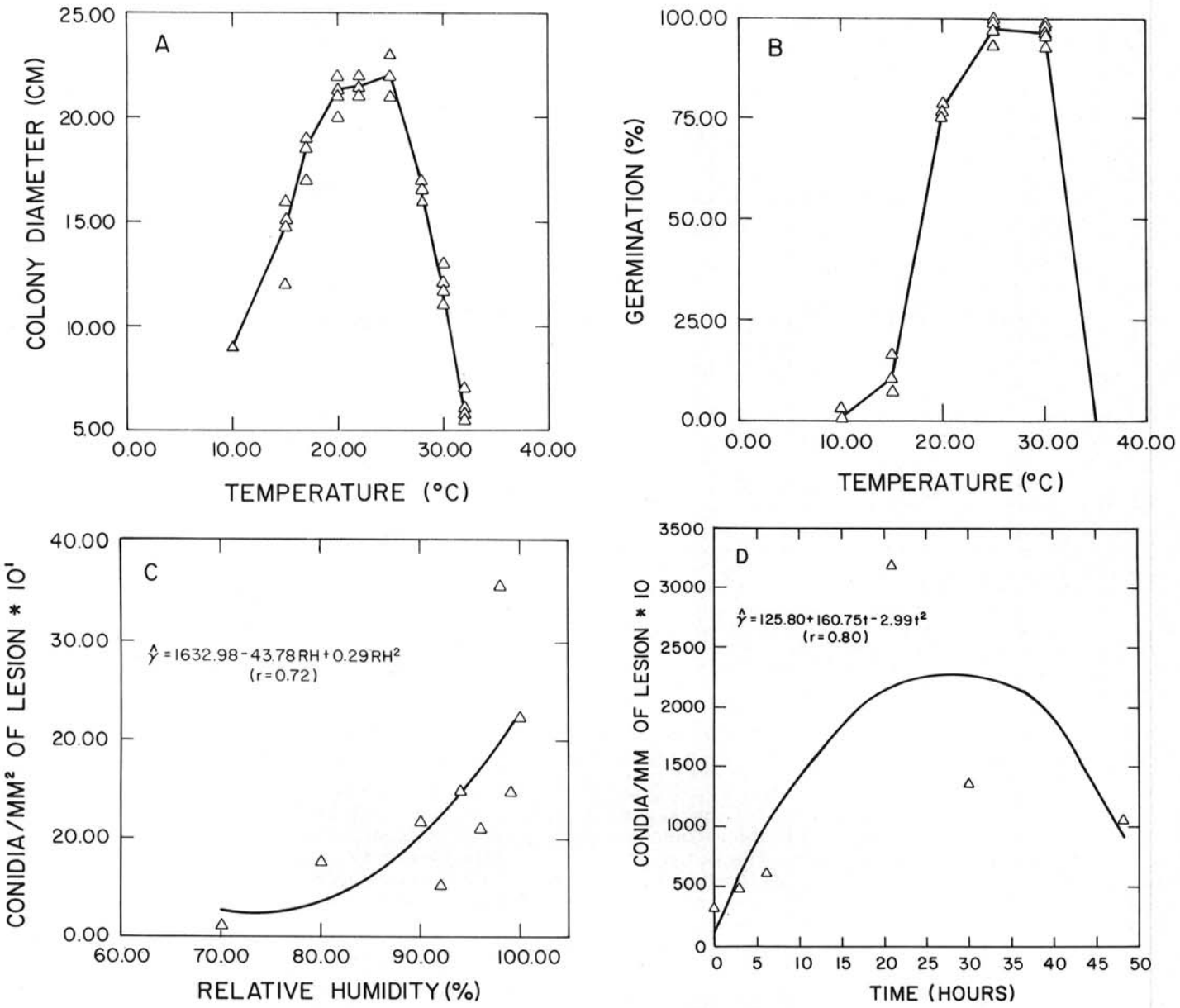

Fig. 1. Effects of temperature and relative humidity on germination, growth, and sporulation of *Cladosporium carpophilum*. **A,** Effects of temperature on growth of *C. carpophilum* on potato-dextrose agar 4 days after inoculation; **B,** germination of conidia of *C. carpophilum* on water agar after 24 hr as affected by temperature; **C,** effects of relative humidity on numbers of conidia produced on twig lesions after 24 hr at 70–100% RH; **D,** rate of sporulation on lesions of twigs incubated at 100% RH for 0–48 hr. Results shown for one representative experiment replicated four times with each data point representing the mean of one observation in A, four in B, and five in C and D.

