Influence of Leaf Position and Maturity on Development of *Botrytis squamosa* in Onion Leaves

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ABSTRACT

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Onion plants inoculated with dry conidia of *Botrytis squamosa*, then incubated in a dew chamber at 20 C for 24 hr, displayed, from youngest (innermost) to oldest (outermost) leaves: increasing numbers of lesions, increasing mean lesion areas, and increasing mean lengths of infection hyphae. However, lesion area was not significantly correlated with lengths of infection hyphae within lesions after inoculation and subsequent incubation in a dew chamber at 20 C for 24 hr. In most lesions, hyphal development was restricted. Lesions developed rapidly, within 24 hr after inoculation, although the rate of development was greater on the older leaves than on the younger ones. Growth of infection hyphae within senescent tissues was about three times faster than in nonsenescent tissues on the same leaf.

Botrytis leaf blight, which is caused by *Botrytis squamosa* Walker, is an important leaf spotting and blighting disease of onion (*Allium cepa* L.) foliage (3,4,6). Leaf spots generally appear as discrete, desiccated spots of restricted size (2–5 mm long by 1–4 mm wide). If leaves remain wet continuously for 2–3 days, some lesions expand rapidly, leading to leaf blighting and collapse of tissues (1,3,4,7). Leaves generally die from the tip downward. More lesions and more extensive blighting occur on outer (older) leaves than on younger (inner) leaves (4,6–8). In previous studies, the development of *B. squamosa* in lesions on leaves of differing maturity was not examined, nor was number of lesions per unit leaf area determined.

Histopathology of infection was studied by Clark and Lorbeer (2). They defined two phases in lesion formation: collapse and separation of mesophyll from the epidermis underneath the germinating spore(s), resulting in cavity formation; and subsequent collapse and degeneration of adjacent tissues. We (1) reported that hyphal development within the 2–5 × 1–4 mm lesion area was restricted in most cases, but in some lesions ramification of hyphae continued into adjacent tissue, and the proportion of lesions where this occurred increased with increasing lengths of continuous dew periods. These lesions (called expanding lesions) led to leaf blighting. However, we did not quantify development of *B. squamosa* within lesions in relation to leaf position or maturity. Shoemaker and Lorbeer (7) showed that numbers of lesions per leaf increased with increasing leaf age, but they did not quantify their data with respect to leaf area. Leaf length varies considerably with leaf age, so that younger leaves would necessarily have smaller leaf surface areas.

Although some aspects of histopathology of infection and lesion development have been documented, there is insufficient knowledge about the influence of leaf age on numbers of lesions per unit leaf area resulting from similar numbers of spores, the development of *B. squamosa* within lesions, and the effect of leaf position and leaf maturity on that development. Since sporulation of *B. squamosa* is associated only with blighted or dead leaf tissues rather than with individual small lesions in otherwise healthy tissue (8,9), knowledge of the effect of leaf age on development of *B. squamosa* within lesions could improve our understanding of onion leaf blighting and of onion leaf blight epidemiology.

The objectives of this study were to examine the effects of leaf maturity on numbers of lesions per unit leaf area, on lesion size, and on development of infection hyphae within lesions on onion leaves.

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MATERIALS AND METHODS

In all experiments a virulent isolate (BSS-4) of *B. squamosa* was used that sporulated prolifically on autoclaved onion leaves on water agar (1). Inoculations were carried out on 1-mo-old onion plants (cultivar Spartan Banner) sprouted from bulbs. Plants were positioned within a cylindrical 61 × 77-cm deep settling tower. Dry conidia (2.5 mg = ~1.35×10^5 conidia, or about 46 conidia per square centimeter) were dispersed near the top of the tower by directing a low velocity stream of air from a pipet tip over the conidia on a piece of weighing paper, while the plants were rotated within the chamber at 5–6 rpm (1). After the conidia were dispersed, the top of the tower was covered for about 5 min to reduce external air currents and allow the spores to settle on leaf surfaces. Dew formed on the plants in less than 1 hr after they were placed in a commercial dew chamber (Percival Mfg. Co., Boone, IA).

The position of leaves on plants was recorded on a scale of one through five or six, in which one represented the youngest (innermost) leaf and five or six represented the oldest (outermost) leaf.

