

**The Influence of Dew Duration, Relative Humidity, and Leaf Senescence on Conidial Formation and Infection of Onion by *Alternaria porri***

K. L. Everts and M. L. Lacy

Former graduate research assistant and professor, Department of Botany and Plant Pathology, Michigan State University, East Lansing 48824.

Present address of first author: USDA-ARS, Department of Plant Pathology, North Carolina State University, Raleigh, 27695-7616.

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**ABSTRACT**

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Formation of conidia by *Alternaria porri* was examined under variable dew duration and controlled relative humidity (RH). Viable conidia produced on lesions increased from 26% after 9 hr of dew to 72, 91, 93, 96, and 96% after 12, 15, 18, 21, and 38 hr of dew, respectively. Conidia formed during dew duration of 12 hr caused flecks when used to inoculate healthy plants, and those formed during dew duration of 16 hr caused typical lesions. Conidia were formed at all RHs tested

(75–100%); numbers were very low at 75–85% RH but increased with increasing RH. Conidia formed on lesions on senescent leaves incubated in dew at 25 C. They formed repeatedly (up to eight cycles) on lesions exposed to alternating low (35–50%) and high (100%) RH in a dew chamber at 25 C. Conidial initials were seen after 9 hr of dew; septa developed after 12 hr; and conidia appeared fully mature after 15 hr of dew when developing conidia were examined with a scanning electron microscope.

*Additional keywords:* epidemiology, onion (*Allium cepa* L.).

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Two of the most important environmental factors that affect sporulation of *Alternaria porri* (Ell.) Cif. appear to be atmospheric humidity and free water on the leaf surface (11,14,15). Fahim (11) found that conidia were produced profusely by *A. porri* on agar media at  $\geq 90\%$  relative humidity (RH), but as humidities decreased below 81%, numbers of conidia decreased and the conidia that formed contained fewer cells. The RH may have varied in this study due to fluctuation in temperature surrounding the jars containing the agar blocks and to equilibration of the blocks. Miller (15) found that  $\geq 11$  hr of leaf wetness and  $\geq 14$  hr of RH  $> 90\%$  were positively associated with increases in the numbers of conidia of *A. porri* that were trapped above an onion field on the following day. Rain or irrigation often preceded increases in airborne conidia of the pathogen above an onion field in Nebraska (14).

The influence of moisture and plant senescence on conidial production and dispersal by the related species *A. solani* (Ell. & Mart) Sorauer (4,16) and *A. dauci* (Kühn) Groves & Skolko (12,17) has been investigated. The relationship of dew duration to conidial formation by *A. solani* was studied in both a moist chamber (5) and in the field (16), where the continuous dew durations necessary for conidial formation were 16 and 8 hr, respectively. Strandberg found that conidial formation by *A. dauci* on carrot tissue did not occur at RH  $\leq 96\%$  and that hours of leaf wetness were correlated with abundance of conidia of *A. dauci* in air over a carrot field (17). Conidial production by *A. solani* on tomato leaves increased as leaves became senescent (4). Thus, while conidial formation by *A. solani* and *A. dauci* has been studied in vivo and in vitro, previous studies of *A. porri* have focused on conidial formation on agar blocks or on the relationship of weather to conidial formation. The present studies attempt to elucidate the influence of moisture and leaf senescence on formation of conidia by *A. porri* on infected plants.

Influence of variable dew duration on maturation of conidia on leaves was also studied to examine the development of conidia in vivo.