We examined effects of RH on sporulation in 1-yr-old lesions on heavily infected peach twigs obtained from experimental orchards in May and June 1979. Thirty-six twigs were attached to glass rods and placed 3–11 cm above solutions of NaCl, granular $CaCl_2$, or granulated sucrose in humidity flasks adjusted to 70, 80, 90, 92, 94, 96, 98, 99, and 100% RH. Numbers of conidia produced per square millimeter of lesion were determined after incubation for 24 hr at 25 C. The total surface area covered by lesions was visually estimated for each twig. The twigs were placed in tubes in 10 ml of distilled water and the conidia were washed off by 5 sec of agitation in a Vortex-Genie mixer with the speed control set at 10. The suspension was filtered through one layer of cheesecloth. To recover the conidia, 2 ml of the suspension were drawn into a hypodermic syringe and filtered through a 0.2-μm (maximum pore size) Metricel membrane filter placed in a Gelman Swinney-type hypodermic adapter (2). The filters were placed on a glass slide, and the conidia were stained with one drop of cotton blue. Filters were examined at ×430, and conidia within an ocular counting grid were counted at five random points on each filter. Each RH treatment was replicated four times and the entire series was repeated three times. Also, numbers of conidia per square millimeter of lesion were determined on four twigs before each experiment began, by using the procedure just described.

Numbers of conidia produced in twig lesions also were investigated in moist chambers. Infected 1-yr-old twigs were obtained from an unsprayed orchard of Redhaven peach trees in May and June 1979, and the number of conidia per square millimeter of lesion was determined. The twigs were placed in two moist chambers consisting of 3-L glass dishes lined with moist paper towels. One chamber, containing 28 3-cm-long twigs, was used to study the effects of wetting twigs before incubation at 100% RH. Chambers were sealed with Parafilm and incubated at 25 C. After 3, 6, 9, 21, 24, 30, and 48 hr, four twigs were removed from each chamber and the total surface area covered by lesions was estimated visually. Conidia then were washed into a test tube, collected on Metricel membrane filters, and counted as previously described. The experiment was repeated three times.

Seasonal development of twig lesions. The appearance of scab lesions on twigs and sporulation in the lesions were studied in two orchards of Redhaven trees. One orchard had received annual fungicide sprays since it was established; the other had not been sprayed for 2 yr and the trees were heavily infected with *C. carpophilum*.

To determine when sporulation began in twig lesions, we collected infected twigs in the fall of 1977 and at 14-day intervals from February to May 1978. The twigs were placed in moist chambers consisting of 3-L glass dishes lined with moist paper towels and sealed with Parafilm. After 48 hr, twig lesions were examined under a dissecting microscope at ×63 for presence of conidiophores and conidia.

Rainwater runoff was collected from infected twigs in an abandoned orchard of Redhaven peaches and examined for the presence of conidia. We attached a 100-ml plastic vial to the end of each of 19 infected twigs on 2 April 1979. The rainwater from each vial was collected after rains on 2, 4, 9, 13, and 26 April and poured together into a large flask. The combined samples were filtered through one layer of cheesecloth and stirred, and a 50-ml aliquot from each sample was diluted with an equal amount of distilled water. Conidia in three 5-cm^3 subsamples were recovered on membrane filters and counted. Counts were converted to numbers of spores per milliliter of water collected.

Spore sampling. The numbers of conidia of *C. carpophilum* in the atmosphere of a peach orchard were monitored daily from March to August 1979 with an Andersen sampler (Model 13-000), which is designed for capturing spores in an agar medium. The sampler was placed on a table 1 m above the ground in an orchard of Redhaven peach trees. The sampler was within 25 cm of foliage of the nearest tree. To avoid confusing *C. carpophilum* with other *Cladosporium* species, benomyl-tolerant strains of *C. carpophilum* which were known to occur in the orchard were captured on plates of PDA amended with 50 μg of benomyl per milliliter. Airflow through the sampler was 28 L/ min. A timer was set for intermittent (30 sec) operation 14 times each hour. Spores were sampled daily at 0400–1000 hours during March and 0300–1500 hours with a change of plates at 0900 hours from March through July. Exposed plates were incubated at 25 C for 1–4 days and colonies of *C. carpophilum* were counted.

RESULTS

Temperature effects. *Cladosporium carpophilum* grew best at 20–25 C, grew slowly at 10 and 32 C, and failed to grow at 35 C (Fig. 1A). The optimum temperature range for germination of conidia was slightly higher than the optimum for growth. Germination occurred at 15–30 C but was best at 25–30 C (Fig. 1B). There was little germination at 10 C and none at 35 C.

Relative humidity effects. Conidia of *C. carpophilum* germinated on glass coverslips when exposed to relative humidities (RH) of 94–100%. Germination was slow and did not exceed 13% on the coverslips. They remained free of condensation water even at high RH. During each of the first 4 days after the test was begun, germination was highest at 98–100%. Germination appeared to decrease with decreases in RH below 98%, but differences were not significant. After 7–10 days, germination at 94–96% RH was about the same as at 98–100%.

