Sources of Primary Inoculum of Botrytis squamosa

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ABSTRACT

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Sclerotia-bearing conidia of *Botrytis squamosa* were found on onion bulbs and leaf debris in cull piles and seed production fields in April and May and on the surface of organic soil from April through July in fields cropped to onions in Orange County, New York. Sclerotia produced conidia in the laboratory over a range of 3-27 C (optimum 9 C) and produced four successive crops of conidia in repeated germination tests. Apothecia of *B. squamosa* were observed in April on sclerotia associated with onion leaf debris on the surface of soil in fields previously cropped to onions. Ascospores from these apothecia were pathogenic to onion leaves. Dormant sclerotia covered with 10-15 cm of soil in April produced apothecia when uncovered at different times from late May through July. *Botrytis squamosa* frequently was isolated from lesions on leaves of sprouted bulbs in cull piles and seed producetion fields in late May and early June. Conidia of *B. squamosa* were trapped from the air over cull piles and seed production fields 2-4 wk prior to the appearance of Botrytis leaf blight in commercial onion fields in 1971-73. Cull piles and seed production fields are, therefore, important sources of primary inoculum for initiation of Botrytis leaf blight epidemics in commercial onion fields in Orange County. Elimination or reduction of these sources of inoculum should delay the development of leaf blight epidemics.

Additional key words: Allium cepa, apothecia, sclerotia.

Botrytis leaf blight of onion (*Allium cepa* L.), caused by *Botrytis squamosa* Walker (*Botryotinia squamosa* Viennot-Bourgin), is an important disease in Orange County, New York where onions are grown on organic soil (8). Each year onions are sprayed routinely with protective fungicides from approximately mid-June until harvest to control the disease. Because the time of initial appearance of Botrytis leaf blight and the ultimate level of disease development vary considerably from year to year, needless sprays often are applied.

Recently, efforts have been made to forecast the development of Botrytis leaf blight so that the need for and timing of fungicide applications can be predicted (12, 20, 21). One aspect of the epidemiology of Botrytis leaf blight that has received little attention concerns the nature and source of inoculum that initiates epidemics. In addition to its importance in disease forecasting, better knowledge of this aspect would make it possible to eliminate or reduce the source of inoculum and effect better disease control.

Walker (23) noted that sclerotia and conidia of *B. squamosa* survived from December to March in situ on onion bulbs placed on a window sill in Wisconsin. Lafon (12) proposed that conidia produced by sclerotia were responsible for initiation of primary infection. McLean and Sleeth (16) suggested that sclerotia produced on infected onions may perpetuate *B. squamosa* from year to year in Texas, and it has been demonstrated that sclerotia perennate in the soil and on the soil surface in association with onion leaf debris in New York (6). There have been no attempts, however, to make direct correlations between the above observations and development of Botrytis leaf blight epidemics.

Cronshey (5) was the first to produce the apothecial stage of *B. squamosa* in culture, and Viennot-Bourgin (22) described and named the fungus. Apothecia have been produced in culture by several other investigators (3, 15), but heretofore they have not been observed in nature. Apothecia of *B. squamosa* were found occurring naturally during this study. A preliminary report of these observations was published earlier (7).

The purposes of this study were to ascertain where and when the buildup of *B. squamosa* inoculum first occurs in the spring, to determine the immediate source and the nature of the primary inoculum, and to relate the appearance of primary inoculum to development of Botrytis leaf blight epidemics.

MATERIALS AND METHODS

Production and sporulation of sclerotia.—Sclerotia were produced on autoclaved onion leaf straw in order to simulate those formed naturally. Bottoms of Pyrex glass petri plates (90 × 15 mm) were covered with onion leaf

straw (about 2 g), 20 ml of water was added, the plates were autoclaved for 30 min, and 2 ml of a *B. squamosa* conidial suspension was spread over the straw surface. The plates were incubated at 18 C in darkness for 3 wk and then incubated under continuous fluorescent light (Sylvania Cool-White F20T12-CW) at 18 C for 3 wk. Sclerotia then were collected as described previously (6).

To test the effect of temperature on formation of conidia, sclerotia were placed in nylon mesh bags and buried 15 cm below the soil surface in an Orange County field from September to May. They then were removed from the bags, washed in distilled water, placed on moistened filter paper in glass petri plates (20 sclerotia per plate), and incubated under continuous fluorescent light (Sylvania Cool-White F20T12-CW) for 3 days at 12 C. Ten plates then were incubated in darkness in 11 different incubators maintained at 3-degree temperature intervals (0-30 C). After 2 wk, five plates were removed from each

