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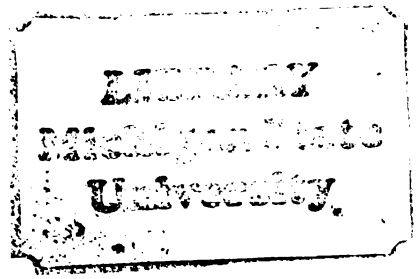

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Melvyn L. Lacy
Major professor

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INFLUENCE OF ENVIRONMENTAL AND HOST FACTORS ON INFECTION OF ONION BY *BOTRYTIS SQUAMOSA* AND *BOTRYTIS ALLII*

By

Stephen Charles Alderman

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ABSTRACT

INFLUENCE OF ENVIRONMENTAL AND HOST FACTORS ON INFECTION OF ONION BY BOTRYTIS SQUAMOSA AND BOTRYTIS ALLII

By

Stephen Charles Alderman

A dry spore inoculation procedure was developed to study infection of onion (Allium cepa L.) leaves by Botrytis squamosa Walker. Dry conidia applied to onion leaves survived for several days. Under continuous dew at 20 C, conidial germination on leaf surfaces began after 2 hours and increased through 20 hours. The first lesions were visible after 8 hours. Lesion production in onion was maximal at 20 C, slower at 15 C and greatly reduced at 25 C. The minimal leaf wetness period for infection was 6 hours, and numbers of lesions increased with increasing leaf wetness duration. Infection hyphae extended beyond lesion borders in relatively few instances. Hyphal development increased with increasing leaf age (tissue maturity), and with increasing periods of continuous leaf wetness. A dry period following inoculation and 6 gours of dew followed by resumed dew reduced lesion numbers. Lesion numbers tended to decline with decreasing humidities during a dry period following a 6 hour post-inoculation wetness period.

Survival studies indicated that B. squamosa can survive as mycelia in colonized, desiccated leaf segments. Conidial formation was observed from such segments after 48 hours under moist

conditions. Sporulation from colonized leaf segments was abundant at 15 or 20 C and nil at 35 C.

Growth of B. allii or B. squamosa on artificial media or on onion leaves at 20, 25, or 30 C increased from 0.9 through -5 to -10 bars, then declined through -90 to -100 bars. At water potentials greater than -30 bars growth was greater at 20 or 25 C than at 30 C, but at potentials below -30 bars growth was similar at all three temperatures. Conidial suspensions of B. allii applied to leaves of field onions caused latent infections and increased neck-rot incidence in storage.

TO

My Late Mother

who helped me start on this path

and

To Dad

who helped me finish

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Since ancient times, the onion (*Allium cepa* L.) has been valued as a food crop. Little is known regarding the origin of *A. cepa*, although reports of its consumption, as early as 3200-2780 B.C., have been found in Egyptian tombs (Jones and Mann, 1963). Reports of onion consumption were also documented by herbalists in ancient Greece, in India during the 6th century B.C., and during the middle ages in Europe (Jones and Mann, 1963). In modern times the onion is commonly found worldwide as a food crop (Jones and Mann, 1963).

Some 43 pathogens of onion have been described (Anonymous, 1960). In the United States the major pathogens attacking *A. cepa* include *Botrytis squamosa* Walker, which causes Botrytis leaf blight; *Botrytis allii* Munn, which causes Botrytis neck-rot; *Peronospora destructor*, Berkeley, which causes downy mildew; *Alternaria porri* (Ellis) Ciferri, which causes purple blotch; *Pseudomonas cepacia* Burkholder, which causes sour skin; *Urocystis colchici* (Schlecht.) Rabenh. (=*U. cepulae* Frost) which causes onion smut; *Sclerotium cepivorum* Berleley, which causes white rot; *Colletotrichum circinans* (Berk.) Vogl., which causes smudge on white-skinned onions; *Pyrenochaeta terristis* (Hansen) Gorenz, Walker, and Larson, which causes pink root; *Fusarium oxysporum* Schlecht. *F. sp. cepa* (Hans.) Snyd. and Hans., which causes basal rot; and the aster yellows mycoplasma, a mycoplasma-like organism, which causes aster

yellows. The studies described in this dissertation were directed towards the epidemiology and histopathology of Botrytis squamosa and Botrytis allii.

Numerous Botrytis species have been described on onion. However, similarity between species of Botrytis and overlapping of symptoms induced in onion by the various species led to ambiguities in the early literature.

Perhaps the first reports of a Botrytis disease of onion were by Sorauer in 1876 and Frank in 1880. Sorauer (1886) identified the conidial state as Botrytis cana (Pers.) Fr. and Sclerotium cepa as the sclerotial state. Frank (1896) published a similar report.

The first report of a Botrytis disease of onion in the United States was by Halsted (1890) who identified the causal agent as Botrytis parasitica Cav. Smith (1900) described a Botrytis disease of onion but considered the pathogen to be an atypical Botrytis cinerea. Clinton (1903) described a neck-rot disease of onion in Connecticut occurring in 1902-1903 and suggested it was the same disease described by Halsted (1890).

In 1917 Munn published a detailed description of a neck rot disease in Michigan and named the pathogen (n. sp.) Botrytis allii. He compared his isolate with literature descriptions or samples of other Botrytis species found on onion and described B. allii as a new species, claiming that it was morphologically different from the other species described on onion. In inoculation experiments, Munn (1917) demonstrated that B. allii was an aggressive pathogen on the bulbs, and an aqueous suspension of B. allii conidia applied to onion leaves also induced leaf lesions.

In 1925 Walker described two additional pathogens of onion, Botrytis byssoidea and Botrytis squamosa. He compared and contrasted both of these species with B. allii Munn. In 1926 Walker published a more detailed account concerning the biological differences between the three Botrytis species. He defined B. squamosa as the cause of small sclerotial neck rot and B. byssoidea as the cause of mycelial neck-rot. Walker (1926) recognized B. byssoidea as the most common bulb pathogen in Wisconsin. At a later date, however, Walker (1969) considered B. allii as the most important bulb pathogen on the world level.

In 1938 Yarwood claimed to have isolated B. cinerea from onion leaves, and induced leaf lesions by applying conidial suspensions to onion leaves. Olgivae (1941) also implicated B. cinerea as the cause of leaf spotting of onion. Thus, by the 1940's B. allii was recognized as the major cause of bulb rot, although 5 species of Botrytis were implicated as causing a foliar disease of onion.

Hickman and Ashworth (1943) reported that B. squamosa and B. cinerea were responsible for leaf spotting and dieback of onion leaves in Great Britain, with B. squamosa reported to be the predominant pathogen. The two species were weakly pathogenic to onion bulbs. Page (1955) and Viennot-Bourgin (1953) also identified B. squamosa as the major cause of leaf spotting and blighting in onion. These studies established B. squamosa as the major cause of leaf spotting and blighting in onion.

Some confusion was created when Segall and Newhall (1960) reported that B. allii was responsible for a leaf spotting and blighting disease of onion in New York. It is possible the Segall and Newhall (1960) either did not recognize B. squamosa as a species of misidentified B.

squamosa as B. allii. The confusion was resolved by Hancock and Lorbeer (1963) who studied symptom expression in onion foliage induced by B. squamosa, B. cinerea, and B. allii. Based on their observations they proposed that the disease caused by B. squamosa be named Botrytis leaf blight, and that caused by B. cinerea be named Botrytis leaf fleck. They did not recognize B. allii as a significant foliar pathogen. B. byssoidea also is not recognized as a significant pathogen of onion foliage (Walker, 1926) or flowers (Ellerbrock and Lorbeer, 1977a).

Recent reports indicated that B. allii may be associated with onion in a more subtle fashion. Studies by Tichelaar (1967) and Maude and Presly (1977a, 1977b) indicated that B. allii could colonize healthy onion leaves asymptomatically. Further, these studies suggested that B. allii could move down the leaves asymptomatically and colonize the bulb tissues. The disease would then appear in bulbs placed in storage.

Recent studies also indicated that B. squamosa may act in association with another pathogen. Maude and Presly (1980) reported that B. cinerea and B. squamosa, occurring jointly, were responsible for a decay of the neck region of overwintered salad onion.

Thus, B. squamosa is a foliar pathogen of onion which causes a leaf spotting and die-back disease, although it may also colonize neck tissues. On the other hand, B. allii causes a storage decay of onion bulbs, although leaf flecking and symptomless colonization of the foliage may occur. Although both diseases are caused by species of Botrytis, the plant parts attacked and the symptoms they induce are sufficiently different so as to recognize them as two distinct diseases. Although B. byssoidea has been associated with bulb decay (Walker, 1926),

I did not observe it among onion bulbs during my course of study. This dissertation addresses leaf blight and neck-rot separately. Within this introduction, and among the Chapters, leaf blight, then neck-rot, will be discussed.

BOTRYTIS LEAF BLIGHT

Occurrence

Botrytis leaf blight has been reported in Belgium (Henebert, 1964), Canada (Page, 1953), England (Maude and Presly, 1980), France Viennot-Bourgin, 1953), Italy (Crotti, 1969), Japan (Takakuw, 1974), the Netherlands (Tichelaar, 1967), New Zealand (Dingly, 1961), Poland (Lutynska, 1968), and the United States (McLean and Sleeth, 1959). The disease is economically important in the northeastern United States, where weather conditions are favorable for disease development.

Symptoms

Detailed descriptions of leaf blight symptoms were first published by Walker (1926), Hickman and Ashworth (1943), and Page (1955). Lesions induced by *B. squamosa* on onion leaves are discrete, greyish-white, desiccated spots, 2-5 mm in length and 1-4 mm in width. Under prolonged warm, moist weather conditions leaf blighting and collapse may occur. Leaves die from the tip downward and older leaves are more severely attacked than younger ones. Sporulation occurs on necrotic or blighted leaves, leaf tips, or, occasionally, from expanding lesions. Segall and Newhall (1960) described leaf blight as a two phased phenomenon where phase one was leaf spotting and phase two was blighting.

Leaf spotting and blighting have been reported to occur most commonly when plants were near maximum foliar development (Small, 1970;

Segall and Newhall, 1960; Swanton, 1977; Lacy and Pontius, 1983). However, leaf blight attributed to B. squamosa was reported on seedlings by Lafon (1962).

Morphology

Morphological characteristics of B. squamosa were described by Walker (1925, 1926). Mycelia of B. squamosa are hyaline, multibranched, septate, and variable in diameter. Conidia are borne on short sterigmata at swollen apical tips of conidiophores. Conidia are ovoid to ellipsoid, smooth, hyaline at first but darkening with age. Most conidiophores are 11-15 X 15-22 um. Following fructification, side branches of conidiophores degenerate to form characteristic accordian-like folds. Microconidia are hyaline, globose, about 3 um in diameter. Sclerotia are roughly circular, flat, scale-like, ½-4 mm in diameter, white at first but darkening with age until black.

Cronshy (1946) was the first to report the perfect state of B. squamosa. Viennot-Bourgin (1953) made a detailed study of the apothecia of B. squamosa and named the teleomorph Botryotinia squamosa. McLean (1960) examined apothecia of B. squamosa and felt that the teleomorph should be named Sclerotinia squamosa (Viennot-Bourgin) Dennis. However, no subsequently published reports that I am aware of have referred to the fungus as Sclerotinia squamosa.

Apothecia of B. squamosa are 2-3 mm tall with a cup diameter of 3-5 mm (Viennot-Bourgin, 1953). Asci were observed by McLean (1960) to be 162.5-200 um X 13.8-16.5 um, each containing 8 ascospores averaging 10.0-12.5 X 15.0-17.5 um. Ascospores were measured by Viennot-Bourgin (1953) as 11.7 X 8.11 um.

Survival and Primary Inoculum

Walker (1926) reported that sclerotia of B. squamosa survived on a window sill at Madison, WI from December-March. Ellerbrock and Lorbeer (1977b) studied sclerotial survival under field conditions and found that 7 and 65% of sclerotia survived for 21 months when buried 3 or 5 cm deep respectively in organic soil. Sclerotia placed on the surface of soil survived from September through May. Conidia survived for only about 2 months in soil under both controlled and field conditions.

Sources of primary inoculum include sclerotia and infested onion debris (Ellerbrock and Lorbeer, 1977c). McLean and Sleeth (1959) and Lafon (1961) suggested that conidia produced on sclerotia could serve as primary inoculum. Segall and Newhall (1960) observed sporulation of Botrytis species on cull onions discarded the previous year. Ellerbrock and Lorbeer (1977b) also observed sclerotia bearing conidia on onion bulbs and leaf debris in cull onions discarded the previous year. They trapped conidia from the air over cull piles and seed production fields 2-4 weeks prior to the appearance of the disease in commercial onion fields. In addition, Ellerbrock and Lorbeer (1977c) observed apothecial production from sclerotia in onion fields on one occasion, although this is extremely rare, and determined that ascospores could induce lesions on onion leaves. Conidia of B. squamosa on seed have not been shown to infect onion seedlings (Ellerbrock and Lorbeer, 1977a).

Secondary Inoculum

The potential of B. squamosa to cause epidemics has been well documented (Page, 1955; McLean and Sleeth, 1959; Swanton, 1977; Lacy and Pontius, 1983). Lorbeer (1966) and Lacy and Pontius (1983) observed a

diurnal periodicity in spores trapped. Spore trap studies by Swanton (1977) suggested that prolonged warm, wet weather conditions followed by increasing temperature and declining relative humidity favored spore release. The analysis of several years of weather and spore trap data by Lacy and Pontius (1983) indicated that warm, humid weather over a consecutive three day period is necessary to induce a large spore release.

Control

Berquist and Lorbeer (1971) examined varieties of *Allium cepa* for resistance to *B. squamosa* but found none. However, *Allium bouddhae* C 598 and *A. schoenoprasum* were found to be immune to *B. squamosa*. They suggested that it may be possible to introduce this resistance into *Allium cepa*.