Leaf position vs lesion numbers. The influence of leaf position on the number of lesions per unit area of leaf induced by a standardized number of conidia was examined. Six replicate onion plants were inoculated, placed in a dew chamber at 20 C for 24 hr, then held in a growth chamber at 20 C for 6 hr. Leaf area was determined and lesions were counted for each leaf. Since calculated leaf area, using the equation for area of a right circular cone ($A = \pi r h$, in which A = area, π = 3.146, r = radius of cone base, and h = cone height), was generally within 1–2 cm^2 of leaf area determined by using a leaf area meter (Li-Cor model 3100, Li-Cor, Lincoln, NB), this equation was used for computing leaf areas. This represented a simple, nondestructive method of estimating leaf area.

Leaf position vs lesion size. The relationship between leaf position on the plant and mean lesion size was examined. Four replicate onion plants were inoculated with 2.5 mg of dry conidia and incubated in the dew chamber at 20 C for 24 hr. Four 1-cm^2 tissue segments were excised from each of four replicate leaves at different positions on the plant. Lesions were fixed in FAA (37% formalin-50% ethanol-glacial acetic acid [1:18:1, v/v]) and stained with 1% aqueous trypan blue prior to examination. Lesions were elliptical, and estimates of lesion area were obtained by multiplying maximum lesion length by maximum width. A minimum of 20 lesions per leaf were examined.

Leaf position vs development of infection hyphae. The relationship between leaf position and mean length of infection hyphae within lesions was examined. Four onion plants were inoculated, then incubated in the dew chamber at 20 C for 24 hr. Four 1-cm^2 tissue segments were excised at random from each of four replicate leaves for each position on the plant. Segments containing lesions were fixed in FAA, stained with trypan blue, and mounted on slides prior to examination. Lengths of infection hyphae were measured by using a microscope equipped with an ocular micrometer.

Influence of tissue maturity on infection hyphae development. Development of infection hyphae in healthy and senescent tissues was examined. Four onion plants were inoculated with 5-μl aliquots of an aqueous suspension that contained 1,000 conidia per milliliter (five conidia per drop). Drops were applied at four sites on each leaf. Plants were incubated in a dew chamber at 20 C for 24 hr. Leaf pieces from inoculated senescing (yellow) and healthy (green) tissues were excised, fixed in FAA, and stained in trypan blue. A minimum of 20 hyphal lengths in senescent or healthy leaves were measured by using a compound microscope fitted with an ocular micrometer. All treatments in experiments were completely randomized, insofar as possible, although leaf position is fixed and cannot be random. Data were analyzed by analysis of variance. All experiments were repeated at least once with similar results.

RESULTS

Leaf position vs lesion numbers. Numbers of lesions per square centimeter of leaf tissue on onion plants uniformly inoculated with conidia of *B. squamosa* increased from the youngest to the oldest leaf (Fig. 1). Significantly ($P = 0.05$) more lesions were produced on the oldest leaf (position 5) than on leaves one through four (LSD test, $P = 0.05$). More lesions were also produced on leaf four than on leaves one and two.

Leaf position vs lesion size. Lesions of the same age were larger on older onion leaves (Fig. 2). Mean lesion areas were significantly greater on leaf six than on leaves one through four. Lesion areas on leaf five were greater than those on leaf one (LSD test, $P = 0.05$).

Leaf position vs hyphal development. Infection hyphae were longer in older onion leaves (Fig. 3). Significantly greater mean lengths of infection hyphae were observed on leaves five or six than on leaves one through four. Greater mean lengths were also observed on leaves three or four than on leaf one (LSD test, $P = 0.05$).

Lesion size vs lengths of infection hyphae. Lesion area correlated poorly with length of infection hyphae within lesions after 24 hr of incubation in the dew chamber. Correlation coefficients (r) for these variables on leaves in positions 1 to 6 were 0.67, 0.36, 0.36, 0.42, 0.36, and 0.27, respectively. After 1 day in the dew chamber, infection hyphae were <100 μm in length, although lesions had developed to near the maximum size for nonexpanding lesions (2–5 × 1–4 mm), indicating that lesions developed well in advance of the hyphae within.