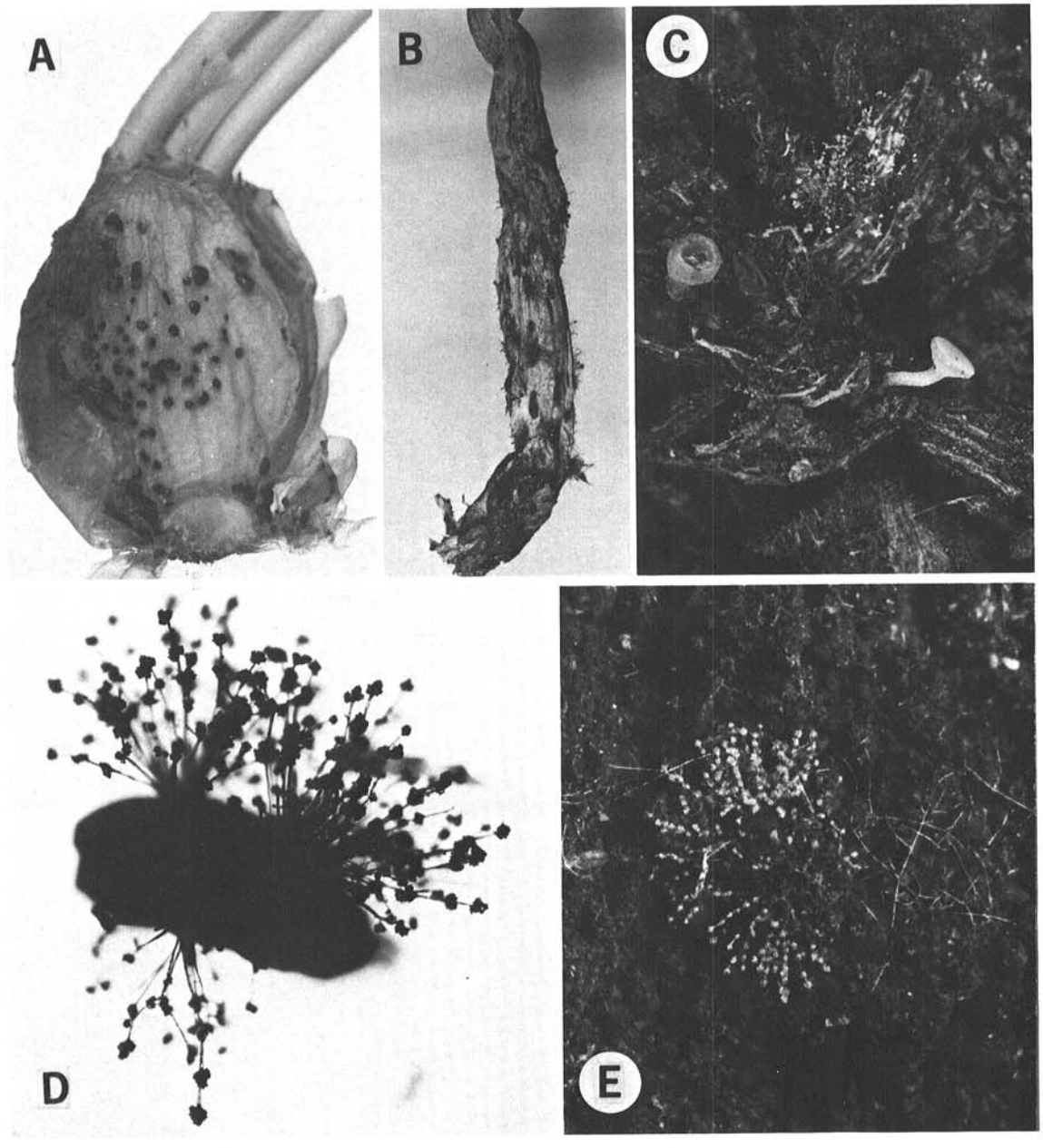

Fig. 1-(A to E). Sclerotia as sources of conidia of *Botrytis squamosa*. **A)** Sclerotia (which later produded conidia) on a sprouted mother bulb in a seed production field in April. **B)** Sclerotia producing conidia on a piece of onion leaf debris in a cull pile in May. **C)** Sclerotium producing conidia and apothecia of *B. squamosa* simultaneously on leaf debris in April on the surface of soil in a field cropped to onions the previous year. **D)** Sclerotium with conidia on the soil surface. **E)** Sclerotium with conidia on the soil surface in June in a field cropped to onions.

incubator, sclerotia for each temperature were placed in 125-ml Erlenmeyer flasks with 50 ml of water, the flasks were shaken on a Burrell Wrist-Action shaker for 30 min, and the number of conidia produced per sclerotium was determined for each temperature with a hemocytometer. This procedure was repeated 2 wk later with the remaining five plates.

To determine ability for repeated production of conidia, air-dry sclerotia were spread over the surface of moistened filter paper in five glass petri plates (1 g of sclerotia per plate). The plates were enclosed in small plastic bags to reduce evaporation and then placed at 9 C in darkness. After 3 wk, the sclerotia from each plate were placed in 250-ml Erlenmeyer flasks with 100 ml of distilled water, the flasks were shaken for 1 hr on a Burrell Wrist-Action shaker, and the number of conidia