Control of leaf blight based on calendar applications of fungicides. Several systems have been developed to reduce the number of spray applications. Shoemaker and Lorbeer (1977) described a critical disease level (CDL) of one lesion per 10 leaves to determine when spraying should be initiated. Swanton (1977) combined the CDL with weather conditions favorable for spore production so as to further reduce unnecessary sprays after the CDL was reached. Dzikowski (1980) developed a similar system based on the CDL but suggested spraying in advance of expected rain. Lacy and Pontius (1983) developed a sporulation prediction model which provided a probability index for sporulation based on three day averages of temperature and vapor pressure deficits.

BOTRYTIS NECK ROT

Occurrence

Botrytis diseases caused by Botrytis allii have been reported in England (Moore, 1948), Denmark (Hellmers, 1943), Holland (Tichelaar, 1967), New Zealand (Brein, 1939), Canada (McKeen, 1951), Norway (Røed, 1952), Israel (Netzer and Dishon, 1966), France (Henebert, 1964), Poland (Lutynska, 1968), and the United States (Munn, 1917).

Symptomatology

Botrytis allii has been found in association with onion bulbs, leaves, scapes, flowers and seeds. Symptoms on bulbs were described by Munn (1917) and Walker (1926). Onion bulb tissues at the early stage of infection appear soft, slightly sunken and water soaked, with a distinct margin. As the fungus develops on the older diseased areas the tissues become greyish in color and a smoke-grey mat of mycelia and conidiophores bearing conidia develops on the surface of the scale. The fungus progresses within the bulb scales more rapidly than between scales. Mycelial mats between scales may fill the space between scales and appear dirty white to brown. Sclerotia develop in the older diseased areas and appear whitish at first, but later turn black. Walker (1926) described sclerotial bodies as hard, black, rounded, spherical, oblong or irregular, varying from 1-3 mm or more in length and which may become aggregrated into crusty masses. Little odor is associated with the bulb decay.

Botrytis allii was reported to cause leaf lesions and blighting of the foliage (Segall and Newhall, 1960), scape (Yarwood, 1938), flower (Ellerbrock and Lorbeer, 1977a) and seedling (McKeen, 1951), although

later studies indicated that B. allii is not a serious pathogen of onion (Hancock and Lorbeer, 1963). Symptomless infections of B. allii in healthy onion leaves were observed by Maude and Presly (1977a, 1977b) and Tichelaar (1967). It is possible that Segall and Newhall (1960) either did not recognize B. squamosa as a species or misidentified B. squamosa as B. allii.

Morphology

Morphological characteristics of B. allii were described by Munn (1917). Mycelia of B. allii varies from 4.5-9.0 um in width. Hyphae branch irregularly. Conidiophores are short and erect, occurring singly or in clusters. Munn (1917) observed that conidiophores were branched in culture and unbranched on the host plant. Conidia are hyaline, oblong in shape, and most are between 7-10 by 5-6 um in size. Unlike B. squamosa, no perfect state has been reported for B. allii.

Overwintering; Primary and Secondary Inoculum

Munn (1917) observed sporulation of B. allii in cull piles in Michigan in April and May and in onion leaves in New York onion fields in April. Reports by Maude and Presly (1977a, 1977b) indicated that B. allii can be seedborne and that B. allii on seed can infect seedlings symptomlessly. They reported survival of B. allii on onion seed during storage.

Munn (1917) suggested that aerial dissemination of B. allii may occur and that the disease is favored by cool wet weather. Munn (1917) stated that the optimal temperature for sclerotial germination was 10 C. Maude and Presly (1977a) believed that long periods of relative

humidities greater than 80% and frequent rains favored conidial production and spread of the disease.

Control

Maude and Presly (1977b) observed that seed infested with B. allii resulted in increased neck-rot in storage. Further, they stated that seed treatments with a systemic fungicide (benomyl) effectively reduced the incidence of neck-rot in bulbs grown from these plants in storage.

Most control efforts have been directed towards the harvesting and storage of onions. Proper handling, curing, and storage of onions has contributed greatly to the reduction of neck rot incidence.

OBJECTIVES

The overall objectives of this research were to determine the influence of temperature and moisture on infection of and development within onion tissues by B. squamosa and B. allii, and to integrate this knowledge with that derived from previous studies so as to more clearly define the biology of these two fungi.

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CHAPTER II

INFLUENCE OF DEW PERIOD AND TEMPERATURE ON INFECTION OF ONION BY *BOTRYTIS SQUAMOSA*

INTRODUCTION

Early investigations concerning leaf blight established that the disease was favored by prolonged moist weather conditions (Clinton, 1903; Page, 1955; McLean and Sleeth, 1959). Subsequent investigations have established that temperature and leaf wetness are important epidemiological parameters in spread of Botrytis leaf blight under field conditions (Segall and Newhall, 1960; Swanton, 1977; Lacy and Pontius, 1983). Lacy and Pontius (1983) identified temperature and humidity as the most important factors in production and release of *B. squamosa* conidia, and devised a disease predictive system based on these factors. Production of inoculum, however, is only part of the disease spread process. Understanding the influence of temperature and leaf wetness on germination and infection of onion by *B. squamosa* is also epidemiologically important, and would allow a more complete predictive system to be synthesized.

Germination of *B. squamosa* conidia has been examined in water on glass slides and on leaf surfaces with conflicting results. On glass slides, Shoemaker and Lorbeer (1977) reported optimal germination at 15 C with no germination above 27 C. Swanton (1977) reported optimal germination at 20 or 28 C with reduced germination at 33 C. McDonald

(1981) reported optimal germination at 24 C. On leaf surfaces, histopathology of conidial germination and subsequent infection was studied by Clark and Lorbeer (1976) at 21 C, but temperature influences on germination were not determined.

Several studies have addressed the effect of temperature and/or leaf wetness on infection of onion by B. squamosa. The optimal temperature for leaf blight development was reported to be 18 C (McDonald, 1981; Tanner and Sutton, 1981), and 20 C (Shoemaker and Lorbeer, 1977; Swanton, 1977). Lesion development was reduced at temperatures above 24 C (McDonald, 1981; Shoemaker and Lorbeer, 1977; Tanner and Sutton, 1981). Lesion numbers increased with increasing leaf wetness duration at 9-25 C (McDonald, 1981; Shoemaker and Lorbeer, 1977; Swanton, 1977; Tanner and Sutton, 1981). Conflicting reports have been published on optimum leaf wetness periods for maximum infection. Using aqueous conidial suspensions, minimal leaf wetness durations for subsequent development of lesions at 18-20 C have reported to be 12 (Tanner and Sutton, 1981), 9 (McDonald, 1981), 6 (Shoemaker and Lorbeer, 1977), or 5 hours (Swanton, 1977) while maximum numbers of lesions were reported to occur after 24 (McDonald, 1981), 48 (Swanton, 1977) or 60 hours (Shoemaker and Lorbeer, 1977) of continuous leaf wetness. At 24 C McDonald (1981) reported maximum lesion production after 12 hours of postinoculation leaf wetness.

Preliminary experiments gave highly variable numbers of lesions when plants were inoculated with aqueous conidial suspensions. Much more consistent results were obtained by applying the conidia in a dry state in a settling tower, so we used the dry conidia inoculation technique throughout these experiments.

The objectives of this study were to determine the effect of length of dew period at various temperatures on conidial germination and subsequent infection and lesion production in onion leaves exposed to a standardized number of conidia under controlled environmental conditions, with the conidia applied in a dry state similar to that occurring in nature; and to determine the effect of extended dew duration on postinfection hyphal development within lesions.

MATERIALS AND METHODS

Production of B. squamosa Conidia

Isolate BSS-4 of B. squamosa, originally isolated from Michigan-grown onions, was used in all experiments because it was highly virulent, and it sporulated somewhat more prolifically on artificial media than other isolates. The fungus was grown on potato dextrose agar (PDA) for 7-12 days, then transferred to sterilized muck soil in test tubes, incubated 7-12 days at room temperature, and stored at 5 C. For conidial production, infested muck soil particles from the soil tubes were placed on autoclaved (85 minutes at 20 p.s.i.) onion leaves placed aseptically on the surface of water agar in 9-cm diameter petri plates. The plates were incubated at 20 C under a 16 hour fluorescent light photoperiod for 5-7 days. Conidia were produced abundantly using this system, but not on other agar media. Cultures survived greater than two years in muck soil tubes and did not lose virulence.

Development of a Dry Spore Inoculation Procedure

Preliminary inoculation experiments using aqueous conidial suspensions of B. squamosa gave highly variable lesion numbers using standardized spore suspensions. Difficulties were encountered in suspending

the conidia in water and in applying a uniform mist of the suspension to leaves due to the hydrophobic nature of onion leaves and of B. squamosa conidia. Aggregations of fine droplets easily ran off the leaves. A wetting agent (Tween 20) aided in suspending conidia in water, but difficulties persisted in the application of suspensions to leaf surfaces. A dry spore inoculation technique was devised to overcome the difficulties associated with aqueous conidial suspensions and their application to leaves.

A galvanized sheet metal cylinder (61 cm diameter by 77 cm deep) mounted on a wooden base was used as a settling tower. Conidia were collected from sporulating cultures using a pasteur pipet connected to a water aspirator. At very low suction conidia were collected in the pipet without being sucked into the aspirator. Conidia were easily tapped out of the pipet onto weighing paper.

The relationship between conidial weight and numbers was determined by suspending known weights of conidia in 70% ethanol and estimating spore numbers using a hemacytometer. Ethanol solution was used since conidia readily entered into suspension in this medium. Amounts of conidia weighing 0.5, 1.0, 1.5, 2.0, and 2.5 mg were determined to contain ca. 2.5-, 4.9-, 7.4-, 10.0-, and 12.5 X 10^5 conidia respectively. These numbers represented the actual conidia, measured by weight, falling on the floor area in the settling tower.

For inoculation, plants were positioned within the settling tower and dry conidia were dispersed near the top of the chamber by directing a low velocity stream of air from a pipet tip over the conidia on a piece of weighing paper. A wooden cover was positioned over the top of the cylinder to reduce air currents and allow the conidia to settle on the plants.

In experiments requiring dew formation on plants, the plants were positioned within a commercial dew chamber (Percival Mft. Co., Boone, IA 50036) for the appropriate dew period. Dew formation was evident on plants within 1 hour of their placement in the dew chamber.

To determine the relationship between inoculum density and lesion numbers, groups of six replicate onion plants were each inoculated with 0.5, 1.5, 2.0, or 2.5 mg dry conidia, then placed in a dew chamber at 20 C for 24 hours. Lesion numbers were counted at the end of the incubation period.

Influence of Dew Period and Temperature on Lesion Production

Groups of 18 onion plants were inoculated with dry conidia and placed in the dew chamber at 15, 20 or 25 C. Six randomly preselected plants were removed from the dew chamber after each period of 4, 8, 12, 16, 24, or 32 hours of continuous dew, respectively, and were then moved to a growth chamber set at the same temperature as the dew chamber set at the same temperature as the dew chamber, with 60 ± 10% relative humidity (RH) and a 16 hour photoperiod. Dew dried from the leaves within 5 minutes. Lesions were counted on each plant after the variable times in the dew chamber and growth chamber totaled 48 hours. The experiment was repeated three times.

Influence of Temperature on Spore Germination and Infection

Four onion plants were inoculated in the settling tower with 2 mg dry conidia and placed in a dew chamber for 24 hours at 20 or 25 C. The third or fourth youngest leaf on each plant was used since lesion size distribution was more uniform on these leaves. Four 1-cm^2 leaf tissue pieces were removed from each of four replicate leaves on four separate

plants, fixed in formalin:acetic acid:50% ethanol (1:18:1 v/v) (FAA), stained in cotton blue, and examined using light microscopy. Conidia which washed off leaves during fixation were collected on a 13-mm-diameter filter membrane (Millipore Filter Corp., Bedford, MA 01730) with 0.33 um pore size, stained with cotton blue, and counted using light microscopy.

Numbers of conidia on leaves which had formed appressoria were counted, as well as spores which had formed both appressoria and infection hyphae. Appressorial counts were based on swollen germ tube tips and infection hyphal counts were based on the observation of infection hyphae within lesions. The experiment was repeated two additional times.

Influence of Dew Period on Infection Hyphal Development

Sixteen onion plants were inoculated and placed in the dew chamber for 2, 4, or 6 days of continuous dew at 20 C with a 12 hour photoperiod. In addition, four replicate plants were given a 2 day dew period at 20 C, followed by incubation in a growth chamber maintained at 20 C, 60 ± 10% RH and a 12 hour photoperiod for 4 days. Leaf sections containing lesions were randomly removed from plants, fixed in FAA, cleared in boiling 70% ethanol, and stained in 1% aqueous trypan blue. Lengths of infection hyphae in each lesion were measured with an ocular micrometer for a minimum of 50 lesions. The experiment was repeated three times.

Longevity of Conidia on Leaves

To determine the longevity of spores on onion leaves following inoculation, two groups of 21 onion plants were inoculated with 4 mg conidia each and placed in a growth chamber at 20 C. after 0, 1, 2, 3,

4, 6, or 8 days, 6 replicate plants were removed from the growth chamber and incubated with continuous dew for 36 hours, then returned to the growth chamber. Numbers of lesions per plant were counted 24 hours after removal from the dew chamber.

RESULTS

Influence of Inoculum Density on Lesion Numbers

Lesion numbers increased linearly up to a maximum of about 280/ plant when 0.5 to 2.5 mg conidia per 0.3 m^2 area of settling tower floor were applied to onion plants subsequently incubated for 24 hours in a dew chamber at 20 C (Figure 2.1).