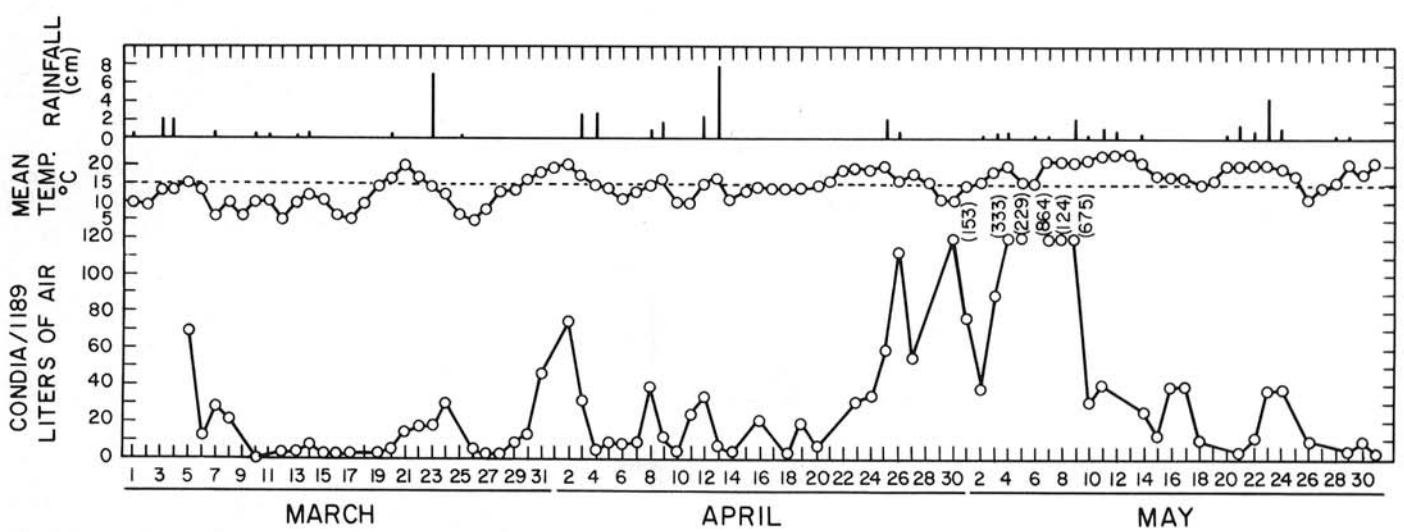

Fig. 2. Numbers of benomyl-tolerant *Cladosporium carpophilum* conidia collected daily in a peach orchard in 1979 with an Andersen spore sampler. Compare with mean daily temperatures and rainfall.

Sporulation on twig lesions increased with RH when infected twigs were exposed to 70–100% RH (Fig. 1C). An increase in conidial production was often noted as early as 3 hr after exposure to 100% RH (Fig. 1D), but in some experiments there was no increase until after 6 hr of exposure. Conidial production increased for the first 20 hr (Fig. 1D). In other experiments, conidial production increased for 30 hr. Conidia were apparently released after 20–30 hr.

Sometimes the fungus in lesions on wetted twigs sporulated sooner than in lesions on unwetted twigs, but not always. The number of conidia produced on twigs wetted before placing them in moist chambers was the same as number of conidia on unwetted twigs. Exposure to wetting seemed not to be a requirement for sporulation if the RH was near 100%.

Seasonal development of twig lesions. In an unsprayed orchard with high inoculum levels, lesions appeared on new twig growth about the same time that they appeared on the fruit. In sprayed orchards, a few lesions were present on new twig growth by mid-July in 1977 and 1978. More lesions appeared by September, but they were not numerous until the following spring. Sporulation on lesions formed in 1977 did not occur until after 1 April 1978.

Conidia of *C. carpophilum* were abundant in the rainwater runoff collected from infected twigs in an abandoned orchard early in April. The numbers of conidia increased from 29 per milliliter of rainwater on 2 April 1979 to 274 on 9 April. Twigs died after 9 April, 6 days before calyx-split, and the experiment was discontinued.