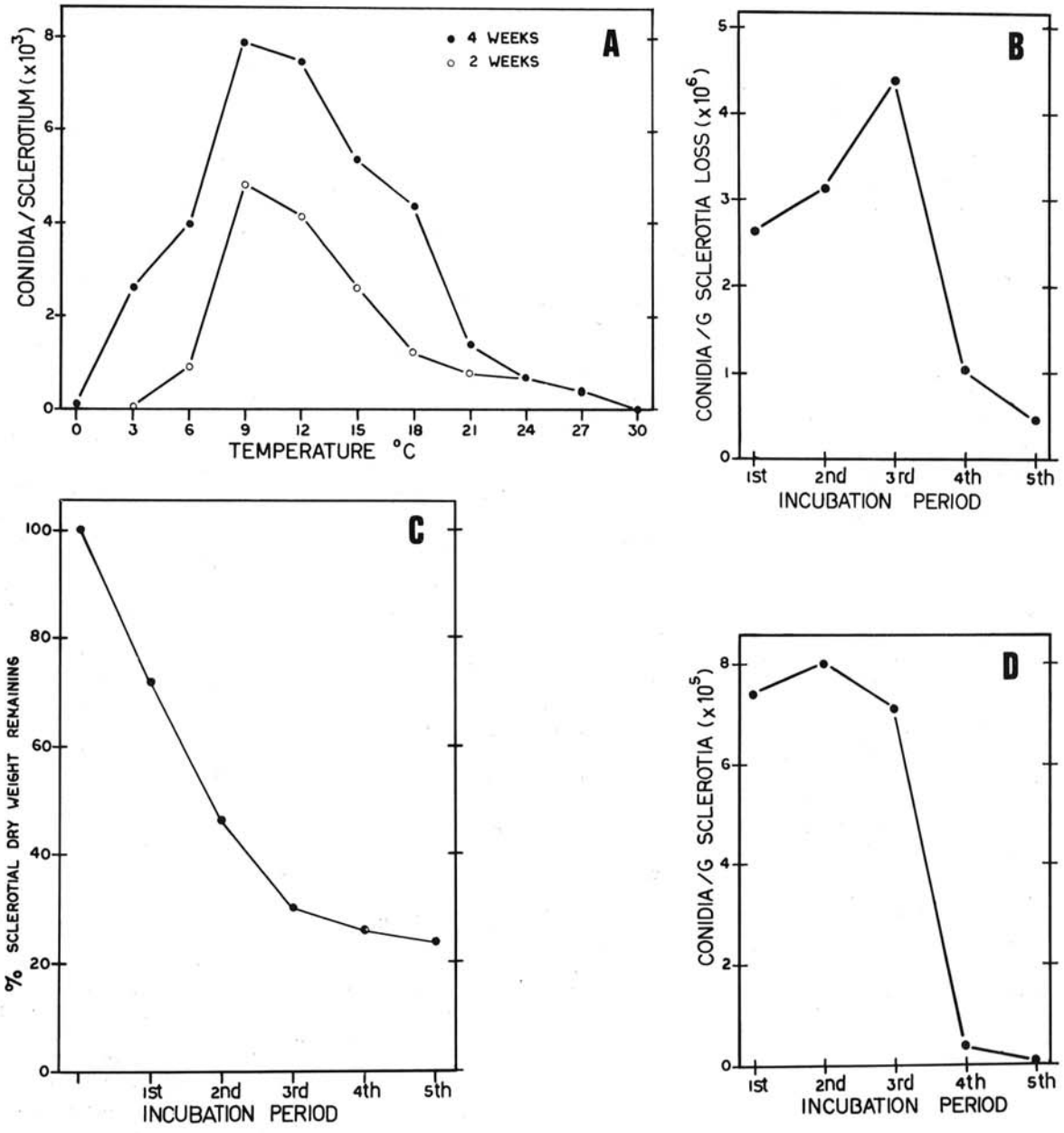

Fig. 2-(A to D). In vitro production of conidia by sclerotia of *Botrytis squamosa*. **A)** Effect of temperature on sporulation. Sclerotia that had overwintered in the field 15 cm below the soil surface were incubated on moistened filter paper in petri plates at the indicated temperatures in darkness for 2 or 4 wk. (B to D) Repeated production of conidia by sclerotia incubated on moistened filter paper in petri plates at 9 C in darkness. Incubation periods lasted 3 wk and conidiophores and conidia were washed from the sclerotia after each period. **B)** Efficiency of conidial production based on air-dry sclerotial weight loss. **C)** Percent of initial sclerotial air-dry weight remaining after each incubation period. **D)** Total conidial production per gram of air-dry sclerotia during each incubation period.

produced was determined as described above. Sclerotia from each flask were weighed after air-drying for 8 hr on paper towels. The sclerotia then were spread again over filter paper in Pyrex glass petri plates, the plates were incubated under continuous fluorescent light (Sylvania Cool-White F20T12-CW) at 18 C for 3 days, the filter paper was moistened, and the plates then were placed in plastic bags at 9 C in darkness for 3 wk. The above procedure was repeated four times.

Fungal isolations.—Onion leaves with typical Botrytis leaf blight lesions were collected from cull piles and seed production fields in late May and early June in 1971, 1973, and 1974. In May 1975, leaves with such lesions were obtained from volunteer onion plants (sprouted bulbs left in the field after harvest in 1974) in commercial onion fields. Leaves were washed in tap water and surface sterilized in a 0.5% sodium hypochlorite solution. Leaves then were rinsed twice in sterile distilled water, small sections were cut from margins of lesions, the sections were plated on acidified potato-dextrose agar (APDA) in petri plates, and the plates were incubated at 16-18C in darkness for 4-6 days. The plates then were examined for fungal growth, and transfers were made to potato-dextrose agar (PDA) slants to confirm identification.

Onion seeds as sources of inoculum.—Since survival of *B. squamosa* on onion seeds has been observed (6), investigations were made to determine if primary inoculum originated from onion seeds planted in the spring.

In one set of experiments, 50 onion seeds (cultivar Early Yellow Globe) were planted in 48 clay pots (10-cm diameter) containing either natural or autoclaved organic soil. The seeds were naturally infested with *B. squamosa* at the rate of 6%, as determined by plating of seeds on APDA. The soil was free of *B. squamosa* propagules. The pots, covered with clear plastic bags to prevent contamination and to maintain a high relative humidity, were placed on a shaded greenhouse bench at 18 C, and the soil was kept moist by subirrigation. After 1 wk, and at weekly intervals thereafter for the next 5 wk, the germinating seeds or seedlings were removed from four pots of both natural and autoclaved soil and examined for the presence of *B. squamosa* sporulation. Portions of the seedlings (either washed in water or surface sterilized) were plated on APDA in petri plates in attempts to isolate *B. squamosa*. The experiment was repeated as described above, except that the pots were maintained in a growth chamber at 85% relative humidity (RH), day and night temperatures of 24 and 18 C, and a 16-hr photoperiod.

In similar experiments, onion seeds (cultivar Elite) were coated with conidia by shaking them over the surface of sporulating cultures of *B. squamosa* grown on onion leaf straw agar (6) in petri plates. The seeds then were treated as described in the above experiments.