Influence of Dew Period and Temperature on Infection

Lesions were produced after 8 but not after 4 hours continuous dew at 20 C. The number of lesions per plant increased in apparent sigmoidal fashion with increasing dew duration, with little increase in lesion numbers after 24 hours of dew (Figure 2.2). Total lesions produced at 20 C or 15 C were approximately equal on plants incubated with constant dew for 32 hours, although the rate of increase in lesion production at incremental dew periods was faster at 20 than at 15 C. Lesion production was severely curtailed at 25 C and did not increase further after 12 hours continuous dew.

To determine the minimum dew period required for infection, 6 replicate onion plants were inoculated with dry conidia and placed in the dew chamber at 20 C. After 4, 5, 6, or 7 hours plants were transferred to a growth chamber set at 20 C and 60 ± 10% RH. Lesions were counted after an additional 24 hour in the growth chamber. No lesions

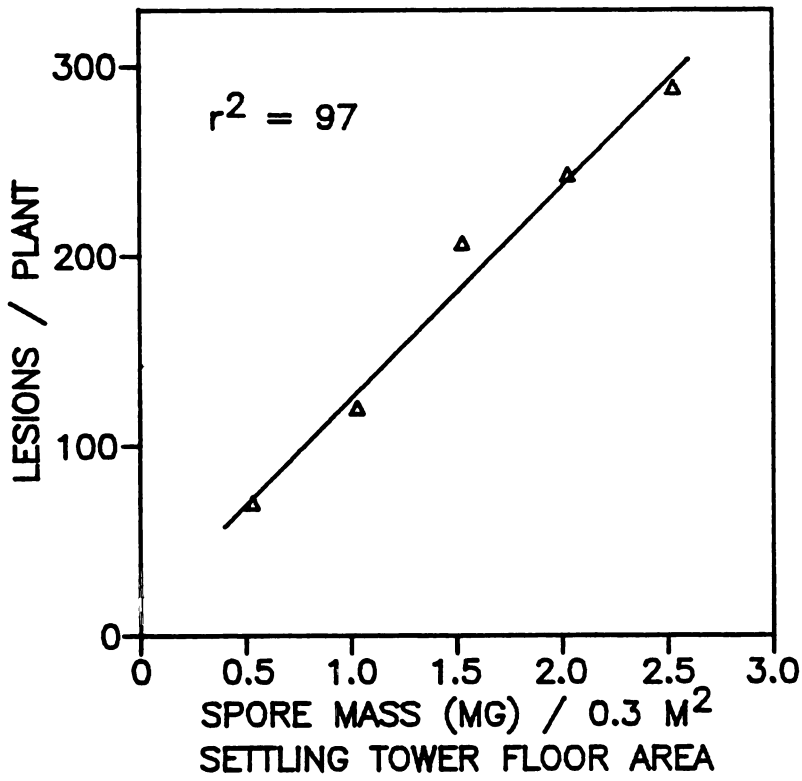

Figure 2.1. Relationship between mass of Botrytis squamosa conidia and lesion numbers of 1-month-old onion plants after incubation in a dew chamber for 24 hours.

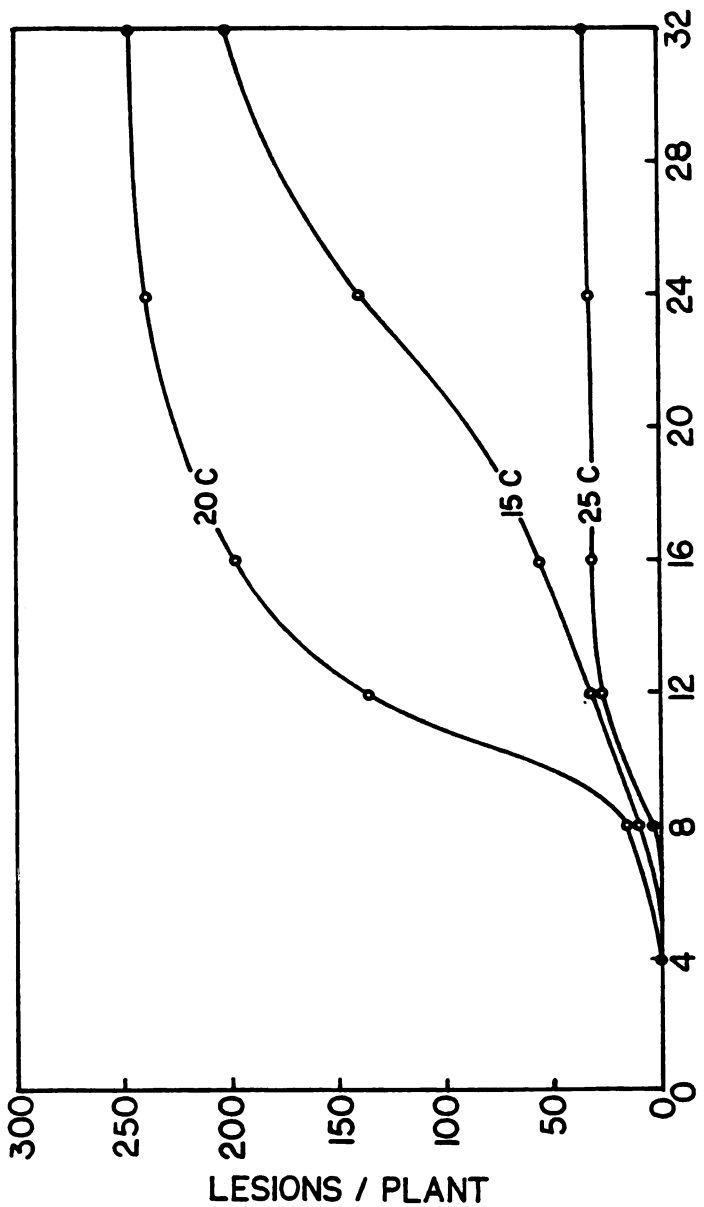

Figure 2.2. Influence of temperature and dew period on lesion development in one-month-old onion plants following inoculation in a settling tower with 2 mg conidia of Botrytis squamosa. Mean values were derived from three replicate experiments; LSD ($P=0.05$) between 8 and 32 hours was 41.

were observed on plants given 4 or 5 hour dew periods. After 6 or 7 hours dew, 4.2 ± 4 and 29 ± 18 lesions per plant, respectively, were observed. Thus, 6 hours was the minimum dew period required for lesion formation.

Influence of Temperature on Spore Germination and Infection

At 20 C, 78 ± 10% of conidia on leaves germinated, while 62 ± 8% induced lesions. At 25 C, 55 ± 5% of the spores germinated while only 27 ± 10% induced lesions. At 20 C, 61 ± 8% of the applied spores formed appressoria and 36 ± 6% formed infection hyphae, compared with 37 ± 1% forming appressoria and 11 ± 6% forming infection hyphae at 25 C.

Influence of Dew Period on Development of Infection Hyphae

Length of dew period following inoculation had a dramatic effect on infection hyphae development within lesions. Lengths of infection hyphae within lesions, after 2 days in the dew chamber were less than 125 um (Table 2.1). After 4 days ca. 30% of the hyphae extended beyond 125 um with the greatest number falling into the 125-150 um catagory. After 6 days of continuous dew ca. 35% of the hyphae extended beyond 125 um, again with the greatest number in the 125-150 um catagory. Infection hyphae in lesions on plants treated with 2 days dew followed by 4 days without dew in the growth chamber remained restricted, with only 4% reaching the 125-150 um catagory, and none falling into the longer catagories.

Longevity of Conidia on Leaves

Numbers of lesions remained relatively constant on leaves held in the growth chamber (60 ± 10% RH and 20 C) for up to 2 days after inoculation, then placed in the dew chamber for a 36 hour dew period

(Figure 2.3). There were ca. 425-450 lesions per plant with a 0-2 day interval between inoculation and exposure to dew. With a 3-4 day interval this declined to ca. 245 lesions per plant, and with a 6 or 8 day interval this further declined to 130 lesions per plant, reflecting a 71% loss in infectivity of conidia after 6 days in the growth chamber prior to dew period. Viability of conidia evidently declined rapidly after the first two days without dew.

Table 2.1. Percentage of infection hyphae of Botrytis squamosa falling within ranges of lengths in lesions on onion leaves after various dew periods at 20 C.

Infection hyphae lengths (um)	Length of dew period (days) 2[a]	4[a]	6[a]	2 days dew + 4 days no dew
1-125	100[b]	72 ± 15	65 ± 14	96 ± 6
125-150	0	13 ± 8	17 ± 9	4 ± 6
250-375	0	7 ± 4	5 ± 5	0
375-500	0	3 ± 3	4 ± 1	0
500	0	5 ± 1	9 ± 3	0

[a]Lesions were examined immediately after the end of the indicated dew period.

[b]Based on the mean of three replicate experiments with each experimental run including a minimum of 50 lesion observations.

DISCUSSION

Previous dew period studies employed aqueous conidial suspensions as inoculum. However, under field conditions B. squamosa conidia are disseminated by wind and deposited on leaves as dry spores (Lacy and Pontius, 1983). Most conidia of B. squamosa are released between 0800

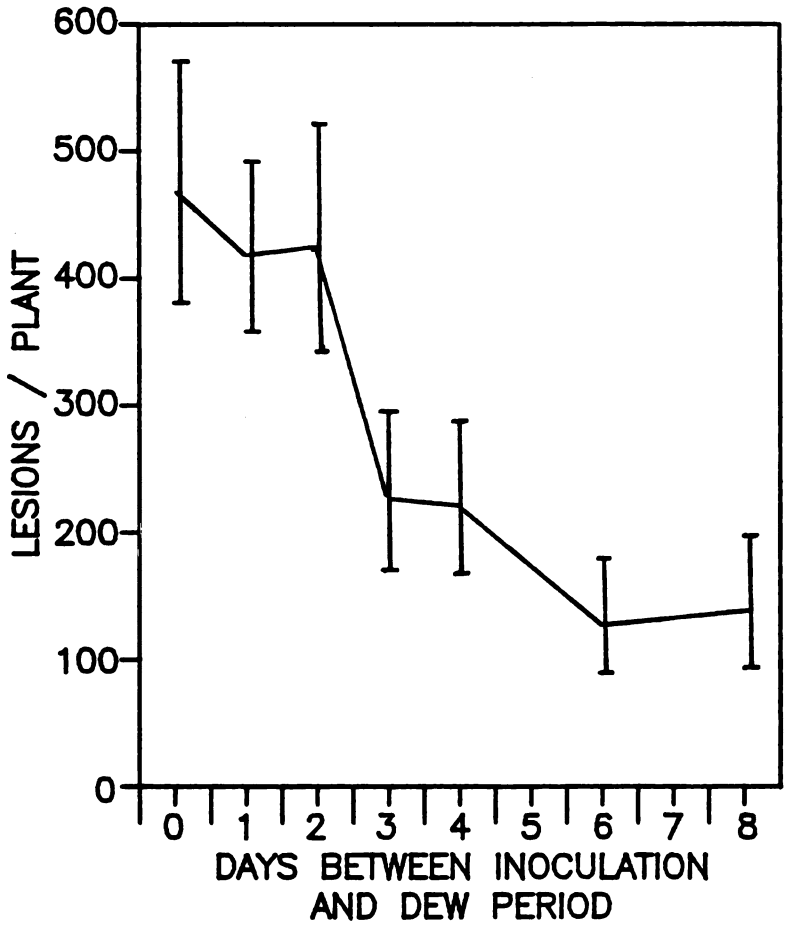

Figure 2.3. Effect of periods of time following inoculation without dew (20 C and 60% RH) prior to exposure to 24 hours continuous dew (20 C) on numbers of lesions per plant. Plants were inoculated in a settling tower using 4 mg dry Botrytis squamosa conidia.

and 1300 hours (Lacy and Pontius, 1983; Sutton et al., 1978) where they must survive in a dry state until a period of leaf wetness occurs. Shoemaker and Lorbeer (1977) reported that dry conidia brushed onto leaf surfaces of plants in growth chambers survived 2 days at 92% RH, although lesion numbers relative to control plants were not quantified. Results of my study suggest that B. squamosa conidia survive well on leaf surfaces for 2-3 days after deposition in the absence of dew or rain without a significant loss of viability, although survival could be shorter on leaves exposed to full sunlight.

Lesion production after a minimum 6 hour dew period was consistent with the observations of Shoemaker and Lorbeer (1977) who, using aqueous conidial suspensions, reported a 6 hour minimum leaf wetness period for lesion development. We observed that relatively few lesions were produced using only 6 hours dew, and a sharp increase in lesion numbers occurred with 12 hours of dew at 20 C (Figure 2.2), which also agreed with Shoemaker and Lorbeer (1977). However, my study more clearly and quantitatively defined the influence of temperature and dew period on lesion numbers induced by B. squamosa.

The slower rate of lesion production at 15 C compared with 20 C could aid in understanding lesion production under field conditions. Dew periods shorter than 16 hours could result in significantly fewer lesions at 15 C than at 20 C (Figure 2.2), and temperatures of 25 C of greater with any dew period could also result in fewer lesions (Figure 2.2)(McDonald, 1981; Shoemaker and Lorbeer, 1977).

Shoemaker and Lorbeer (1977) reported that spore germination on glass slides declined from 80% at 21 C to 20% at 24 C, while McDonald (1981) and Swanton (1977) reported optimal germination at 24 C on glass

slides. I observed a sizable reduction in spore germination on leaves at 25 C compared to 20 C. The reduced spore germination and even greater reduction in production of infection hyphae could account for the reduced lesion numbers noted at 25 C.