## MATERIALS AND METHODS

**Inoculum production.** Muck soil was infested with *A. porri* by placing a 0.5 cm diameter agar block colonized with *A. porri* (isolate AAP-3) in a test tube containing 8 g of sterile muck soil. Tubes were shaken daily for 3–5 days at 25 C, then placed in the refrigerator for storage. Inoculum was prepared by placing 0.1–0.2 g of infested soil on V-8 agar (9). The plates were incubated at 22–24 C in darkness for 6–8 days, exposed to ultraviolet (UV) light (peak wavelength = 360 nm) for 24 hr to initiate conidiophore formation, and placed in the dark for 1–3 additional days. To collect the conidia, sterile water was poured over the colonies, agitated with a glass rod, and filtered through a membrane filter of 0.8  $\mu\text{m}$  pore size (Millipore Filter Corp., Bedford, MA, 01730). The conidia were then dried for 24 hr over anhydrous  $\text{CaSO}_4$  before use.

**Inoculation procedure.** Onion plants were grown from bulbs forced in the greenhouse. Plants at the five-to-six leaf stage were inoculated in a settling tower that consisted of a metal cylinder (61 cm in diameter by 77 cm deep) mounted on a wooden base. The plants were placed on a turntable operated at 5–6 rpm in the base of the tower, and 2.5 mg of conidia (except where otherwise noted) were dispersed over the plants by gently blowing them from weighing paper and allowing them to settle for 30 min. About 3,750 conidia per square centimeter of the base were deposited with each 2.5 mg of conidia.

**Effect of dew duration on conidial size.** Inoculated plants were placed in a water-saturated atmosphere in a dew chamber (Percival model I-35DL, Percival Manufacturing Co., P.O. Box 249, Boone, IA 50036) for 24 hr (25 C), then placed in a growth chamber (Sherer Model MG-8, Sherer-Gillett Co., Marshall, MI 49068) at 23–24 C, RH = 35–50%, with a 12-hr day length for 1 wk. Then they were again incubated in the dew chamber for 9, 12, 15, or 18 hr (25 C) to promote sporulation. Lesions were excised immediately after each dew duration and were shaken for 3 min in tubes containing distilled water and 1 drop of Tween 20. Conidial suspensions were plated on water agar, and 60 individual conidia were measured at a magnification of 200. All experiments were repeated once.

**Effect of dew duration on conidial germination and infectivity of conidia.** Plants were inoculated as previously described, except that 10 mg of conidia were used to increase lesion numbers. Plants were placed in a dew chamber (25 C) for 24 hr, then moved to the growth chamber (23–24 C) for 5 days. Plants were then placed back in the dew chamber (25 C) for 9, 12, 15, 18, 21, or 38 hr. Lesions were excised, and developing conidia were collected as described above, plated onto water agar, and incubated in the dark for 4 hr at 24 C. Conidia were stained with 0.1% cotton blue in 85% lactic acid and examined for germination. Conidia were considered to be germinated if germ tubes were at least 10  $\mu\text{m}$  long.

The infectivity of conidia formed during dew durations of 12, 16, or 20 hr was determined by washing conidia from lesions immediately after each dew duration, and their concentration in suspension was determined with a hemacytometer. Conidia formed after a 9-hr dew duration were small and incompletely formed, and the conidial concentration could not be determined; these conidia were not further evaluated. For inoculation tests, leaves of four onion plants were wiped with cheesecloth to remove surface wax (this made the leaves more easily wettable but did not injure the tissue), then sprayed to runoff with an aqueous suspension of  $2.0 \times 10^4$  conidia per milliliter. These plants were placed in the dew chamber for 24 hr (24 C), then moved to the growth chamber (23–24 C). Lesions and flecks were counted after 6 days.

**Effect of RH on conidial formation by *A. porri*.** Onion plants were inoculated, placed in the dew chamber (25 C) for 24 hr, then moved to the growth chamber (23–24 C) for 7 days. Plants

were placed under near-UV light (peak wavelength = 360 nm) for 3 hr; then leaves containing lesions were excised and incubated in sealed glass jars above a reservoir of water or saturated salt solutions of  $\text{K}_2\text{SO}_4$ , KCl, or NaCl (9) at 24 C. The RH in the jars after 24 hr of equilibration was 100, 97.5, 85.5, or 75%. Tissue (0.5  $\text{cm}^2$ ) was excised from the center of each lesion after 48 hr of incubation, and conidia were washed off and suspended in 80% ethanol. Conidia were collected on a Millipore filter and counted.