Spore trapping.—To determine the location of sources of air-borne inoculum of *B. squamosa* and when inoculum buildup was occurring, many different suspected sites were monitored. This was logistically impossible with conventional spore traps; moreover, trapping methods based on microscopic recognition of spores on glass slides were not feasible because of the simultaneous occurrence of similar conidia of other fungi at some of the monitored locations. Therefore, a method was devised which involved exposing petri plates containing a previously described agar medium selective for *B. squamosa* (6). Although this method was less sensitive than use of a continuously sampling spore trap, it facilitated monitoring of many sites, made possible positive identification of trapped propagules, and provided verification of the viability of the trapped propagules. Four plastic petri plates (90 × 15 mm), containing 12-15 ml of the selective medium, were attached to a wood stake 15, 40, 65, and 90 cm above the soil surface. Plates were exposed with the agar surface in a vertical position, facing into the wind when there was noticeable air movement and into the direction of prevailing winds when the air appeared calm. Four plates were exposed daily for 10 min at each location. Time of exposure usually was between 0800 and 1300 hours, the period of the day when maximum numbers of air-borne *B. squamosa* conidia were expected (19); however, plates occasionally were exposed later in the day when rain or other circumstances precluded earlier exposure. The plates then were incubated at 16-18 C for 5-6 days in darkness and *B. squamosa* colonies were counted. It was assumed that the colonies originated from conidia since ascospores and mycelial fragments of *B. squamosa* either do not germinate or do not give rise to colonies on the selective medium.

In 1971, six locations in Orange County were monitored from 2 June through 23 August. These included a large cull pile (covering about 1 hectare) that had been a dumping site for discarded onion bulbs and associated debris for at least 5 yr and in which onion bulbs and plants were in all stages of growth and decay during this study, a field in which onion seed was produced from sprouted mother bulbs, a field cropped to lettuce in which numerous sclerotia of *B. squamosa* were returned to the soil after harvest in 1970, and three direct-seeded fields for commercial fresh bulb production. Five locations monitored from 15 June through 16 August in 1972 included the cull pile monitored in 1971, a seed production field, and three commercial fields. In 1973, trapping was begun on 25 May and continued until 15 August. The seven locations monitored included the cull pile monitored in 1971 and 1972, a smaller cull pile where bulbs had been periodically discarded for 2 yr, two seed production fields, and three commercial fields.

RESULTS

Production of conidia by sclerotia in nature.—Sclerotia bearing conidia of *B. squamosa* frequently were found in April and May in Orange County on cull piles where onion bulbs and associated debris were discarded throughout the year. Sclerotia with conidia most commonly were present on segments of bulb necks that were separated from bulbs during grading following removal from storage (Fig. 1-B). Sclerotia also were observed on bulb scales and on blighted leaves that were not removed by mechanical harvesters. On several occasions, sclerotia with conidia were found in cull piles on bulbs that originated outside Orange County. Similarly, sclerotia were observed on the scales (Fig. 1-A) and neck regions of bulbs planted in March for seed production. These sclerotia usually produced conidia by early May.

Sclerotia on the soil surface of fields in which sclerotia had been incorporated into the soil after harvest the previous year produced conidia when the soil surface was moist (Fig. 1-D, E). They were observed with varying frequency in many fields from May through July and usually were found only where the onion leaf canopy provided sufficient shade to retard drying of the soil surface. Sclerotia of *B. squamosa* giving rise to conidiophores and/or apothecia also were observed in early April when onion leaf debris had overwintered on the soil surface (Fig. 1-C).

Effect of temperature on production of conidia by sclerotia.—Maximum in vitro production of conidia by sclerotia of *B. squamosa* occurred at 9 C after incubation for either 2 or 4 wk (Fig. 2-A). Moderate-to-profuse production of conidia occurred at 3-18 C after 4 wk. No conidia were evident after 2 wk at 3 C, but moderate development of conidia had occurred after 4 wk. Production of conidia was only slight at 21-27 C and none were produced at 30 C.

Repeated production of conidia by sclerotia.—Similar numbers of conidia were produced on sclerotia during each of the first three incubation periods (Fig. 2-D). The number of conidia produced decreased sharply during the fourth incubation period and production was almost nil during the fifth. Loss of sclerotial dry weight was about 60% during the first two incubation periods, but then was less in the third period and decreased very little during the remaining incubation periods (Fig. 2-C). Sclerotia produced conidia until about 70% of the sclerotial air-dry weight had been dissipated. The number of conidia produced per unit of dry weight lost was greatest during the third incubation period, followed by the second and first incubation periods, respectively (Fig. 2-B).