Spore sampling. The benomyl-amended acidified medium used with the Andersen sampler was effective for suppressing growth of most other airborne fungi and bacteria. Often nearly pure cultures of *C. carpophilum* were obtained. This procedure made it possible to study fluctuations of numbers of airborne *C. carpophilum* conidia without confusion with morphologically similar fungi. Conidia of the pathogen were present when sampling began on 4 March 1979 (Fig. 2), but they were few until 20 March. During 20 March–19 April spore numbers increased only when mean daily temperatures approached or exceeded 15 C. Numbers of airborne conidia increased sharply on 26 April, 2 wk after the calyx-split stage of development of peach. Counts were highest May 4–9, 3–4 wk after calyx-split. More than 1,000 colonies formed after the 7 May sampling date. Fewer than 25 colonies per day were recorded in mid-July.

In studies of sporulation on twig lesions in May–June 1979, large numbers of conidia were produced when twigs were incubated in moist chambers for 1–2 days. Fungi in the lesions sporulated the most profusely in May, and numbers of conidia declined sharply in early June (Fig. 3).

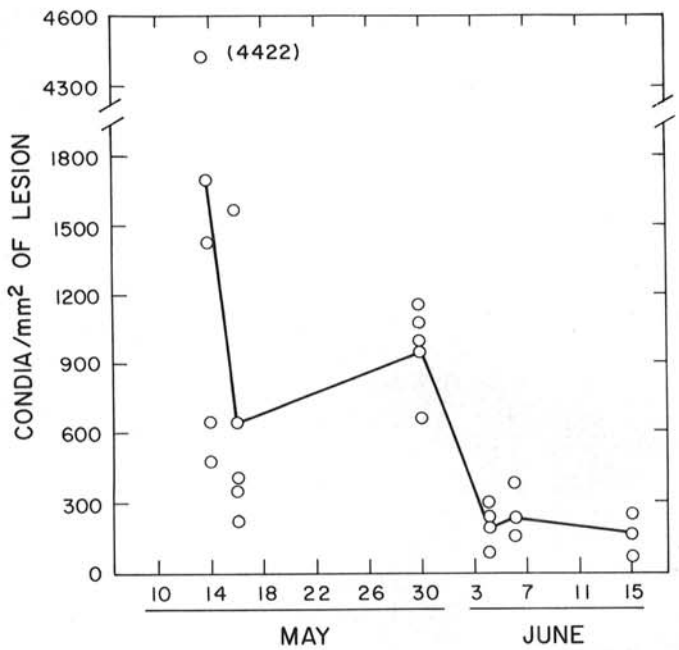

Fig. 3. Numbers of conidia of *Cladosporium carpophilum* produced on infected peach twigs obtained from the field and incubated at 100% relative humidity for 24 hr.

DISCUSSION

The optimum temperature for germination and growth of *C. carpophilum* in the laboratory was 20–30 C. Temperatures in South Carolina peach orchards, therefore, are favorable for fungus development during most of the season from the petal-fall stage onward. Since this fungus grows well at high temperature, but does not sporulate in midsummer to late summer, temperature does not seem to be the critical factor. The fungus may continue to grow during the winter, since growth was observed at 10 C, and was reported by Keitt (3) to occur slowly at 2 C. Higher temperatures appear to be important for germination; the optimum temperature for germination was 22–30 C, while the optimum range for growth was 20–25 C. Temperature might be a limiting factor for germination and fruit infection during early stages of fruit development. Increased numbers of airborne conidia and conidia on twigs coincided with temperatures near the optimum for growth, but effects of temperature on sporulation in vivo have not been determined.

The higher percentage of germination on water agar than on dry coverslips probably was due to greater availability of free water. However, poor germination on coverslips might have been due in part to mutual inhibition of germination by conidia clustered together, since conidia germinated more readily when few were present.

The occurrence of conidia in the air during dry weather as well as during moist periods has important implications for disease control in peach orchards. The large numbers of conidia on lesions and in the air 2–6 wk after calyx split indicate that this period is especially critical for fungicide protection. This conclusion also is supported by fungicide tests, which show this period to be especially important for fungicide protection (5). Although moisture is important for spore production and germination, dew might provide sufficient moisture for infections to occur even during periods of drought. This tentative conclusion is supported by field observations of severe scab development on fruit when rainfall is well below normal (*unpublished*). There appear to be few opportunities for reducing fungicide use during this critical period. Although measurement of spores on twigs and in the atmosphere might be used for determining periods of maximum infection potential, stage of plant development appears to be a more reliable guide for the application of sprays.

We used benomyl resistance as a genetic marker to enable us to distinguish between *C. carpophilum* and other *Cladosporium* spp. in the air. Although this procedure was useful, it assumes that the responses of benomyl-sensitive strains and benomyl-resistant strain(s) are similar and that the other *Cladosporium* spp. are sensitive to benomyl. The airborne spores of the resistant strain(s) corresponded to patterns of sporulation of all strains on scab lesions, and we therefore have confidence in this procedure. The pattern of highest sporulation also corresponded to the most critical period previously reported for fungicide protection (5).

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