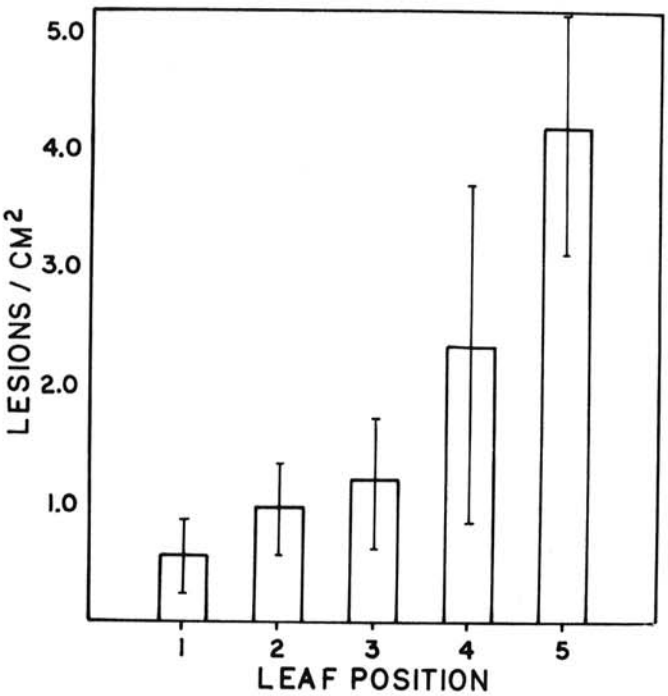

Fig. 1. Influence of leaf position on numbers of lesions on onion after inoculation with *Botrytis squamosa* and incubation in a dew chamber at 20 C for 24 hr (leaf position 1= youngest, innermost leaf).

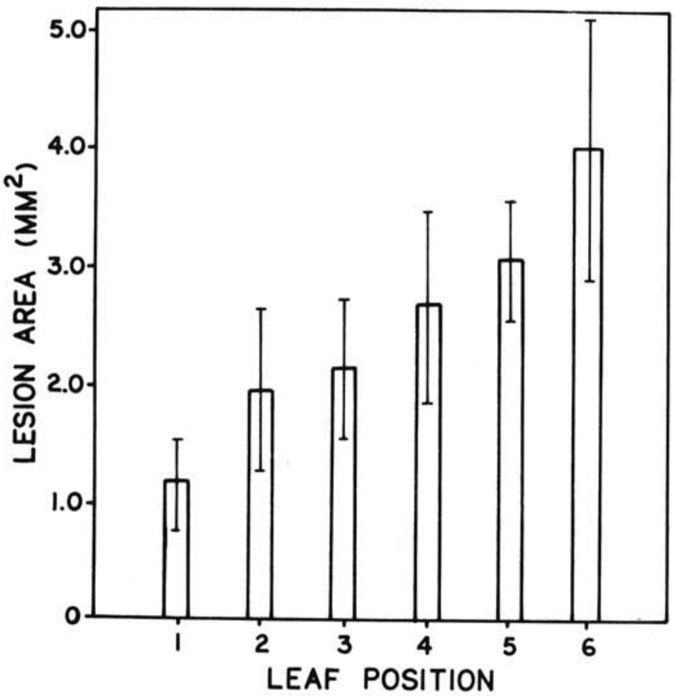

Fig. 2. Influence of leaf position on lesion size on onion after inoculation with *Botrytis squamosa* and incubation in a dew chamber at 20 C for 24 hr (leaf position 1 = youngest, innermost leaf).