**Ability of conidia to form repeatedly on a single lesion and to form on lesions on senescent leaves.** Plants that had been inoculated were placed in the dew chamber for 24 hr (25 C), moved to the growth chamber (23–24 C) for 6 days, exposed to near-UV light for 6 hr, and finally incubated in the dew chamber again for 24 hr. Thirty sporulating lesions on 10 plants were marked with tags so that they could be subsequently identified. Conidia were then removed from the lesions with compressed air, and the plants were returned to the growth chamber for three additional days at RH 35–50%, which was unfavorable for spore production. After a 3-day dry period, plants were again exposed to near-UV light for 6 hr, examined to ensure that no conidia were present, and incubated in the dew chamber for 24 hr. The same 30 lesions were then examined for conidial formation.

Infected onion plants with well-developed lesions were placed in the dew chamber for 24 hr, then moved to the growth chamber. One lesion on each of 10 leaves was examined for conidia and tagged. All conidia were removed with a small brush. After 24 hr in the growth chamber, the plants were replaced in the dew chamber for 24 hr and examined for conidial formation; the conidia were removed. This process was repeated for seven cycles to determine how many cycles of conidial formation could occur.

To determine whether conidia of *A. porri* formed on senescent leaves, plants were inoculated with 5.0 mg of conidia of *A. porri*, placed in the dew chamber for 24 hr (25 C) for infection to occur, then placed in the growth chamber (23 C) for 14 days. Senescent leaves that were completely dead and dry were detached from the plants, exposed to UV light for 6 hr, then placed in the dew chamber for 24 hr. One lesion on each of 20 leaves was examined for conidia.

**Studies on conidial ontogeny of *A. porri* using the scanning electron microscope.** Onion plants infected with *A. porri* were maintained in the growth chamber (23 C, 12-hr daylength) as purple blotch lesions expanded. Plants were then placed in the dew chamber (24 C) in the dark to induce conidial formation. Small tissue samples were cut from lesions at 3-hr intervals beginning 9 hr after placement in the dew chamber. These were vapor-fixed with  $\text{OsO}_4$  for 96 hr in a closed chamber, air-dried for two days, and then mounted, coated, and examined for conidial development in a JEOL JSM-35 CF scanning electron microscope.

## RESULTS

**Effect of dew duration on conidial size.** Average widths and lengths of conidia tended to increase when dew duration increased from 9 to 18 hr (Fig. 1). Conidial length (Fig. 1A) increased concurrently with width (Fig. 1B) and beak length (Fig. 1C). The longest conidium was 125  $\mu\text{m}$ .

**Effect of dew duration on conidial germination and infectivity of conidia.** Incidence of germination increased from 26% for conidia formed during 9 hr of dew duration to a maximum of 96% for those formed in 21 hr of dew duration (Fig. 2). Numbers of the small nonexpanding flecks or both the flecks and expanding lesions that formed on plants inoculated with conidia increased as dew duration increased. Plants inoculated with conidia from lesions incubated for 12, 16, or 20 hr in dew developed 60, 687, and 1,026 flecks and 0, 10, and 15.5 lesions, respectively.

**Effect of RH on conidial formation by *A. porri*.** Conidia were formed at all humidities tested (75–100%); few (<175/ $\text{cm}^2$ ) were formed at 75–85% RH, but the number increased with increasing humidity (Fig. 3). The number of spores that formed at each RH tested was highly variable in these experiments.