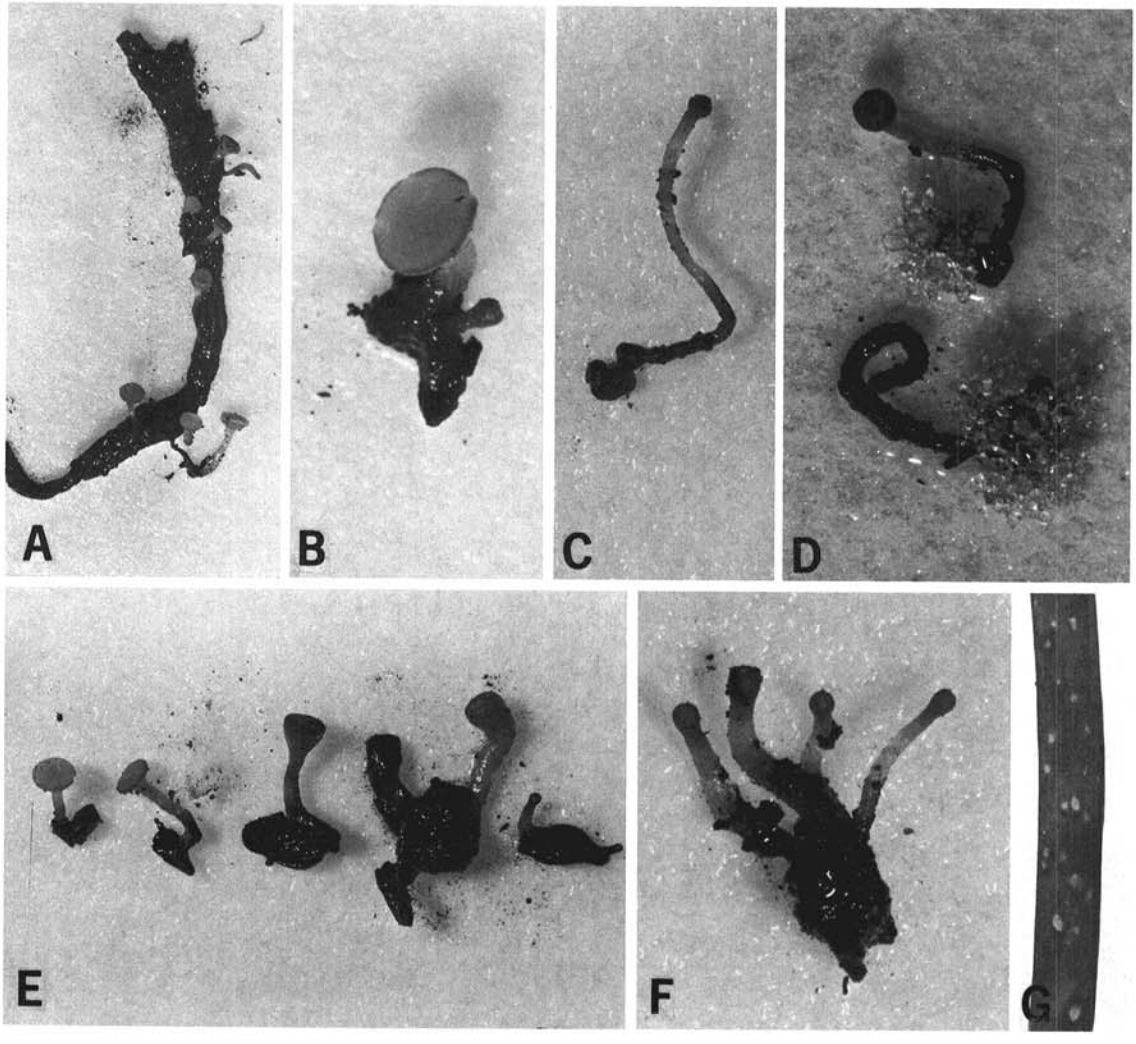

Fig. 3-(A to G). Gross morphology of naturally occurring apothecia of *Botrytis squamosa* and symptoms induced by ascospores on onion leaves. **A)** Apothecia arising from sclerotia still attached to an onion leaf fragment. **B)** Mature, well-developed apothecium. **C)** Poorly developed apothecium with elongated stipe. **D)** Sclerotia giving rise both to apothecia and conidiophores. **E)** Apothecia at different stages of development. **F)** Sclerotium giving rise to four apothecia. **G)** Lesions that developed following discharge of ascospores onto an onion leaf.

Efficiency of conidial production decreased markedly during the last two incubation periods.

Natural occurrence of apothecia.—Apothecia of *B. squamosa* were found in April 1975 on the soil surface of fields cropped to onions in 1974. The apothecia were borne on sclerotia that had overwintered on the underside of aggregated mats of dead onion leaves. The leaf mats had dried somewhat and had separated slightly from the soil surface. This allowed sufficient light to enter at the leaf mat margins to stimulate apothecial formation. The moist soil-leaf mat interface provided the following conditions conducive to apothecial development: (i) high relative humidity, (ii) subdued light, and (iii) sufficiently high temperatures. Although air temperatures at the soil surface in early April were 0-5 C, temperatures within the leaf mats were 15-20 C on sunny days. Naturally-formed apothecia usually were smaller (disks 0.5-2.5 mm in diameter) than those formed in pure culture (22). Apothecia were most frequent near the peripheries of leaf mats and were attached to sclerotia on onion leaf fragments (Fig. 3-A). Apothecia that had formed near the center of a mat often were nonfunctional and had elongated stipes (Fig. 3-C). In contrast, those near the periphery of a mat, where light intensity was greater, were well-developed (Fig. 3-B). Sclerotia usually gave rise to a single apothecium (Fig. 3-E), but occasionally two or more developed from a sclerotium (Fig. 3-F). Sclerotia bearing conidia were present at the same time apothecia were observed (Fig. 1-C). Occasionally sclerotia gave rise to conidiophores and apothecia simultaneously (Fig. 3-D). Monoascosporic cultures gave rise to typical sclerotial colonies of *B. squamosa* on PDA slants.

Fields were prepared for planting shortly after apothecia were observed. The leaf debris, still bearing many dormant sclerotia, was incorporated into the soil. Presumably, some of these sclerotia later were returned to the surface during routine tillage. To determine if such sclerotia could give rise to apothecia, a large amount of leaf debris was collected in April and covered with 10-15 cm of soil along the edge of a field. Portions of this material (about 0.5 m^2) then were uncovered at weekly intervals from May through July. The uncovered material was partially shaded by wood laths to simulate an onion leaf canopy. The uncovered material was examined frequently for apothecia during the ensuing 2-wk period.

TABLE 1. Frequency of isolation of different fungi from lesions on onion leaves collected from cull piles and seed production fields in Orange County, New York in May and early June

Fungi isolated	Percentage of total isolations[a]		
	1971	1973	1974
Botrytis squamosa	13	47	17
Botrytis alli	36	11	30
Botrytis byssoidea	19	2	14
Botrytis cinerea	4	5	5
Alternaria sp.	12	15	17
Stemphylium sp.	3	0	6
Other unidentified fungi	13	20	11

[a]Total isolations were 319, 85, and 252 in 1971, 1973, and 1974, respectively.

Apothecia developed in all but one instance (8 July). Largest numbers of apothecia were formed in May and June, probably because the soil became quite dry by July.