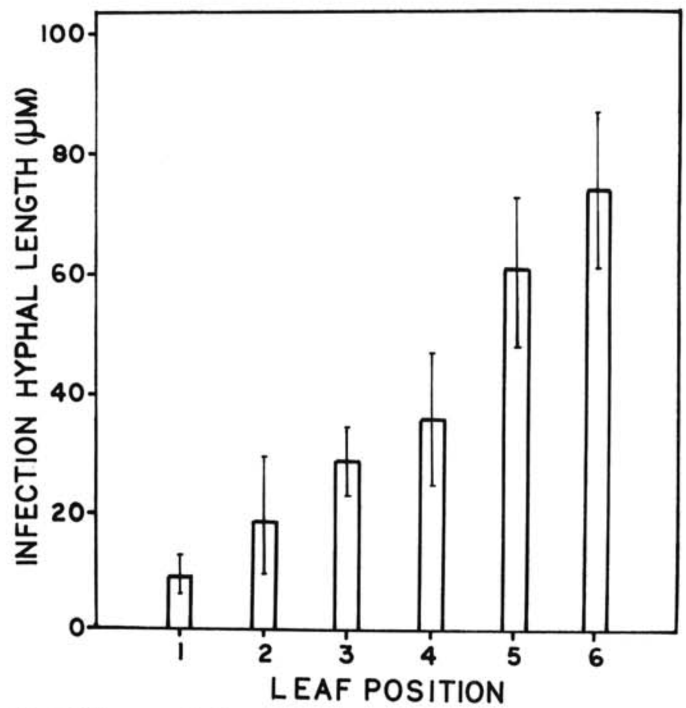

Fig. 3. Influence of leaf position on the length of infection hyphae within lesions on onion after inoculation with *Botrytis squamosa* and incubation in a dew chamber at 20 C for 24 hr (leaf position 1 = youngest, innermost leaf).

Influence of tissue maturity on infection hyphae development. Hyphae grew almost three times as fast in senescent as in healthy tissue. Lengths of infection hyphae of *B. squamosa* growing in senescent tissues at 20 C for 24 hr after inoculation were 207 ± 9 μm compared with lengths of 77 ± 15 μm in nonsenescent tissue.

DISCUSSION

The very rapid formation of lesions, which were observed within 8 hr following inoculation and subsequent incubation under dew at 20 C, indicates that germination and penetration occur quickly. Hancock and Lorbeer (3) believed that lesions developed in response to pectic enzymes released during spore germination, and that pectic enzymes alone could cause leaf spots. We observed that lesions were always associated with conidia from which a germ tube had penetrated but not with conidia from which a germ tube had not penetrated. It appears that germination must be followed by penetration to induce lesions. In addition, the lack of a significant correlation between lesion area and lengths of infection hyphae within lesions on leaves suggests that lengths of infection hyphae may be independent of the age of the leaf on which the lesion occurs.

Initial lesion formation occurs rapidly and involves cavity formation. Expanding lesion development does not result in continued cavity formation, but involves hydrolysis and collapse of the mesophyll as the fungus extends beyond the lesion border (2). Clark and Lorbeer (2) and Alderman and Lacy (1) reported formation of expanding lesions under conditions of continuous leaf wetness 72–96 hr after inoculation. While prolonged leaf wetness periods favored development of *B. squamosa* on leaves of intermediate age in a previous study (1), the work reported here demonstrated that a greater proportion of expanding lesions were present at leaf positions five or six (older) and fewest at positions one through three (younger).

Onions normally produce 15–16 leaves in a growing season, but rarely have more than ten at one time (5). Older leaves begin to senesce and die naturally, even without the effects of disease, as plants begin to mature and bulbs to form (5). When infected, older leaves provide a substrate in which a greater proportion of the lesions expand. The importance of senescing leaf tissue as a substrate for *B. squamosa* and for epidemic development is further indicated by field observations (8,9) that *B. squamosa* appears primarily as a mid- to late-season pathogen. Leaf blight generally does not become serious until bulb initiation (8,9) when senescing tissues begin to appear, even though environmental conditions may be favorable for blight development early in the season.

Senescing tissues were found in this study to be especially favorable for the growth of *B. squamosa*. Clark and Lorbeer (2) reported greater superficial growth of hyphae of *B. squamosa* when plants were inoculated with conidia suspended in water containing nutrients than in water alone. Thus, greater availability of nutrients and lack of a resistance response may account for the aggressive colonization of and sporulation on senescent tissues by *B. squamosa*. We observed longer germ tubes and more stomatal penetrations when conidia were placed on senescing tissues, although this was not quantified. Much shorter germ tubes were observed on younger leaves than on older leaves.

During leaf blight epidemics the outermost leaves are rapidly blighted. Since sporulation occurs only on blighted or dead tissues, and not on lesions in green living tissue, premature senescing or blighting of leaves greatly facilitates development of the epidemic. If large amounts of inoculum are produced, even young leaves can be severely damaged with large numbers of small lesions.

This study suggests that *B. squamosa* acts as a weak pathogen on young, healthy onion foliage, inducing a host reaction resembling the hypersensitive response (rapid collapse of cells within 6–12 hr after penetration). However, *B. squamosa* growing within mature or senescent tissues or within expanding lesions can result in destructive leaf blighting.

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