Extent of pathogen development within lesions was dependent, at least in part, upon length of continuous dew period. After 4 days of continuous dew some lesions were expanding, initiating the leaf blight phase of the disease (Table 1), similar to observations of Clark and Lorbeer (1976). Hyphal length observations revealed that hyphae continued to grow in 5-15% of the original lesions and were responsible for the rapid and destructive leaf blighting phase of the disease. The reason for some lesions expanding and others remaining static in size is not understood as yet, nor is the effect of extended dew periods on expansion of a larger proportion of lesions understood. It is possible that biochemical or physiological characteristics, which could aid in overcoming host resistance mechanisms may be inherent in 5-15% of the conidia. Quantitative studies using other isolates of B. squamosa, or mutants of B. squamosa may identify cultures which differ in ability to induce expanding lesions. Such cultures may be useful in elucidating why some lesions expand and other do not.

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CHAPTER III

INFLUENCE OF INTERRUPTIONS OF DEW PERIOD ON NUMBERS OF LESIONS PRODUCED ON ONION BY BOTRYTIS SQUAMOSA

INTRODUCTION

Botrytis leaf blight is a leaf spotting and blighting disease which is especially severe under prolonged moist conditions at temperatures of 15-24 C (Lacy and Pontius, 1983; Segall and Newhall, 1960; Sutton et al., 1978; Swanton, 1977). Studies relating dew period and temperature to leaf blight development indicated that lesion production was optimal at 18-20 C and that lesion frequency increased with increasing leaf wetness duration through 24-48 hours (McDonald, 1981; Shoemaker and Lorbeer, 1977; Swanton, 1977; Tanner and Sutton; 1981). Under field conditions, leaves are wet or dry for variable time periods, depending on dew or rain conditions. The influence of the length of and timing of interruptions in leaf wetness on lesion development in Botrytis leaf blight has not been well defined.

McDonald (1981) reported reductions in lesions when leaf wetness periods were interrupted for 4 hours after a 4 or 8 hour leaf wetness period compared with those given a 24 hour leaf wetness period. Swanton (1977) examined the influence of interruptions in leaf wetness after 2 and 8 hours. His data suggested that interruptions longer than 10 hours after 8 hours leaf wetness resulted in reduced lesion numbers. Dzikowski (1980) observed greater reductions in percent leaf area

diseased when plants were interrupted for 1-4 hours after 5 hours of leaf wetness than after 2 hours leaf wetness. The timing of dew period interruptions and influence of interruption duration needs further clarification, and the influence of humidity during interruption has not been examined.

The objectives of this study were to determine the influence of timing of initiation of post-inoculation interruptions in leaf wetness, the influence of duration of the interruptions, and the influence of humidity during the interruption, on lesion numbers produced on onion leaves by Botrytis squamosa.

MATERIALS AND METHODS

Botrytis squamosa was grown and spores were collected as described in Chapter II. In all experiments 1-mo-old onion plants (cv. Spartan Banner, Granada, or Yellow Sweet Spanish) sprouted from bulbs were used. For inoculation, plants were positioned within a cylindrical 61 cm diameter by 77 cm deep settling tower with a rotating base. Dry conidia (2.5 mg, ca. 1.25×10^6) were dispersed near the top of the tower by directing a low velocity stream of air from a pipet tip over conidia on a piece of weighing paper, while the plants rotated in the chamber at 5-6 r.p.m. After the conidia were dispersed, a cover was placed over the top of the tower for about 5 minutes to reduce external air currents and allow the spores to settle on leaf surfaces. Dew was produced on plants within 1 hour after placing them in a commercial dew chamber (Percival Mfg. Co., Boone, IA 50036).

Influence of Timing of Dew Period Interruption on Lesion Production

Forty-two onion plants were inoculated in each of 2 groups of 21 plants, then were incubated within the dew chamber at 20 C for 2, 4, 6, 8, 10, 12, or 24 hours. These incubation periods were followed by 2 hours without dew in a 20 C growth chamber at 65 ± 10% relative humidity (RH), then plants were moved back to the dew chamber for the remainder of the 24 hour incubation period. Inoculated and noninoculated controls remained in the dew chamber for 24 hours. Numbers of lesions per plant were determined after an additional 6 hours in the growth chamber. The experiment was conducted three times.

Influence of Interruption Duration on Lesion Production

Thirty-six onion plants were inoculated in each of 2 groups. Plants were incubated in the dew chamber for 6 hours then were transferred to a growth chamber at 20 C. After 0.3, 0.7, 1.0, 1.3, or 1.7 hours in the growth chamber, 6 replicate plants for each interruption period were returned to the dew chamber for the remainder of the 24 hour incubation period. Noninoculated controls remained in the dew chamber for 24 hours. Lesions were counted after an additional 6 hours in the growth chamber.

In a separate set of experiments 24 onion plants were inoculated in each of two groups. Plants were incubated in the dew chamber at 20 C for 6 hours, then were transferred to a growth chamber at 20 C and 65 ± 10% RH. After 4, 8, or 24 hours in a growth chamber, 6 replicate plants from each interruption period were returned to the dew chamber for an additional 44 hour incubation period. Noninoculated controls remained in the chamber for 44 hours. Experiments were conducted twice.

Influence of Humidity During an Interruption

Onion plants were inoculated with 2.5 mg conidia. After 6 hours in the dew chamber, 6 replicate plants were removed and placed in a growth chamber at 30, 60, or 90 ± 10% RH for 20 minutes, then returned to the dew chamber for the remainder of the 24 hour period of the experiment. Six control plants remained in the dew chamber continuously for 24 hours. Humidity and temperature were monitored with a recording hygrothermograph. The experiment was conducted three times.

Spore Germination Rate on Leaf Surfaces

Two groups of fourteen onion plants were inoculated and placed in the dew chamber at 20 C. After 1, 2, 4, 6, 8, 10, 12, or 24 hours, the 3rd or 4th youngest leaves of 4 replicate plants were sampled, since lesions produced on these leaves were the most uniform in size. Four 1-cm^2 leaf tissue pieces were removed from each leaf, fixed in formalin-50% ethanol-glacial acetic acid (1:18:1, v/v), stained with cotton blue in lactic acid solution (28 mg aniline blue, 20 ml water, 10 ml glycerol, and 10 ml 85% lactic acid), and examined using light microscopy. Conidia which washed off leaves during fixation were collected on 13-mm-diameter membrane filters (pore size = 0.33 um), stained, and counted as above.

RESULTS

Influence of Timing of Dew Period Interruptions on Lesion Production

Plants given an interruption in dew period of 2 hours following 2-12 hours of dew had fewer lesions than those continuously in the dew chamber for 24 hours prior to interruption (Figure 3.1). Fewest lesions were produced on those plants provided with 6 hours dew, 2 hours of

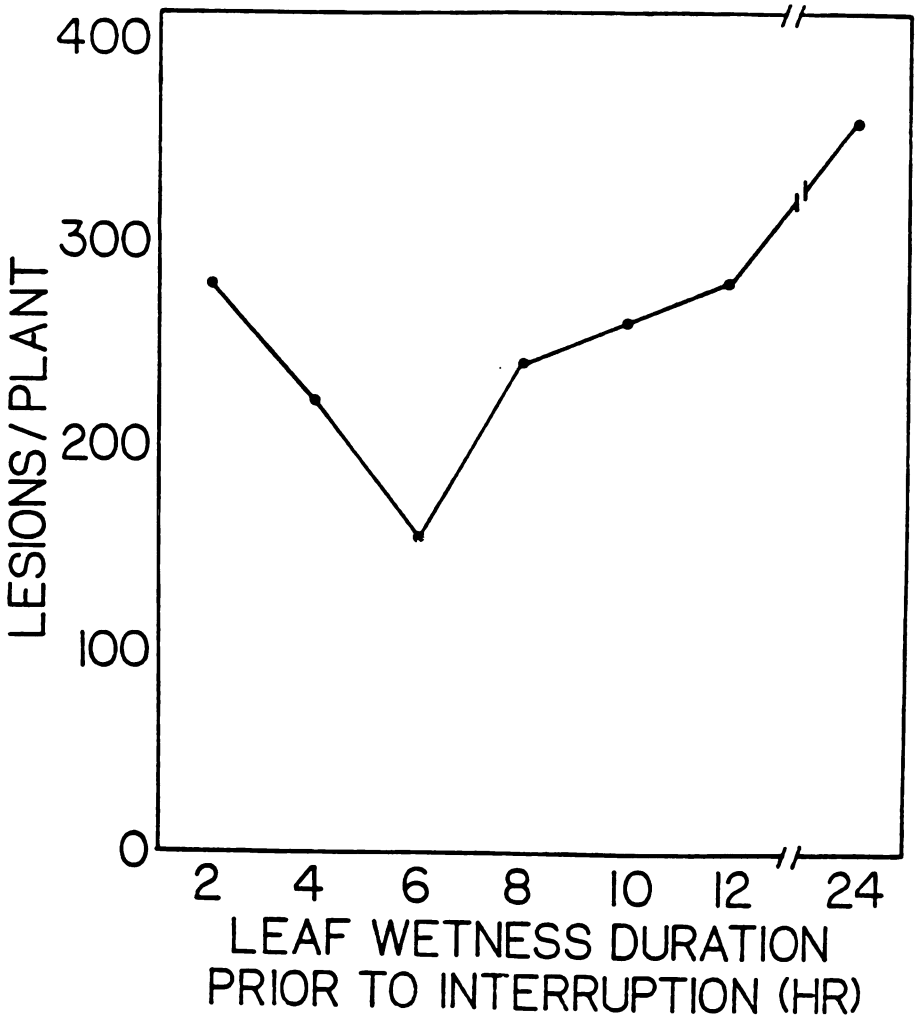

Figure 3.1. Influence of leaf wetness duration prior to a 2-hr interruption (dry period), followed by rewetting plants for a total of 22 hr total wetness period, on lesions/plant. Mean values were derived from three replicate experiments, LSD ($\underline{P}$=0.05)=74.

interruption (dry period), and 18 hours of dew after the interruption. Numbers of lesions decreased with increasing initial wetness duration from 2 through 6 hours, then increased as time of wetness increased through 24 hours. Since maximum lesion production occurred by 24 hours (Chapter II), plants which received 24 hours dew prior to removal from the chamber served as control plants.

Influence of Interruption Duration on Lesion Production

Onion plants provided 6 hours dew followed by interruption durations of 0.3-1.7 hours, within a 24 hour incubation period, displayed fewer lesions than uninterrupted control plants (Table 3.1). An abrupt drop in lesion numbers was apparent after a 0.3 hour (20 minute) interruption, and there was a tendency toward decreasing lesion numbers as the length of dry period increased, but differences were not significant.

Table 3.1. Effect of interruption duration of dew period on infection of onion by *Botrytis squamosa*.

Treatment Duration (hour)			Lesions/Plant	
			Trial Number	
Wet	Dry	Wet	I	II
6	0.0	18.0	[x]343 ± 119	450 ± 90
6	0.7	17.7	229 ± 72	316 ± 20
6	0.7	17.3	233 ± 75	294 ± 51
6	1.0	17.0	209 ± 24	266 ± 23
6	1.3	16.7	174 ± 105	274 ± 55
6	1.7	16.3	140 ± 43	216 ± 101

[x]Mean of 6 replicate onion plants.

Lesion numbers were greatly reduced on plants incubated under conditions of dew for 6 hours, then interrupted for 4 or 8 hours, compared to noninterrupted controls (Table 3.2). Significant reductions in lesion numbers were evident on those plants which were interrupted for 24 hours following 6 hours dew.

Table 3.2. Influence of interruption after a 6 hour dew period at 20 C followed by an additional 24 hour dew period.

Treatment Duration (hour)			Lesions/Plant	
			Trial Number	
Wet	Dry	Wet	I	II
6	0	24	[x]413 ± 64	380 ± 105
6	4	24	152 ± 76	126 ± 60
6	8	24	147 ± 55	167 ± 62
6	24	24	107 ± 55	90 ± 25

[x]Mean of 6 replicate onion plants.

Influence of Humidity During Interruptions

There were fewer lesions on plants where dew period was interrupted by incubations at 90, 60, or 30% RH as humidities decreased (Table 3.3), although differences in most cases were not statistically different from each other or from those where dew period was not interrupted, according to Duncan's multiple range test ($P = 0.05$). Fewer lesions were produced at 30% RH than under continuous dew in one experiment.

Table 3.3. Influence of a 6 hour dew period, a 20 minute dew interruption at 30, 60, or 90% RH, followed by an 18 hour dew period, on infection of onion by Botrytis squamosa.

	[x]Lesions/Plant			
	Trial Number			Mean
Humidity (%)	I	II	III	Response
no interruption	[y]388 a	[y]426 a	[y]376 a	[y]397 a
90 ± 10%	284 ab	326 a	306 a	317 ab
60 ± 10%	270 bc	321 a	273 a	288 ab
30 ± 10%	104 c	303 a	231 a	213 b

[x]Mean of 6 replicate onion plants.

[y]Values in a column followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

Spore Germination on Leaf Surfaces

Spore germination on leaves began after 2 hours in the dew chamber, and percent germination increased rapidly between 6-12 hours dew with maximum germination occurring by 20 hours (Figure 3.2).