**Ability of conidia to form repeatedly on a single lesion and**

to form on lesions on senescent leaves. *A. porri* sporulated more than once on all 30 lesions examined. Six of 10 leaf segments containing lesions and placed in the dew chamber continued to support sporulation after eight alternate wet-dry periods. However, after two cycles, conidial density became increasingly sparse. Fewer than 50 conidia per square centimeter of lesion tissue formed on lesions that had sporulated through eight cycles, whereas  $10^4$ – $10^5$  conidia per square centimeter were commonly produced during the first cycle. Conidia formed profusely ( $\geq 10^4/\text{cm}^2$ ) on all lesions on senescent leaves.

**Studies on conidial ontogeny of *A. porri* using the scanning electron microscope.** After 9 hr in a water-saturated atmosphere, conidiophores observed in the scanning electron microscope were well developed and conidial initials could be seen (Fig. 4A). Even at this early stage, the orientation of conidia was evident because the bases of the conidia were larger than the tips. After 12 hr of dew, the conidial body and beak could be clearly distinguished (Fig. 4B); however, conidia were observed to be in different developmental stages at this time. A few transverse septa could be seen in the bodies of most conidia. After 15 hr of dew, the conidia had taken on the shape characteristic of *A. porri* (Fig. 4C). Both transverse and longitudinal septa were present, and the beaks were generally longer than the bodies of the conidia. Conidia appeared fully mature with long appendages (beaks) when exposed to 15–24 hr of dew (Fig. 4D).

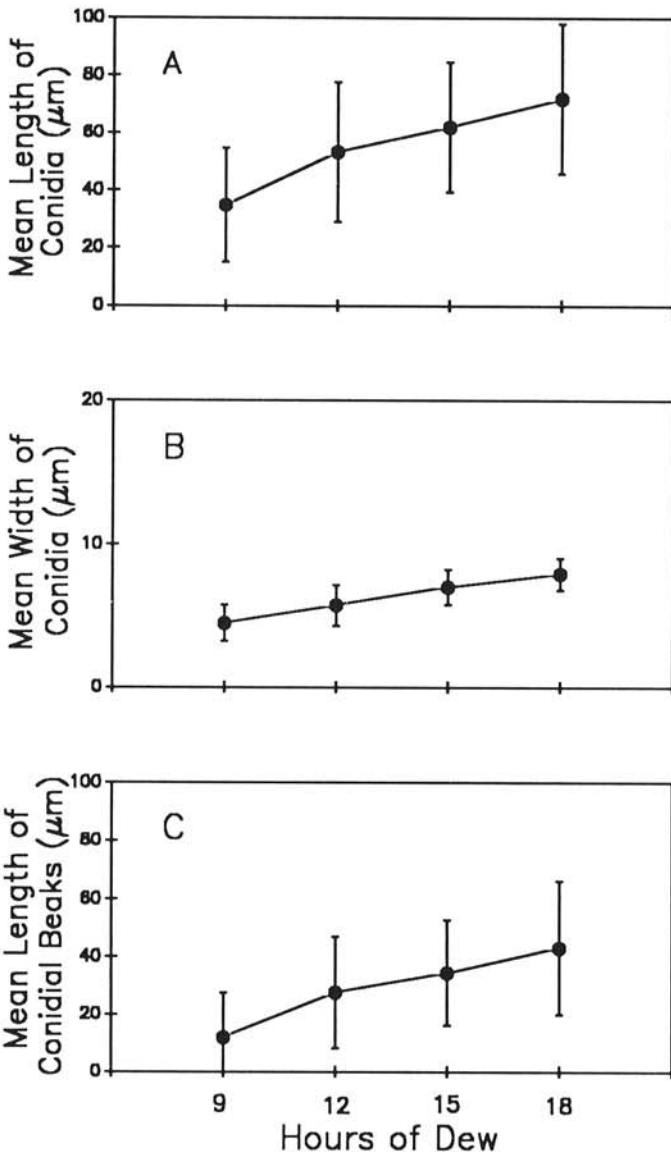


Fig. 1. Effect of dew duration on the total length (A), width (B), and beak length (C) of conidia of *Alternaria porri*. Bars indicate the standard error of the mean.