Samples of the leaf debris bearing dormant sclerotia were collected in April 1975 and stored at 2-4 C in darkness for 6 mo. When placed in a dew chamber (19) at 20-22 C under fluorescent and incandescent lighting at a 14-hr photoperiod, sclerotia often gave rise to apothecia and/or conidiophores after 7-10 days. The daily maximum temperature of the shaded soil surface in an onion field during 1972 exceeded 22 C only once prior to 15 July. This suggests that apothecia can form on uncovered sclerotia during the onion growing season, especially when wetter and cooler weather conditions prevail. Nevertheless, no apothecia were observed in cultivated onion fields after the soil was disturbed by plowing in April. No apothecia were found later in the

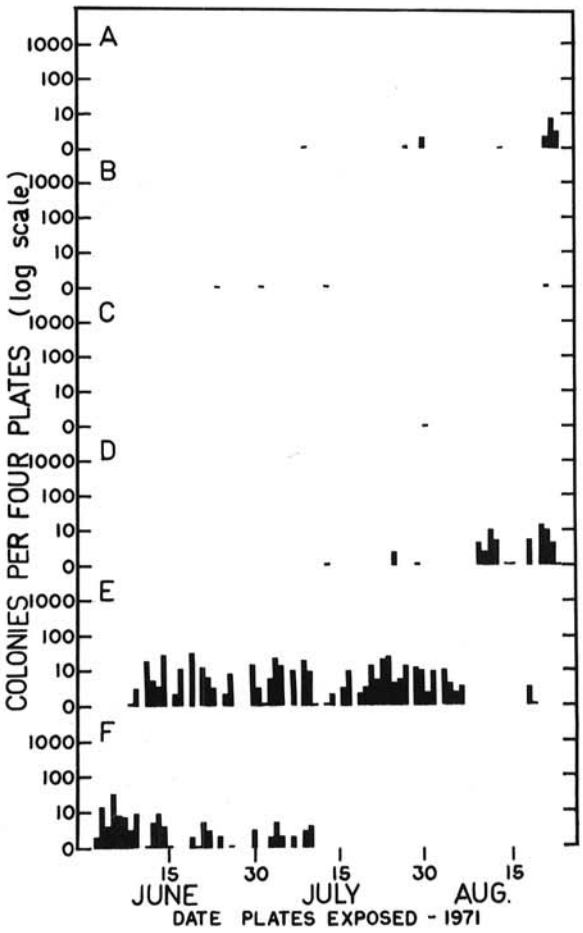

Fig. 4-(A to F). Numbers of conidia of *Botrytis squamosa* trapped from the air at selected locations in Orange County, New York, in 1971. **A, C, D)** Commercial seeded onion fields. **B)** A field cropped to lettuce in which large numbers of *B. squamosa* sclerotia had been returned to the soil following harvest of an onion crop in 1970. **E)** An onion seed production field. **F)** A large cull pile where onion bulbs and associated debris had been dumped periodically for at least 5 yr.

growing season probably because none or only a few were formed.

Pathogenicity of ascospores.—Field-collected apothecia were attached with petrolatum to plastic petri plate lids and suspended above onion plants (cultivar Elite) in a dew chamber (19) at 20-22 C, with fluorescent and incandescent lighting at a 14-hr photoperiod. Ascospores thus were discharged onto the onion leaves. Similar plants, separated by a plastic barrier from the inoculated plants, served as controls. After 3-4 days, lesions with water-soaked margins, typical of those incited by conidia of *B. squamosa*, were evident on leaves of inoculated plants (Fig. 3-G). After 5-7 days, leaf blighting occurred and conidia were produced on necrotic leaf tips.

Onion seeds as sources of inoculum.—*Botrytis squamosa* never was isolated from any of the onion seedlings nor was sporulation ever observed in seed transmission tests. Post-emergence damping-off of seedlings did occur in natural soil, but it was not caused by *B. squamosa*. *Fusarium* spp. and other unidentified fungi frequently were isolated from surface-sterilized seedlings showing disease symptoms. *Botrytis cinerea* Pers. and *B. allii* Munn were observed several times sporulating on sloughed leaves of the Early Yellow Globe seedlings in autoclaved soil. *Botrytis cinerea* and *B. allii* were found sporulating on sloughed leaves of onion seedlings in the field under wet conditions, but *B. squamosa* never was observed under such conditions.

Isolations from cull piles seed fields and volunteer plants.—*Botrytis squamosa* frequently was isolated from lesions on leaves of sprouted bulbs in cull piles and seed fields in late May and early June in 1971, 1973, and 1974 (Table 1). Other fungi that were isolated included *B. allii*, *B. byssoidea* Walker, *B. cinerea*, *Alternaria* sp., and *Stemphylium* sp. *Botrytis squamosa* constituted 13, 47, and 17% of 319, 85, and 252 total fungal isolations in 1971, 1973, and 1974, respectively (Table 1). *Botrytis squamosa* also was the fungus most commonly isolated from lesions on leaves of volunteer plants in May 1975.