DISCUSSION

Timing of and duration of interruptions of the dew period influenced lesion production in onion by B. squamosa. Humidity during the interruption also influenced lesion production significantly in one experiment. Our results indicated that B. squamosa was most sensitive to a desiccation period after 6 hours dew. Dzikowski (1980) also found greater reductions in lesion numbers after a 5 hour initial leaf wetness period than after a 2 or 22 hour leaf wetness period. However, he did not quantify treatment responses in terms of lesion numbers and it is

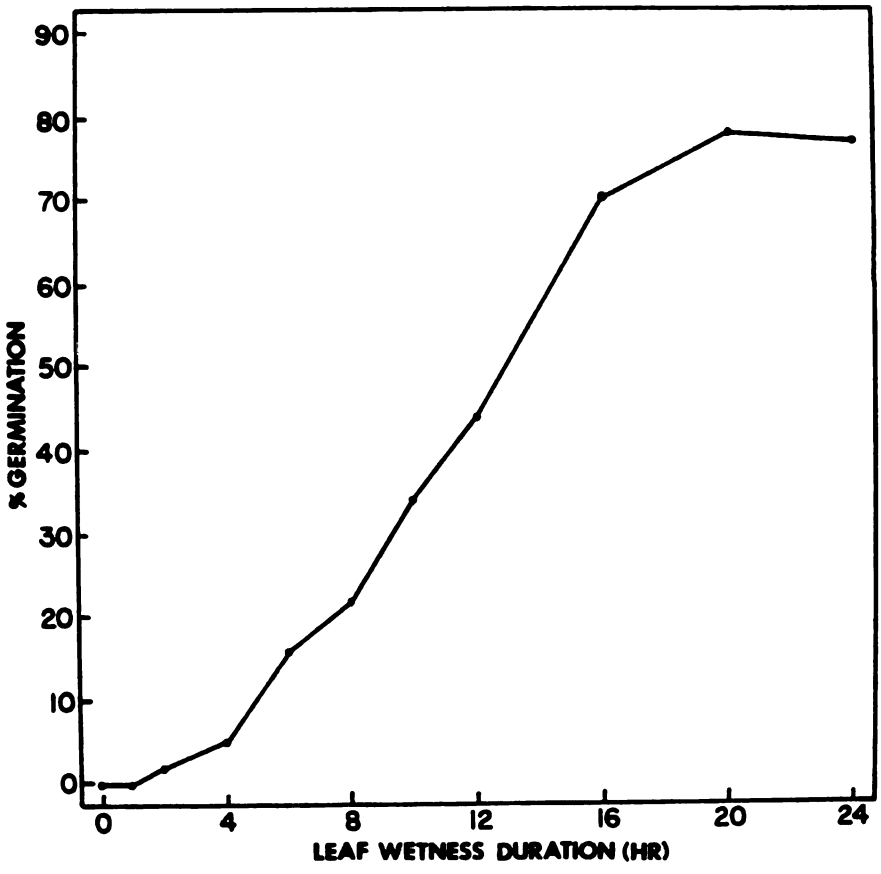

Figure 3.2. Percent germination of Botrytis squamosa conidia on leaf surfaces at 20 C and continuous leaf wetness.

not clear how his treatment differences, quantified in terms of percent leaf area diseased, were obtained.

Fungi such as Stemphylium botryosum f. sp. lycopersici (Bashi and Rotem, 1974), Alternaria porri (Bashi and Rotem, 1974), Cercospora musicola (Goos and Tschirch, 1963) or Botrytis cinerea (Gooda and Zathurecky, 1967) can withstand a dry period prior to their establishment within the host. B. squamosa appeared to be sensitive to desiccation during a dry period occurring prior to penetration into host tissue. Sensitivity to a dry period was also reported for germinating conidia of such fungi as Coccomyces hiemalis (Eisensmith et al., 1982), Glomerella cingulata (Leben and Daft, 1968), and Alternaria longipes (Norse, 1973).

Germination of B. squamosa conidia began after 2 hours and increased through 20 hours on wet leaf surfaces at 20 C. After 6 hours, which is the postinoculation wetness period when conidia were most sensitive to a dry period, less than 20% of the conidia had visible germ tubes, suggesting that the conidia were sensitive to a dry period at a stage prior to the formation of germ tubes.

Previous studies concerning survival of B. squamosa conidia on onion leaf surfaces (Chapter II) and in soil (Ellerbrock and Lorbeer, 1977) indicated that B. squamosa can survive for several days on continually dry leaf surfaces or for several weeks in continually moist soil. However, conidia placed in soil and subjected to wet-dry regimes exhibited enhanced mortality over those exposed to continually moist or dry treatments. In my studies, very few lesions developed on plants provided with 6 hours dew, then interrupted for 24 hours (5-10 per plant). Placing these plants subsequently in the dew chamber for 24 hours

resulted in increased lesion numbers, suggesting that the ungerminated spores had survived on leaf surfaces and could still cause lesions.

Under field conditions B. squamosa conidia probably survive movement by air currents and deposition during the day, and are stimulated to germinate under conditions of leaf wetness at night. Conidia probably do not survive more than one or two periods of dew insufficient for infection. As previously defined (Chapter II; Shoemaker and Lorbeer, 1977) minimal leaf wetness for infection would be 6-12 hours. It is possible that a lower rate of germination on onion leaves may occur under some conditions in nature. For example, Clark and Lorbeer (1977) reported less germination in the presence of some bacterial isolates in the phyllosphere of onion leaves. In addition, other factors such as solar irradiation may also influence the survival of conidia or their ability to infect onion leaves.

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CHAPTER IV

INFLUENCE OF LEAF POSITION AND MATURITY ON DEVELOPMENT OF *BOTRYTIS SQUAMOSA* IN ONION LEAVES

INTRODUCTION

Botrytis leaf blight, caused by *Botrytis squamosa*, is recognized as a leaf spotting and blighting disease of onion foliage (Hickman and Ashworth, 1943; Page, 1955; Hancock and Lorbeer, 1963). Leaf spots are discrete, desiccated spots, 1-10 mm long by 1-2 mm wide. Under prolonged moist conditions, some lesions may expand and girdle leaves, causing collapse of tissues and leaf blighting. Leaves die from the tip downward. Greater lesion numbers and blighting occur on outer leaves than on younger, inner leaves (Hickman and Ashworth, 1943; Page, 1955; Small, 1971; Shoemaker and Lorbeer, 1977).

Histopathology of infection was studied by Clark and Lorbeer (1976). They defined 2 phases in lesion formation: (i) collapse and separation of mesophyll from the epidermis, resulting in cavity formation and (ii) subsequent collapse and degeneration of adjacent tissues. However, little is known regarding growth of *B. squamosa* within lesions, especially relative to leaf position or leaf maturity.

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

MATERIALS AND METHODS

In all experiments isolate BSS-4 was used since this isolate was highly virulent and sporulated more prolifically than other isolates. One-month-old onion plants, sprouted from bulbs, were inoculated using a dry spore inoculation technique as described in Chapter II. Following inoculation, plants were incubated in a commercial dew chamber (Percival Mfg. Co., Boone, Iowa 50036) at 20 C for the specified duration of leaf wetness.

The position of leaves on plants was defined using a number system from 1 through 6 where 1 represented the innermost (youngest) leaf and 6 represented the outermost (oldest) 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 hours, then held in a growth chamber at 20 C for 6 hours. 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 ($A = \pi rh$, where A = area, $\pi = 3.146$, 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 68504), 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 and mean lesion size was examined. Four replicate onion plants were inoculated and incubated in

the dew chamber at 20 C for 24 hours. Four 1-cm^2 tissue segments were excised from each of 4 replicate leaves. The segments containing lesions were fixed in FAA and stained with trypan blue prior to examination. Relative lesion area was estimated by multiplying length by width. A minimum of 20 lesions per leaf were examined. The experiment was repeated twice.

Leaf Position vs. Infection Hyphae Development

The relationship between leaf position and mean length of infection hyphae within lesions was examined. Four onion plants were inoculated with 2.5 mg conidia, then incubated in the dew chamber for 24 hours. Four 1-cm^2 tissue segments containing lesions were excised from each of 4 replicate leaves. Segments were fixed in FAA and stained with trypan blue prior to examination. Infection hyphal lengths were measured using a microscope equipped with an ocular micrometer.

Lesion Size vs. Infection Hyphae Development

The relationship between lesion size and length of infection hyphae within lesions was examined. Onion plants were inoculated, then incubated in the dew chamber at 20 C for 1, 3, or 5 days. Lesions in the leaf positions 1 through 6 were sampled. Four 1-cm^2 tissue segments were excised from each of 4 replicate leaves. Lesions were fixed (and stored) in FAA and stained with trypan blue at room temperature, then were rinsed and mounted in water prior to examination. Infection hyphal lengths were measured using a light microscope equipped with an ocular micrometer.

Rate of Lesion Development

The rate of increase in lesion size with time on leaves of different ages was determined. Sixteen onion plants were inoculated with 2.5 mg B. squamosa conidia, then placed in the dew chamber. After 8, 12, 16, and 24 hours, four replicate plants were removed from the chamber. Lesion sizes on each leaf were estimated using a rating scale from 1-4 where 1 represented small ($\leq$ 1 mm long) lesions and 4 represented large ($\geq$ 4 mm long) lesions.

Influence of Tissue Maturity on Infection Hyphae Development

The influence of tissue maturity (senescence) on infection hyphae development was examined. Four onion plants were inoculated with single 5-ul drops of a 1 X 10^3 conidia per ml suspension at 4 sites on each leaf. Plants were incubated in a dew chamber at 20 C for 24 hours. Leaf pieces from inoculated senescing (yellow) and healthy (green) tissues were excised, fixed in FAA, and stained in trypan blue. Hyphal lengths were measured using a microscope fitted with an ocular micrometer.

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 (Figure 4.1). Significantly ($P=0.05$) higher numbers of lesions were produced on the outermost leaf (position 5) compared with leaves one through four (LSD test, $P=0.05$). Greater number of lesions were also produced on leaf four compared with one and two.

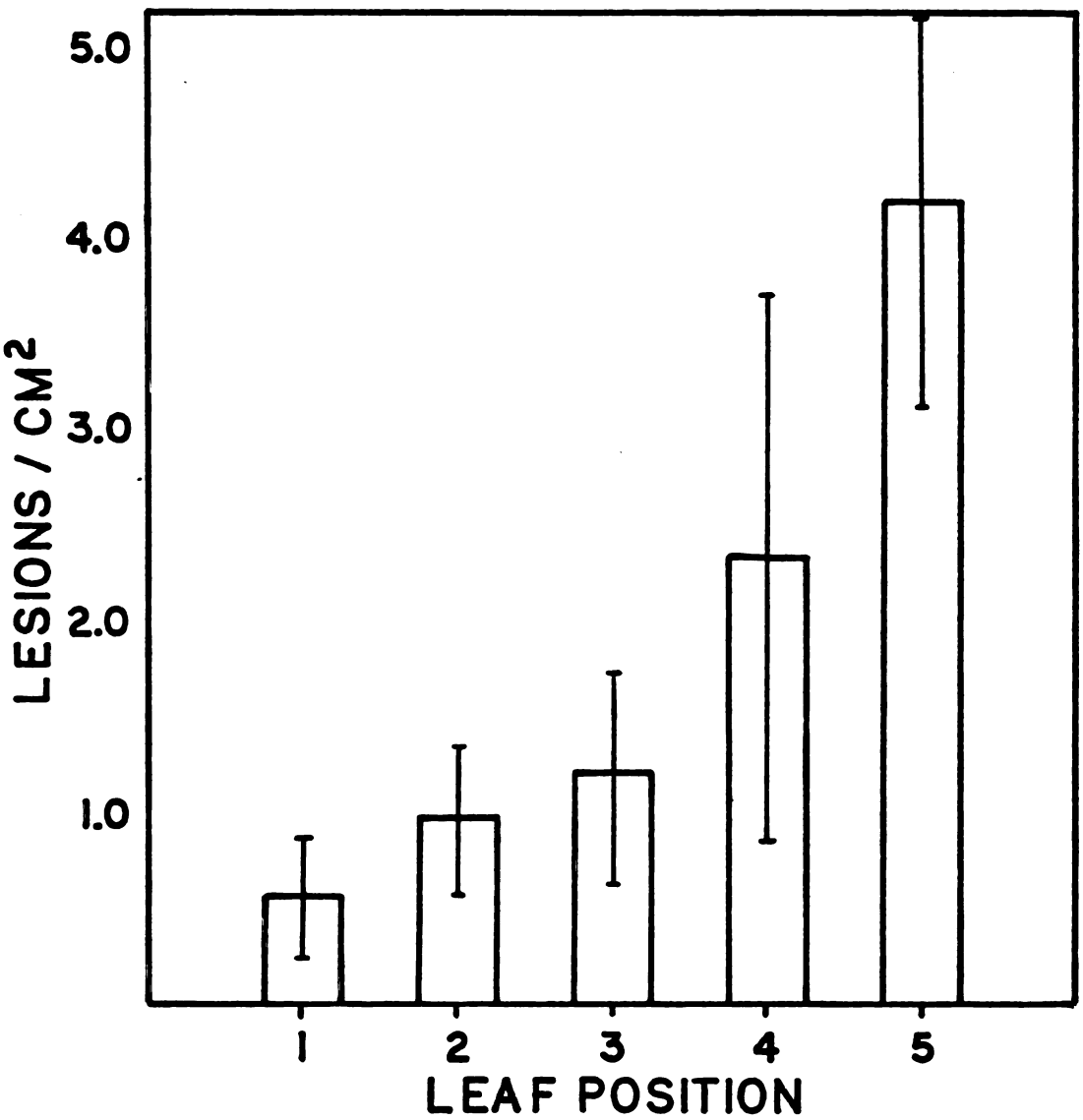

Figure 4.1. Influence of leaf position on lesion numbers on onion after inoculation with B. squamosa and incubation in a dew chamber at 20 C for 24 hours (leaf position 1 = youngest, innermost leaf).

Leaf Position vs. Lesion Size

The mean size of Botrytis leaf blight lesions of the same age increased with an increase in age of onion leaves (Figure 4.2). Mean lesion areas were significantly greater on leaf six compared with one through four. Leaf five contained greater lesion areas than leaf one (LSD test, $P=0.05$).

Leaf Position vs. Infection Hyphae Development

Mean infection hyphal lengths within lesions increased with increase in age of onion leaves (Figure 4.3). Significantly greater mean infection hyphal lengths were observed on leaves five or six compared with one through four. Greater mean lengths were also observed on leaves three or four compared with leaf one (LSD test, $P=0.05$).