## DISCUSSION

Dew period requirements for conidial formation and infection of onion leaves by *A. porri* were similar to those of the closely related species *A. solani* and *A. dauci* (5,12,16,17). Three hours after conidial initials formed, we observed septa, and after 6 hr, conidia appeared to be mature. Lukens and Horsfall (13), who examined *A. solani*, observed septa 3 hr after initials formed but did not see mature conidia until 10 hr later. However, conidiophores were 2 days old at the time of spore inducement, which may have affected maturation, and the size of conidia produced varies a great deal (Figs. 1 and 4) (10). In these studies, dew duration of 9 hr resulted in conidia able to germinate and dew duration of 12–16 hr in conidia able to cause either flecks or lesions. Similarly, conidia of *A. dauci* formed after 10 hr of

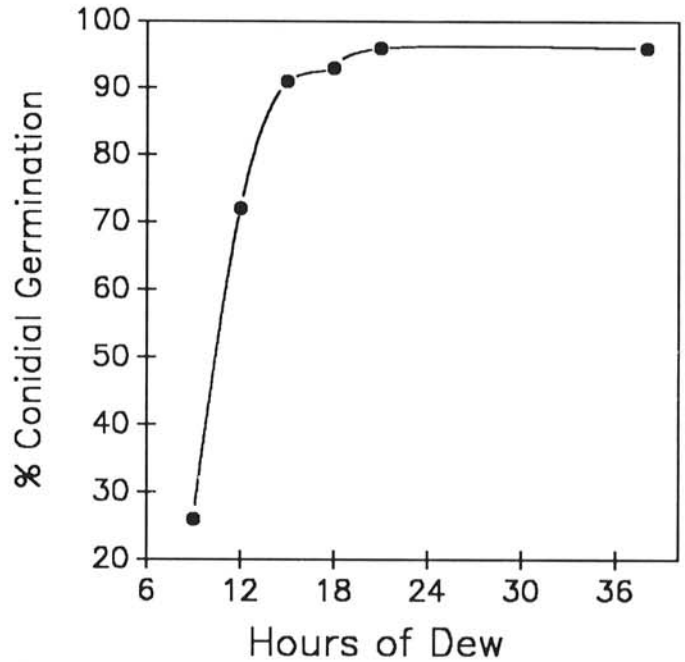


Fig. 2. Percentage of germinable conidia of *Alternaria porri* that formed on onion leaves after 9, 12, 15, 18, 21, or 38 hr of dew.