Patterns of inoculum buildup.—Conidia of *B.*

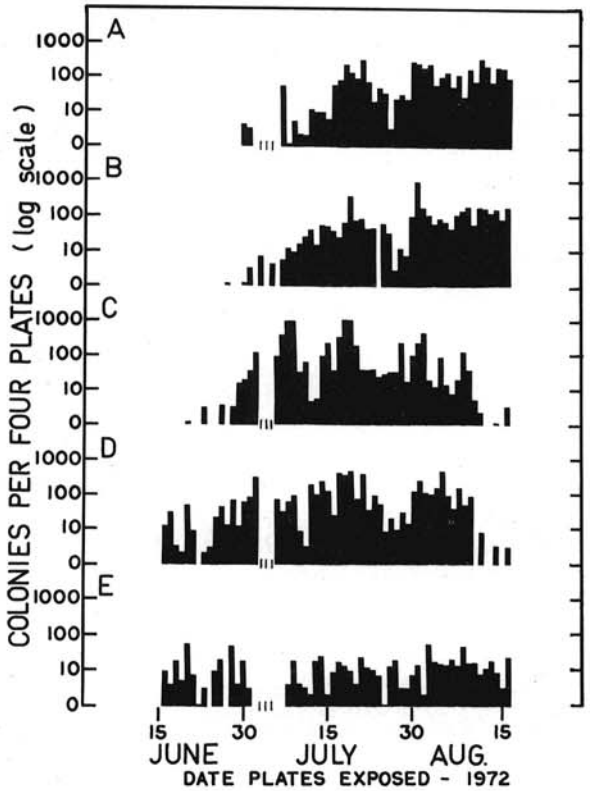

Fig. 5-(A to E). Numbers of conidia of *Botrytis squamosa* trapped from the air at selected locations in Orange County, New York, in 1972. **A to C)** Commercial seeded onion fields. **D)** An onion seed production field. **E)** The same cull pile monitored in 1971. III = no data.

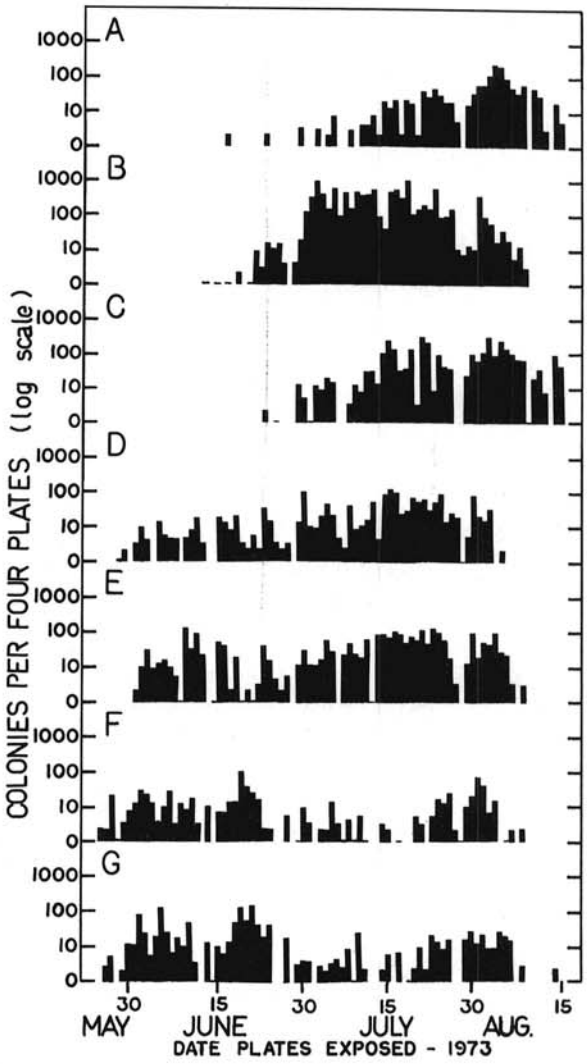

Fig. 6-(A to G). Numbers of conidia of *Botrytis squamosa* trapped from the air at selected locations in Orange County, New York in 1973. **A to C)** Commercial seeded onion fields. **D, E)** Onion seed production fields. **F)** A small cull pile where onion bulbs and associated debris had been dumped periodically for 2 yr. **G)** The same large cull pile monitored in 1971 and 1972.

squamosa were trapped from the air over the cull pile from 2 June in 1971 until early July when dry weather conditions apparently precluded further sporulation (Fig. 4-F). Conidia were first detected over the seed field on 8 June and were trapped from then until harvest on 6 August (Fig. 4-E). The fact that a general epidemic of Botrytis leaf blight failed to develop in most commercial fields in 1971 is reflected in the observation that conidia were trapped very rarely in commercial fields (Fig. 4-C), or only from mid- to late August (Fig. 4-A, D), when moderate leaf blight developed in some fields. Only four conidia were trapped over the lettuce field during the entire period (Fig. 4-B). Since numerous sclerotia of *B. squamosa* had been returned to the soil after harvest in 1970, the dearth of conidia in 1971 suggests that the sclerotia did not become prolific sources of air-borne conidia.

In 1972, conidia again were present over the cull pile when monitoring was begun on 15 June and were trapped until 16 August (Fig. 5-E). Similarly, conidia were detected over the seed field during the entire period (Fig. 5-D). A severe epidemic of Botrytis leaf blight developed throughout commercial fields in Orange County in 1972, but buildup of air-borne conidia and leaf blight did not occur in the three commercial fields until late in June [Fig. 5-(A to C)]. In contrast, air-borne conidia were present over the cull pile and seed field during the 2-wk period preceding epidemic development in the commercial fields.

In 1973, conidia were trapped during the last week in May over both cull piles and were present until early August at both sites (Fig. 6-F, G). Conidia appeared about 1 wk later over both seed fields and were present until harvest in early August (Fig. 6-D, E). A severe epidemic of Botrytis leaf blight developed in commercial fields in 1973, but as in 1972, buildup of air-borne conidia and leaf blight did not occur until late June in commercial fields [Fig. 6-(A to C)]. Therefore, air-borne conidia of *B. squamosa* were present over the cull piles and seed fields for about 1 mo prior to development of the epidemic in commercial bulb fields.

No attempts were made to correlate suspected dissemination of conidia from the cull pile and seed field sources with time of or incidence of development of lesions in nearby commercial fields. On two occasions, however, a gradient of lesion incidence was observed in a commercial field adjacent to a seed field source of conidia, with lesion incidence decreasing with distance from the seed field. Cull piles were too distant from commercial fields for detection of such gradients. When the wind velocity was 1-3 meters/sec, however, viable conidia of *B. squamosa* were trapped 150 m downwind from a cull pile.