Lesion Size vs. Infection Hyphae Lengths

Lesion area correlated poorly with length of infection hyphae within lesions after 24 hours incubation in the dew chamber. Correlation coefficients (r) for leaves in positions 1-6 were 0.67, 0.36, 0.36, 0.42, 0.36, and 0.27, respectively. After one day in the dew chamber infection hyphae were <100 um in length, although lesions had developed to near the maximum size for nonexpanding lesions (2-5 X 1-4), indicating that lesions developed well in advance of the hyphae within.

Rate of Lesion Development

The rate of lesion appearance and expansion within the dew chamber was slower on the innermost leaf than on the outermost leaf (Table 4.1). Rates of development were similar in leaf positions three through six.

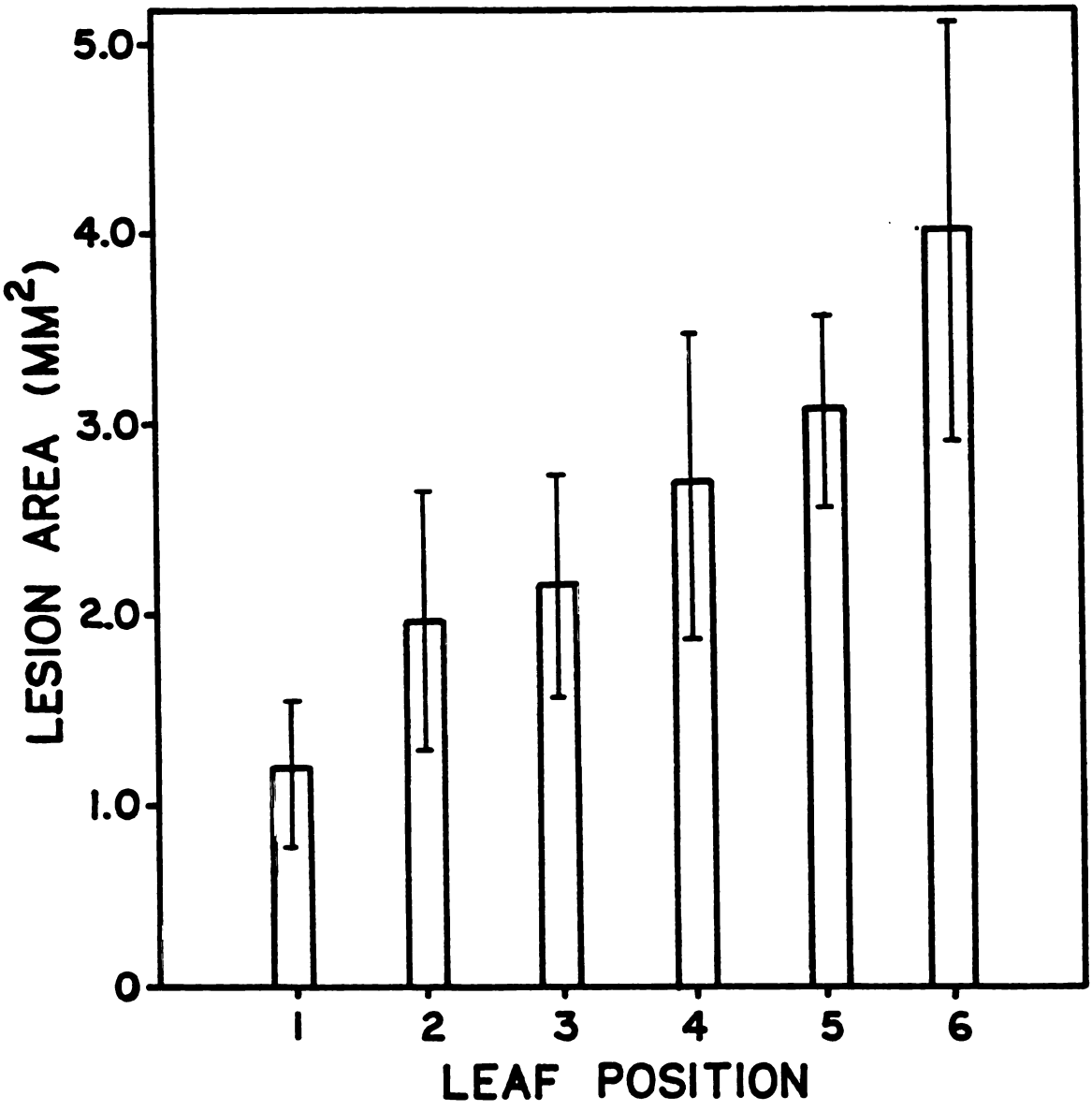

Figure 4.2. Influence of leaf position on lesion size on onion after inoculation with B. squamosa and incubation in a dew chamber at 20 C for 24 hours.

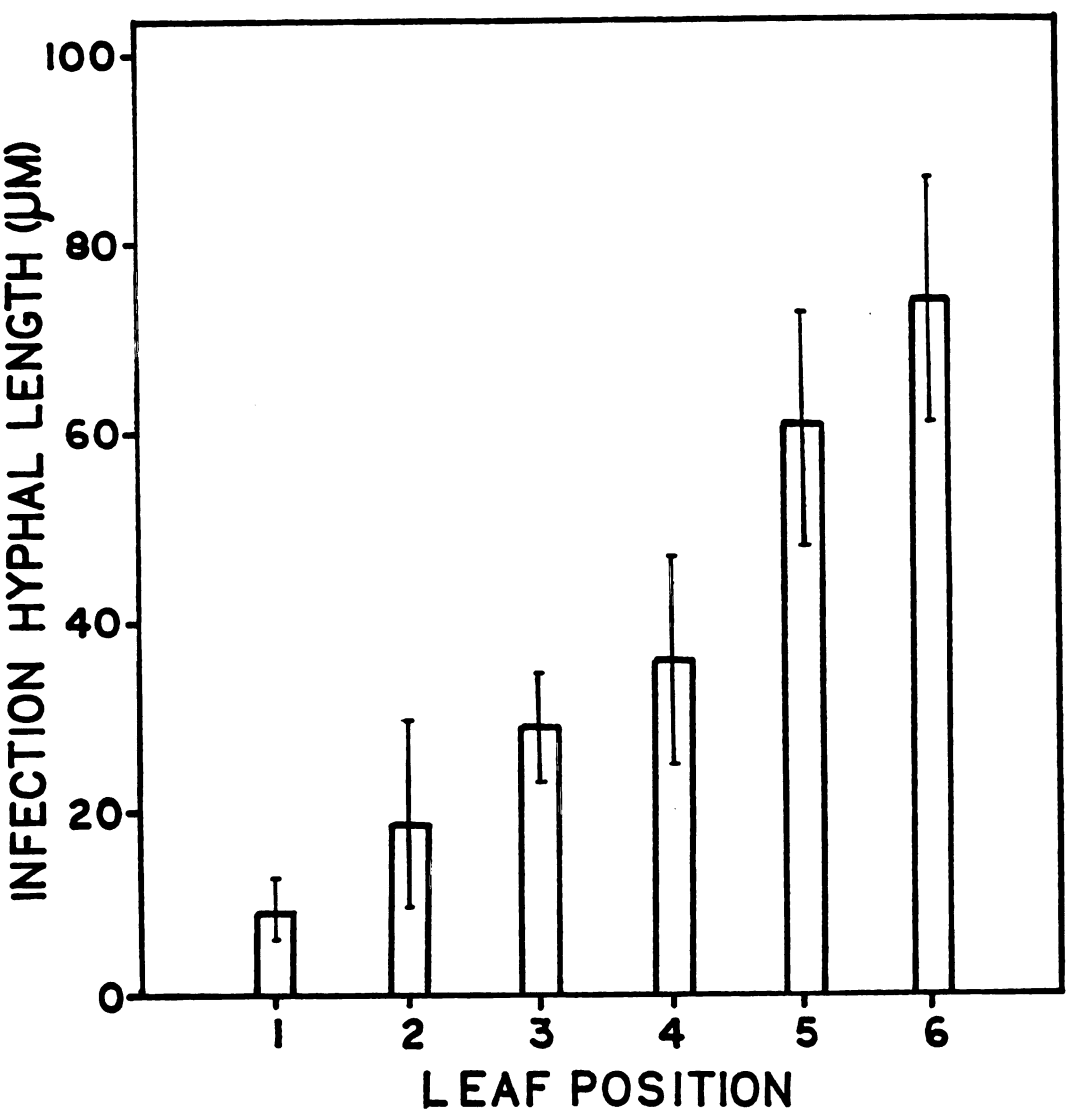

Figure 4.3. Influence of leaf position on infection hyphal lengths within lesions on onion after inoculation with B. squamosa and incubation in a dew chamber at 20 C for 24 hours.

Table 4.1. Relationship between leaf position and incubation period in a dew chamber on lesion size following inoculation of onion plants with B. squamosa.

Dew Period (hour)	Lesion size[a] Leaf position 1[b]	2	3	4	5	6
8	0.0[c]	0.3	0.9	0.5	0.7	0.8
12	0.9	1.0	1.4	1.6	1.7	1.6
16	0.6	1.0	1.4	1.4	1.8	2.0
24	1.0	2.0	2.3	2.3	2.5	2.4

[a]Lesion size was visually rated using a rating scale of 1-4 where 1 represented less than 1 mm diam and 4 represented lesions equal to or greater than 4 mm long.

[b]Youngest leaf.

[c]Based on 20 lesions per leaf from 3 replicate leaves.

Influence of Tissue Maturity on Infection Hyphae Development

Hyphae grew almost three times as fast in senescent as in healthy tissue. Mean infection hyphal lengths of B. squamosa growing in senescent tissues at 20 C for 24 hours after inoculation were 207 ± 9 um compared with lengths of 77 ± 15 in nonsenescent tissues.

DISCUSSION

Although both lesion size and lengths of infection hyphae increased with increasing age of leaves on any given leaf in position 1-6, a poor correlation was found between lesion area and lengths of infection hyphae. Lesions developed rapidly on both young and old leaves. By 24 hours lesions were near their maximum size yet infection hyphae were only 1-100 um in length (Chapter II). This suggests that lesions development

may result from penetration rather than hyphal development within the lesion, with leaf maturity being the primary determinant of lesion size.

Hancock and Lorbeer (1963) believed that lesions developed from enzymes released during spore germination, but prior to penetration. In my observations of spore germination on leaf surfaces, germinated spores were observed which were not associated with lesions. Lesions were generally associated with those conidia which germinated and penetrated. We further observed that most infection hyphae remained restricted in their development within lesions (Chapter II), except on the outermost leaves where expanding lesions occurred under conditions of prolonged leaf wetness.

In the initial lesion there is a rapid and extensive collapse of cells beneath the epidermal layer which results in an empty cavity extending across most of the leaf. The epidermal layer generally remains intact. Twenty four to 72 hours after lesion onset some wall thickening can be observed under the light microscope in cells bordering the lesion. When the leaf senesces and the green leaves begin to fade and turn yellow, a green ring is often observed surrounding the lesion. After the initial 24 hour period, the cavity area does not further expand. In some lesions, hyphae grow beyond the lesion border toward healthy tissues. As hyphae grow toward and into the healthy tissues, there is water-soaking and softening of the tissues in advance of the hyphal progression. Upon drying, these tissues tend to collapse, have a blighted appearance, and may become desiccated ("expanding" lesions). Increasing the leaf wetness period following inoculation resulted in an additional increase in numbers of expanding lesions, primarily on the outermost leaves. Although numbers of expanding lesions were small, even

under extended periods of wetness, only a few of these lesions could cause extensive collapse and blighting.

Since onions normally produce 15-16 leaves in a growing season, but rarely have more than 10 at any one time (Jones and Mann, 1963), older leaves begin to senesce and die naturally, even without the effects of disease, as plants begin to mature and bulbs form. The senescing leaves provide a substrate where expanding lesions and sporulation occurs commonly. If large amounts of inoculum are produced, even young leaves can be severely damaged with very large numbers of small lesions.

Clark and Lorbeer (1976) reported development of expanding lesions 72-96 hours after inoculation. This study demonstrated that extent of development of B. squamosa depended mostly on tissue maturity. Greatest development of B. squamosa was evident on senescing tissues. Botrytis squamosa also grew well in dead onion leaves.

Clark and Lorbeer (1967) reported greater superficial growth of B. squamosa when plants were inoculated with conidia in water containing nutrients than in water. Thus, greater availability of nutrients and lack of a resistance response may account for the aggressive colonization of senescent tissues by B. squamosa. We observed longer germ tubes and more stomatal penetrations on the senescing tissues. Very short germ tubes were observed on young leaves.

This study suggests that B. squamosa acts as a weak pathogen on young, healthy onion foliage, inducing a host reaction resembling that of the hypersensitive response. However, B. squamosa growing within senescent tissues or expanding lesions can result in destructive leaf blighting.

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CHAPTER V

INFLUENCE OF TEMPERATURE AND WATER POTENTIAL ON GROWTH AND SPORULATION OF *BOTRYTIS SQUAMOSA*

INTRODUCTION

Environmental parameters important in leaf blight development include temperature, leaf wetness duration (McDonald, 1981; Tanner and Sutton, 1981; Shoemaker and Lorbeer, 1977; Swanton, 1977), and air humidity, expressed as relative humidity (RH) (Swanton, 1977; Sutton et al., 1978) or as vapor pressure deficits (Lacy and Pontius, 1983). Lesion production is optimal at 18-20 C and lesion numbers and leaf blighting is generally more severe on outer, older leaves (Hickman and Ashworth, 1943). Sporulation occurs only on senescent or necrotic tissues (McDonald, 1981; Small, 1970; Swanton, 1977).