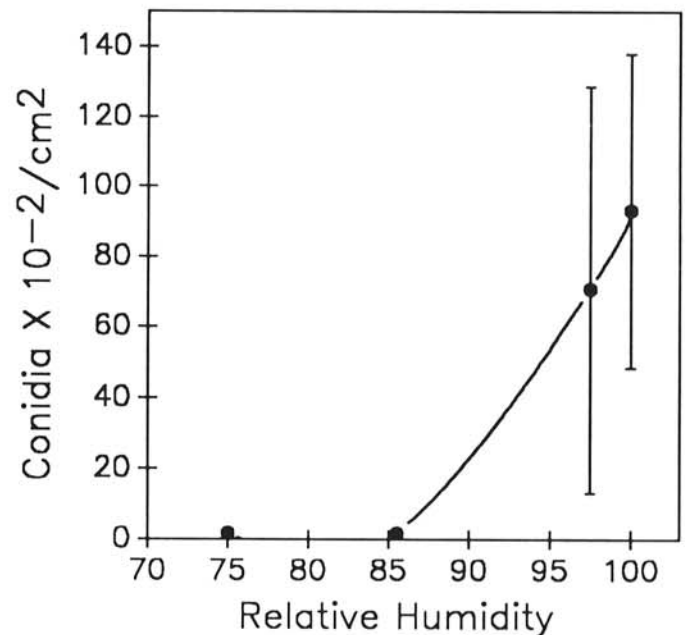
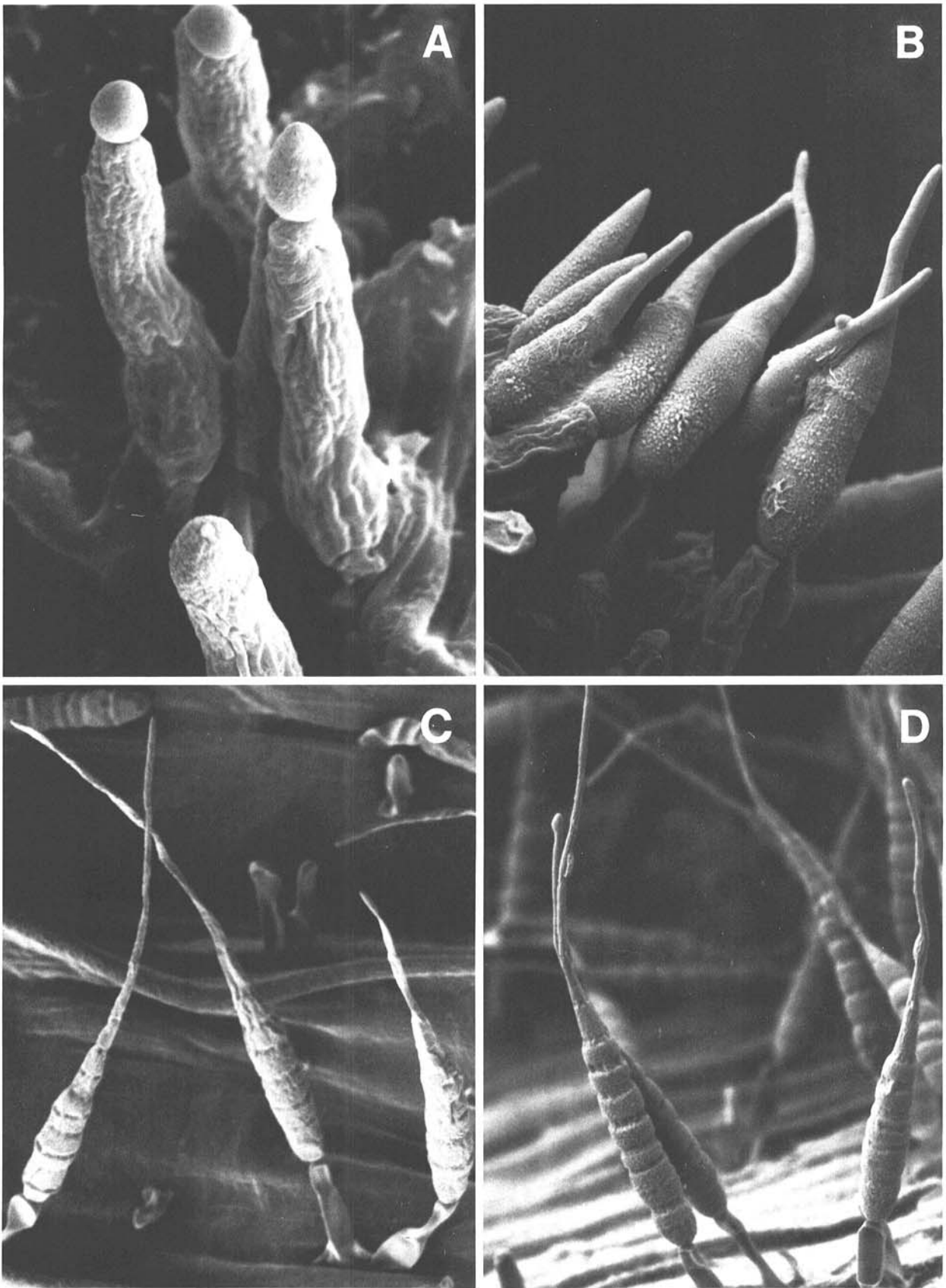


Fig. 3. Influence of relative humidity on numbers of conidia of *Alternaria porri* formed per square centimeter of lesions on onion leaves. Bars indicate the standard error of the mean.