DISCUSSION

Cull piles and seed production fields are sites of sporulation by *B. squamosa* during late May and June in Orange County. They are prolific sources of primary inoculum of the fungus which can initiate epidemics of Botrytis leaf blight. Cull piles and seed fields are especially effective inoculum sources because of their proximity to commercial bulb fields. Sclerotia in cull piles and on mother bulbs in seed fields produce conidia in early spring. These conidia cause infection of leaves on sprouted bulbs, as demonstrated by the isolation of *B. squamosa* from lesions on leaves in cull piles and seed fields. Conidia produced on blighted leaves initiate secondary cycles. The dense, tangled growth of leaves from masses of discarded bulbs in cull piles creates conditions conducive to infection and sporulation by *B. squamosa*, namely, little air movement and attendant high relative humidity. A similar, but slightly later, buildup of inoculum occurs in seed fields. During 1971-73, conidia of *B. squamosa* were present over cull piles and seed fields at least 2 wk prior to development of Botrytis leaf blight symptoms in commercial fields.

Conidia produced by sclerotia on the soil surface in commercial fields are probably a common, albeit relatively unimportant, source of inoculum throughout the growing season. Such sclerotia produce conidia in vitro over a wide temperature range; thus soil moisture is the limiting factor for production of conidia. The ability to germinate repeatedly enables sclerotia to produce conidia over a long period of time. Capacity for repeated production of conidia also has been reported for sclerotia of *B. convoluta* (11), but occurrence of this phenomenon in nature has not been demonstrated. Repeated production of conidia may not be common, however, since Willets (25) has observed that sclerotia are much more susceptible to mycoparasitism after they have germinated.

The natural occurrence of *B. squamosa* apothecia confirms the report that both mating types of the fungus are present in Orange County (3). The role of the sexual stage of *B. squamosa*, if any, in the development of Botrytis leaf blight epidemics remains to be elucidated. Apothecia occurred frequently in April. Although it was demonstrated that apothecia can form in the field throughout most of the growing season and that ascospores are pathogenic to onion leaves, apothecia were never found occurring naturally in fields at the time of epidemic development. Although failure to find apothecia after April does not eliminate the possibility of their presence, it seems unlikely that they occur very frequently during the growing season. Thus, it appears that ascospore-induced infections play a minor role in development of Botrytis leaf blight epidemics. The sexual stage may be very significant if ascospores do successfully incite infections and give rise to genetically new forms of *B. squamosa*. Lafon (12) alluded to the presence of pathogenic races of *B. squamosa*, and Bergquist (2) was able to distinguish three pathogenic races among his isolates of *B. squamosa*.

Viennot-Bourgin (22) suggested that *B. squamosa* may have been introduced into France on onion seed, but he offered no supportive evidence. Page (18) reported infection of onion seedlings by applying conidia of *B. squamosa* to seeds before planting. His observations disagree with our results which failed to demonstrate infection of onion seedlings or colonization of organic debris by seed-borne *B. squamosa*. His use of surface-sterilized seeds, which probably eliminated antagonists, may account for the different results. Page (18) stated that infection of seedlings probably occurred very rarely, if ever, in nature. It appears, therefore, that seeds are not important sources of *B. squamosa* inoculum in New

York. Our observation that *B. allii* developed with germinating seeds and colonized sloughed leaves of seedlings agrees with Maude and Presly's report (14) that *B. allii* is seed transmitted.

Elimination of cull piles, seed production fields, and conidia-producing sclerotia on the soil surface as sources of inoculum probably would disrupt the life cycle of *B. squamosa*. Although complete eradication of inoculum sources probably is impossible, a reduction would delay development of epidemics, reduce fungicide usage, and increase bulb yield. Greatest losses in yield occur when onion leaves are damaged at the time the plant is undergoing maximum bulb formation (1), and direct-seeded onions are generally at this stage of growth when leaf blight becomes epidemic in Orange County. Yields of crops started from sets and transplants are seldom affected by leaf blight because they mature before significant leaf damage occurs (17).

Epidemics of potato late blight in Maine were controlled or delayed when cull pile sources of *Phytophthora infestans* were destroyed (4). Various techniques including use of herbicides, burning, or periodic disking may be practical for preventing buildup of inoculum in cull piles. Sprout inhibitors have been used effectively to prevent buildup of inoculum of *P. infestans* in potato cull piles (13). Discarding bulbs and debris at one common, isolated site distant from bulb fields would reduce the significance of inoculum produced. Isolation of seed production fields at a distance from commercial onion fields should minimize their role as sources of inoculum. Fungicide spray programs in seed fields to control leaf and flower infections also should reduce inoculum buildup.

Reduction of populations of sclerotia in the soil in commercial fields would eliminate another source of primary inoculum. Since sclerotia can survive long periods when incorporated into the soil (6), preventing their formation or destroying them immediately after harvest would appear to be the most practical control measure. Sclerotia form most abundantly on onions left in the field late in the growing season (6). Avoidance of late harvest would reduce the incidence of formation of sclerotia, and destruction of the crop residue by plowing or other means would eliminate them as sources of inoculum. Burning of crop residue to destroy sclerotia has given good control of ergot and blind-seed of perennial ryegrass (9) and stem rot of rice (24).

Hirst (10) has pointed out that spore traps are usually of little value in forecasting diseases that begin from minute local sources since they are not sufficiently sensitive to detect the presence of spores before symptoms are observed. This is the case with Botrytis leaf blight when widely scattered sclerotia are the sources of inoculum. When cull piles and seed fields are sources, however, spore traps may be useful. Monitoring of these sources in June would facilitate issuance of reliable recommendations concerning the need for protective sprays when wet weather occurs. If inoculum levels were high, protective sprays probably would be required at the onset of wet weather. If inoculum levels were low, some latitude could be allowed for protective spray action. Routine assessment of sclerotial formation and incorporation into the soil in fields after harvest would make it possible to estimate this potential for production of inoculum in following years.

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