Growth of *B. squamosa* was reported by Swanton (1977) to be most rapid between 15-33 C. Shoemaker and Lorbeer (1977) reported that the optimal temperature for radial growth was 24 C. Segall and Newhall (1960) reported maximum growth as dry weight at 21 C.

The influence of polychromatic light, carbohydrate source, and pH on conidiation of *B. squamosa* was studied by Berquist et al. (1972). Maximum sporulation occurred at Ph 5.5. Starch, dextrin, or potato extract were more effective than mono- or disaccharides in stimulating conidial production. A 14 hour photoperiod of fluorescent near UV was optimal for conidial produciton.

No studies of which I am aware have examined the influence of water potential on growth and sporulation of B. squamosa. Since air humidity and temperature are important in spore production in the field (Lacy and Pontius, 1983), and understanding of the influence of these parameters on growth of B. squamosa could be epidemiologically important.

The objectives of this study were (i) to examine the influence of moisture and temperature on growth and sporulation of B. squamosa, and (ii) to determine the influence of moisture period and interrupted moisture period on sporulation of B. squamosa from onion leaf tissues.

MATERIALS AND METHODS

Influence of Temperature and Water Potential on Growth Rates on PLY Agar

Prune extract-lactose-yeast extract agar (PLY) was used as the basal medium, the osmotic potentials of which were adjusted using various concentrations of KCl, NaCl, sucrose, or polyethylene glycol (PEG) 8000. Measurements of the water potentials were made using a Wescor dewpoint hygrometer and C-52 sample chambers (Wescor, Inc., Logan, Utah 84321). Agar plates were inoculated by placing a 4-mm-diameter plug from the advancing margin of a 72-hour-old culture of B. squamosa onto the center of each PLY agar plate. Four replicate plates per treatment were then incubated at 20, 25, or 30 C. Colony diameters were measured daily over 4 days and growth rates were calculated over days 2-4 since growth was linear during this time.

Influence of Temperature and Water Potential on Dry Weight in PLY Broth

Flasks containing 50 ml of PLY broth adjusted to various water potentials with KCl, sucrose, NaCl, or PEG 8000 were each inoculated with three 4-mm-diam X 5-mm plugs from the advancing margin of 72-hour-old

cultures of B. squamosa. Flasks were incubated at 20, 25, or 30 C in still culture for 4 days. Dry weights were determined by collecting the contents of each flask on separate 7-cm-diameter glass microfiber filter papers (Whatman Ltd., England). Each filter was rinsed with 1000-1500 ml water to ensure complete removal of media and osmotica, then air dried in a forced air drier at 60 C until a constant weight was obtained.

Influence of Temperature and Water Potential on Growth in Onion Leaves

Water potentials of sterilized (autoclaved for 85 minutes, 20 p.s.i.) green onion leaves were adjusted by positioning leaves over distilled water, in sealed cahmbers, containing various concentrations of NaCl. Seven days were allowed for equilibration prior to inoculation of each leaf piece with a 4-mm-diameter plug from the advancing margin of a 3-day-old culture of B. squamosa. Radial growth on leaves was measured after 3 days incubation at 20, 25, or 30 C. Water potentials of the leaves were determined using a Wescor dewpoint hygrometer.

Influence of Desiccation Period and Temperature on Sporulation

The influence of desiccation period and temperature on sporulation of B. squamosa was examined using infected leaf tissue segments. One-cm^2 onion leaf segments were placed on water agar and a 4-mm-diameter plug from the advancing margin of B. squamosa growing on PLY agar was transferred to each leaf segment. After 3 days incubation at 20 C in darkness, the agar plugs were removed, leaves were transferred from the agar to a sterile plastic surface, air dried for two hours in a laminar flow hood, then were placed in sealed chambers over $CaCl_2$.

To determine the influence of desiccation period on sporulation, dried, infested leaf segments were incubated over $CaCl_2$ for 3, 6, 9, or 12 days at 22 C then were transferred to water agar. The plates were incubated at 20 C under a 12 hour light photoperiod in fluorescent light and examined for sporualtion over a subsequent 5-day incubation period.

Since under field conditions temperature fluctuations would govern rate of sporulation, the influence of temperature on sporulation from the infested leaf segments was examined. After 3 days under desiccation at 22 C, leaf segments were transferred to water agar plates and incubated at 15, 20, 25, or 30 C under a 12 hour photoperiod in fluorescent light. After 3 days incubation, conidia were collected from each of 4 replicate plates for each temperature.

Since sporulation from leaf segments occurred over a period of several days, the dynamics of sporulation was examined to determine when the bulk of conidia were produced. Onion leaf segments were inoculated, incubated, and dried as described above, then were transferred to water agar plates. The plates were incubated at 15 C and conidia were collected daily through 6 days.

The influence of cyclic wet-dry-wet periods on sporulation was examined. Colonized, dried leaf segments were placed on water agar and incubated at 20 C under a 12 hour fluorescent light-12 hour dark regime. At the beginning of each 24 hour period, leaves were removed and dried for 1-2 hours in a laminar flow hood, then placed over $CaCl_2$ in sealed chambers. After drying periods (flow hood + sealed chambers) of 4, 8, 12, or 16 hours, leaves were placed on water agar and returned to the incubator for the remainder of the 24 hour cycle. Conidia were collected immediately prior to each drying period, on each of six consecutive

days. For each treatment 4 replicate plates were used and conidial numbers were quantified as conidia/cm^2 colonized leaf tissue.

Under field conditions onion leaves supporting sporulation frequently lie on the ground and are influenced by moisture conditions at the soil-air interface. The influence of moisture in such a corresponding situation was examined using agar plates adjusted to various water potentials as the model system. Colonized, dried leaf segments were placed on water agar adjusted to various water potentials with KCl. Potentials of the agar were determined using a Wescor dewpoint hygrometer as previously described. After incubating plates at 20 C for 5 days under a 12 hour fluorescent light-12 hour dark regime,conidia were removed as previously described (Chapter II) and quantified using a hemacytometer.

RESULTS

Influence of Temperature and Water Potential on Growth Rate on PLY Agar

Growth of B. squamosa on PLY agar adjusted to various water potentials with osmotica were similar at 20 or 25 C but much lower at 30 C (Figure 5.1). In the presence of KCl, sucrose, or PEG at 20, 25, or 30 C growth rates of B. squamosa increased from -0.9 through -5 to -20 bars, then declined to zero below -100 bars. In the presence of NaCl growth rates increased from 0.9 through -5 to -10 bars only at 20 C, then declined and ceased by -60 bars. At 25 and 30 C growth, in the presence of NaCl, declined and was not observed at lower than -60 bars. Below -30 bars, there was no significant differences in growth at the three temepratures used, with any osmoticum.

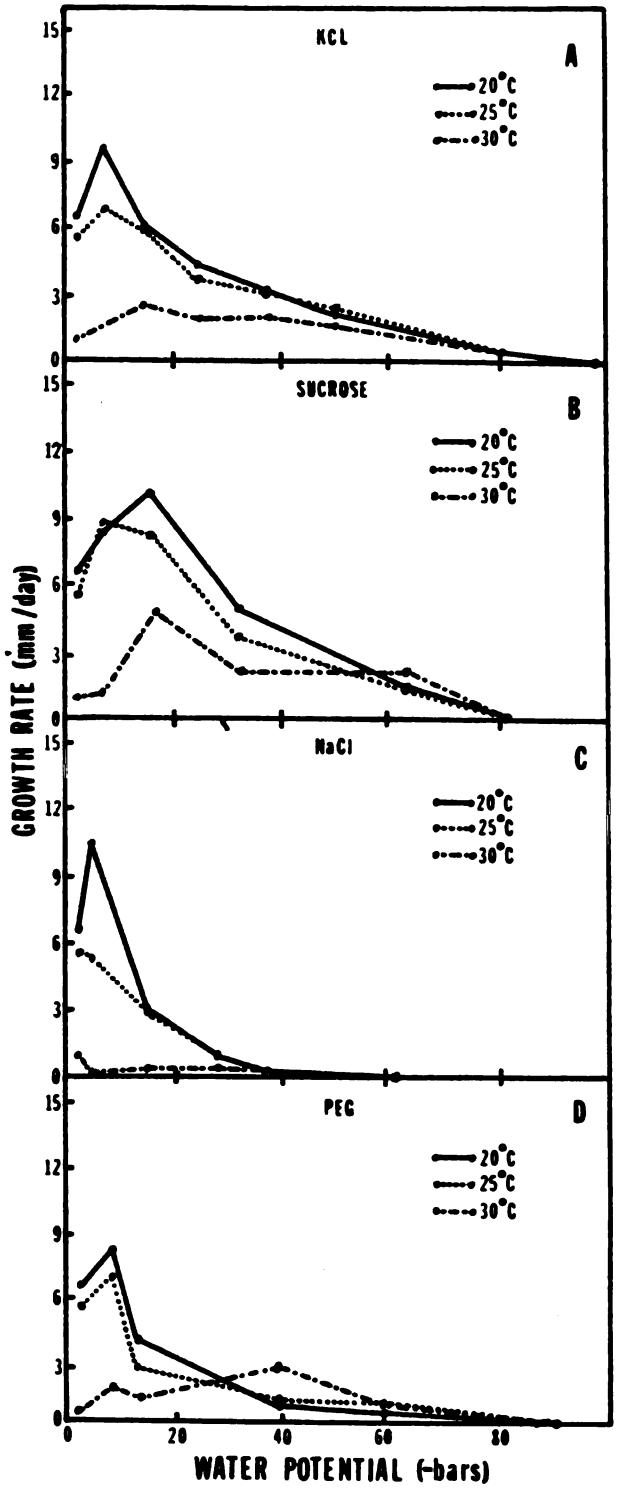

Figure 5.1. Growth rate of B. squamosa at 20, 25, and 30 C on PLY agar adjusted to various water potentials with KCl, sucrose, NaCl, and PEG 8000.

Influence of Temperature and Water Potential on Dry Weight in PLY Broth

Growth of B. squamosa in PLY broth adjusted to various water potentials with osmotica were similar at 20 or 25 C but much lower at 30 C (Figure 5.2). Growth was stimulated when potentials were lowered -5 to -20 bars but over lower potentials growth declined. In the presence of KCl, sucrose, or PEG 800 growth was not detected at -70 to -95 bars and with NaCl growth was not detected below -40 bars.

Influence of Temperature and Water Potential on Growth in Onion Leaves

Growth of B. squamosa in onion leaves was similar at 20 or 25 C and greatly reduced at 30 C (Figure 5.3). At 20 or 25 C radial growth was similar from -0.9 through -24 bars, then declined through -90 bars (Figure 5.3). At 30 C radial growth was similar to that at 20 or 25 C at -9 to -79 bars.

Influence of Desiccation Period and Temperature on Sporulation

When leaf segments, infested with B. squamosa, were desiccated for 3-12 days, then placed on water agar plates at 20 C for 3 days, B. squamosa was observed growing equally well from all of the leaf segments. The duration of the desiccation period through 12 days did not appear to influence the extent of sporulation.

Sporulation of B. squamosa from infested leaf segments placed on water agar for 4 days was greater at 15 or 20 C, less at 25 C, and nil at 35 C (Figure 5.4).

The sporulation dynamics of B. squamosa was monitored over 6 days. No sporulation was evident from leaf segments placed on water agar at 15 C for 24 hours. Large numbers of conidia were produced after 2 or 3 days on leaf segments placed on agar (Figure 5.5). Conidial production

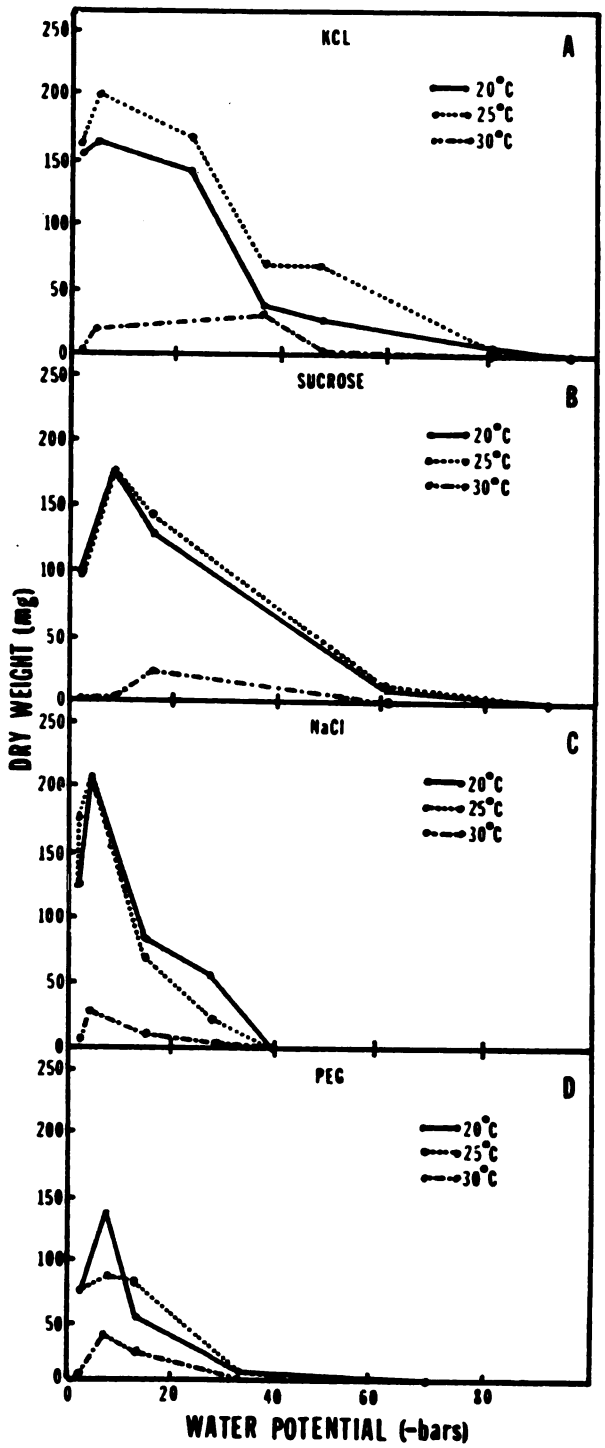

Figure 5.2. Dry weight of B. squamosa at 20, 25, and 30 C after 4 days in PLY broth, adjusted to various water potnetials with KCl, sucrose, NaCl, and PEG 8000.