**Fig. 4.** Photomicrographs of formation of conidia by *Alternaria porri* on onion leaves. **A**, Conidiophores with conidial initials after plants were incubated for 9 hr in the dew chamber (1 cm = 3.66  $\mu$ m). **B**, Conidia with definite beaks after a 12-hr dew period (1 cm = 8.77  $\mu$ m). **C**, Conidia with both longitudinal and transverse septa after 15 hr of dew (1 cm = 14.8  $\mu$ m). **D**, Mature conidia of *A. porri* with characteristic long beaks (1 cm = 17.5  $\mu$ m).

dew duration on already existing conidiophores, and 90% of these conidia germinated (17). Rotem and Reichert (16) found that 16 hr of leaf wetness was necessary for conidia formation on plants kept in growth chambers, but fewer hours were necessary for plants in the field. They attributed this to the ability of conidia of *A. solani* to form over several short interrupted dew periods (5). Conidia formed when the continuous and interrupted dew durations were the same total length of time (for example two 6-hr dew durations compared to one 12-hr dew duration). We observed that conidia would form repeatedly on lesions when dew was interrupted, up to eight wet-dry cycles.

We observed low levels of conidial formation of *A. porri* on onion leaves at 75–85% RH, similar to the formation of conidia on agar blocks (11). However, these results must be carefully interpreted since RH at the leaf surface, where conidial formation occurs, may have been higher than that in the surrounding air. We never observed conidial formation on lesions on plants incubated at ambient RH (35–50%). Strandberg (17) found that conidia of *A. dauci* formed on infected attached and excised carrot leaves maintained at 96–100% RH. Further studies on rates of conidial development by *A. porri* under fluctuating RHs and interrupted dew periods are needed to understand their influence on conidial formation, especially in a field setting, where humidities are fluctuating daily and dew durations shorter than 9 hr are common.

Leaf senescence did not influence conidial formation and may indicate that all tissue supporting sporulation is functionally dead. Bashi and Rotem (3,6) found that the number of conidia formed by *A. solani* increased as tomato leaves became increasingly senescent and died. Also, formation of conidia increased each subsequent night of a 6-day period studied, probably due in part to the increasing leaf senescence and lesion expansion. Many purple blotch lesions, especially on older leaves, expand rapidly, girdling the leaves and causing them to become senescent and die (2). These leaves may eventually fall off the plant. When weather is conducive to conidial formation, conidia can readily form on these lesions.

Little information is available on why flecks form on onion leaves inoculated with *A. porri*. Bock (7) believed that low RH during infection as well as a 6-day postinfection period of low RH (<80%) favored fleck formation over lesion formation. However, many lesions formed at high RHs and may have “masked” the flecks. Flecks may be the result of immature conidia that are not aggressive enough to colonize the onion leaf before encountering a plant resistance response. Expanding and nonexpanding lesions have also been observed in infection of onion by *Botrytis squamosa* Walker and *B. cinerea* Pers. ex Fr. However, nonexpanding lesions associated with these *Botrytis* spp. differ from flecks caused by *A. porri* in that hyphae of *B. squamosa* and *B. cinerea* infect but are restricted in length (1,8). Cavity formation may occur in advance of infection hyphae (8).

Conidia of *A. porri* can always be seen at the centers of flecks, and onion epidermal and palisade cells within flecks are vacuolated but no mycelia are visible (7). Nonexpanding lesions caused by *Botrytis* spp. may be the onion plant's response to ingress of the pathogens, whereas fleck formation caused by *A. porri* may be the plant's response to the presence of the pathogen on the leaf surface.

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