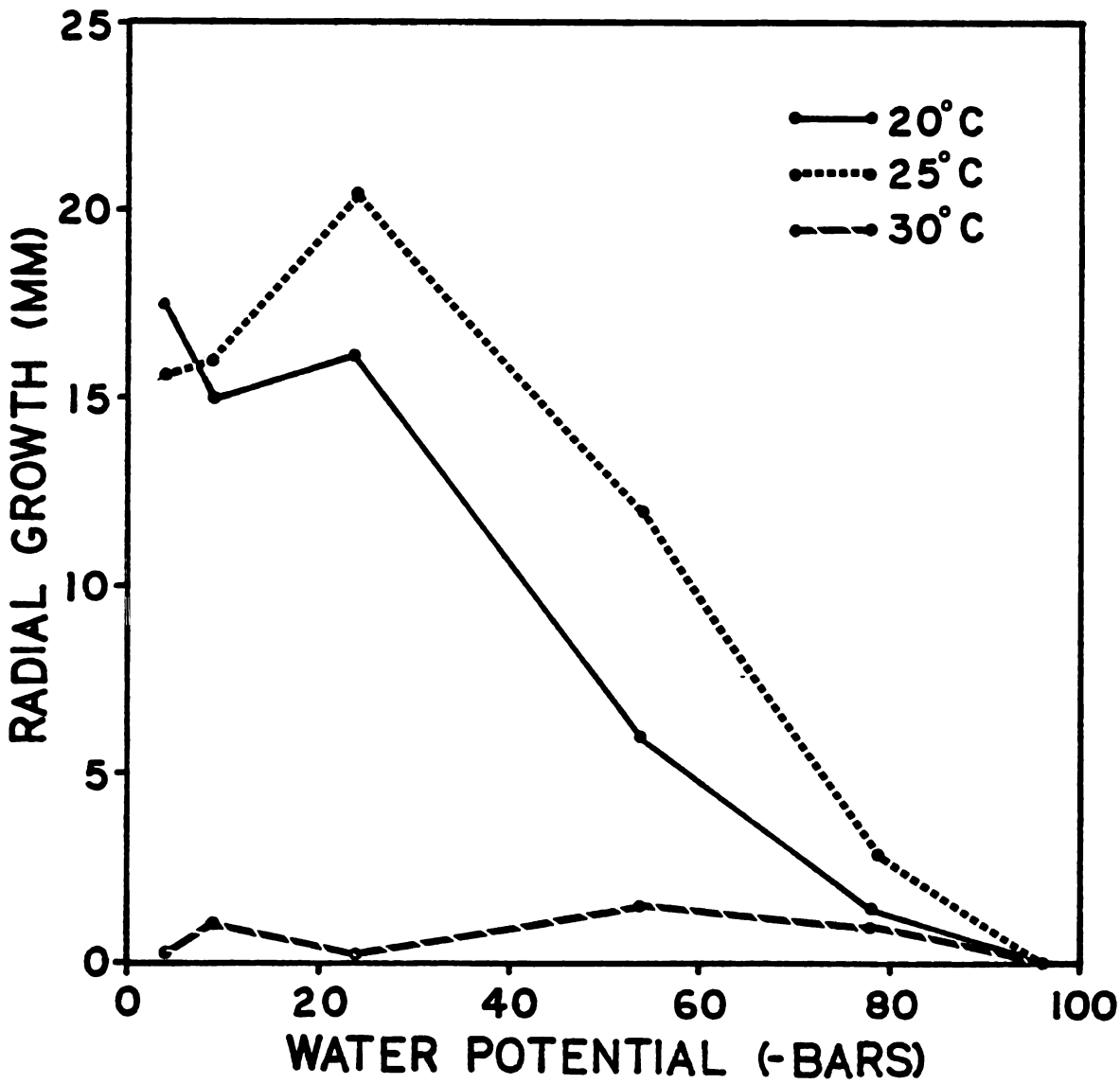

Figure 5.3. Radial growth of B. squamosa in onion leaves at 20, 25, and 30 C at various water potentials.

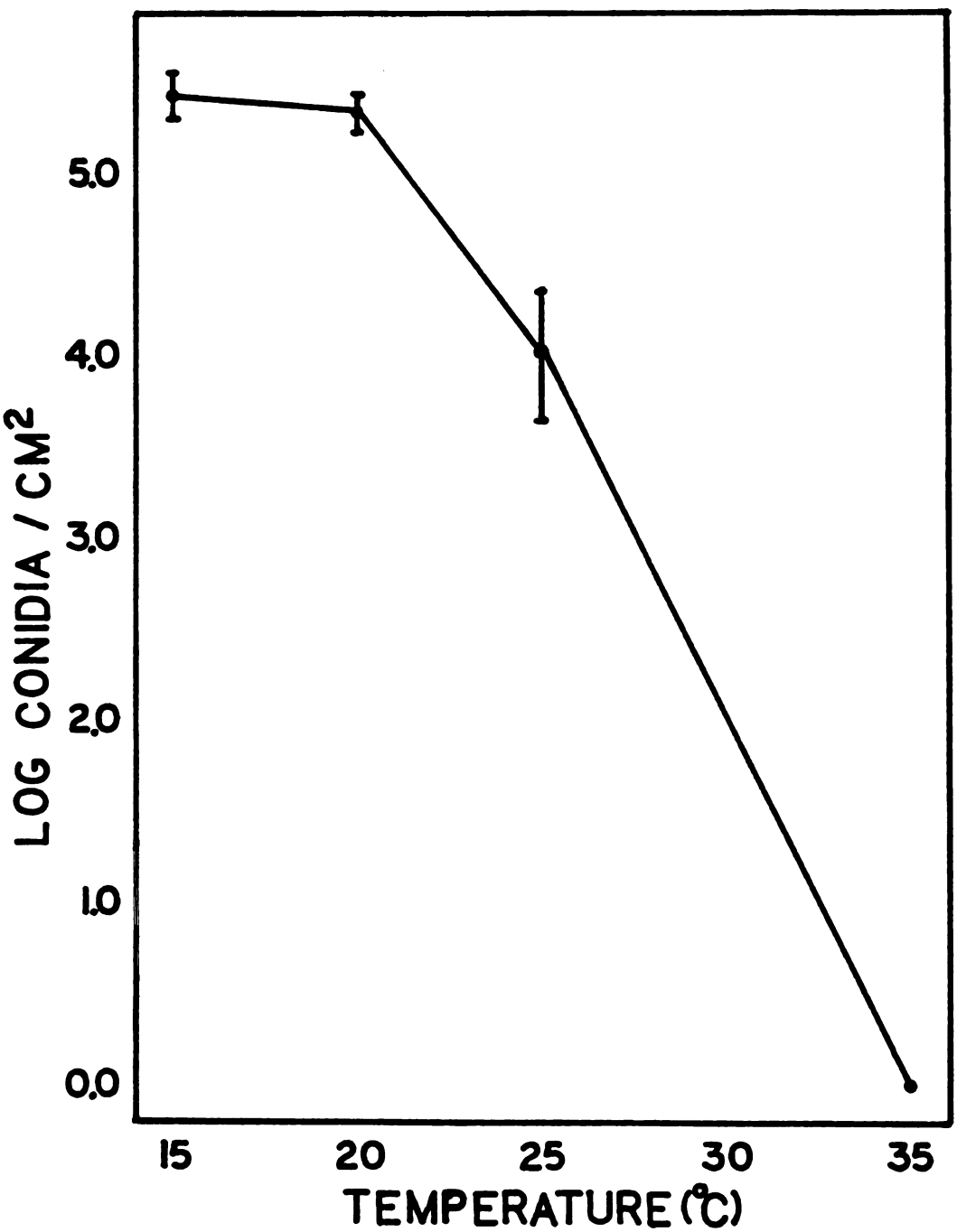

Figure 5.4. Numbers of conidia of B. squamosa collected from onion leaf segments after 4 days incubation on water agar at various temperatures.

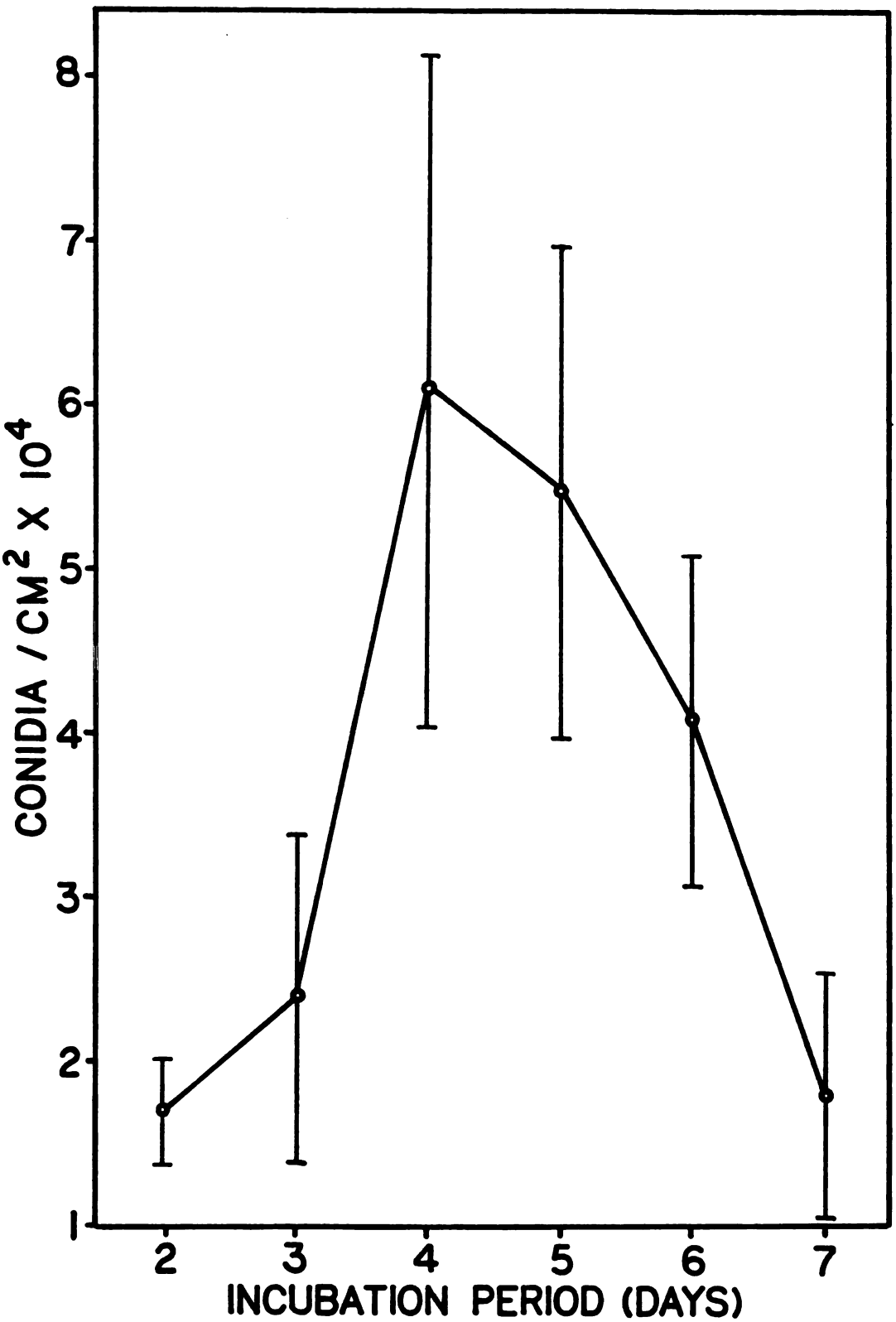

Figure 5.5. Numbers of conidia collected after 1-6 days from B. squamosa-colonized onion leaf segments incubated at 20 C.

declined during the period from day 3 to day 6. Additional leaf segments contained on separate plates, from which conidia were not collected until after 6 days, yielded numbers of spores per cm^2 similar to the sum of conidia daily collected over days 2-6 (7.8×10^5 vs. a sum of 7.0×10^5).

Interruption duration studies revealed that for each 4 hour time period added to the dry duration, conidial numbers were reduced by about half (Figure 5.6). Sporulation with a 16 hour dry period within each 24 hour period resulted in relatively few conidia per cm^2. Similar observations were reported in spore trap studies in field onions (Lacy and Pontius, 1983)

Sporulation of B. squamosa from onion leaf segments placed on water agar adjusted to various water potentials revealed greatest sporulation at 20 C, less at 25 C, and almost none at 30 C. At 20 and 25 C growth declined from -10 through -95 bars (Figure 5.7).

DISCUSSION

Growth and development of B. squamosa was favored at temperatures of 15 or 20 C, but was much less at 30 C or above. In the Northeastern United States, where leaf blight is important, prolonged periods above 30 C are not common, especially during evening hours, so high (30 C) temperatures may not normally be a limiting factor in leaf blight development in this part of the country.

Growth of B. squamosa was not evident at water potentials below -95 to -100 bars within a 4 day period, indicating that growth of B. squamosa is sensitive to moisture stresses which could be common in the aerial environment of the plant. Leaf wetness or very high humidities

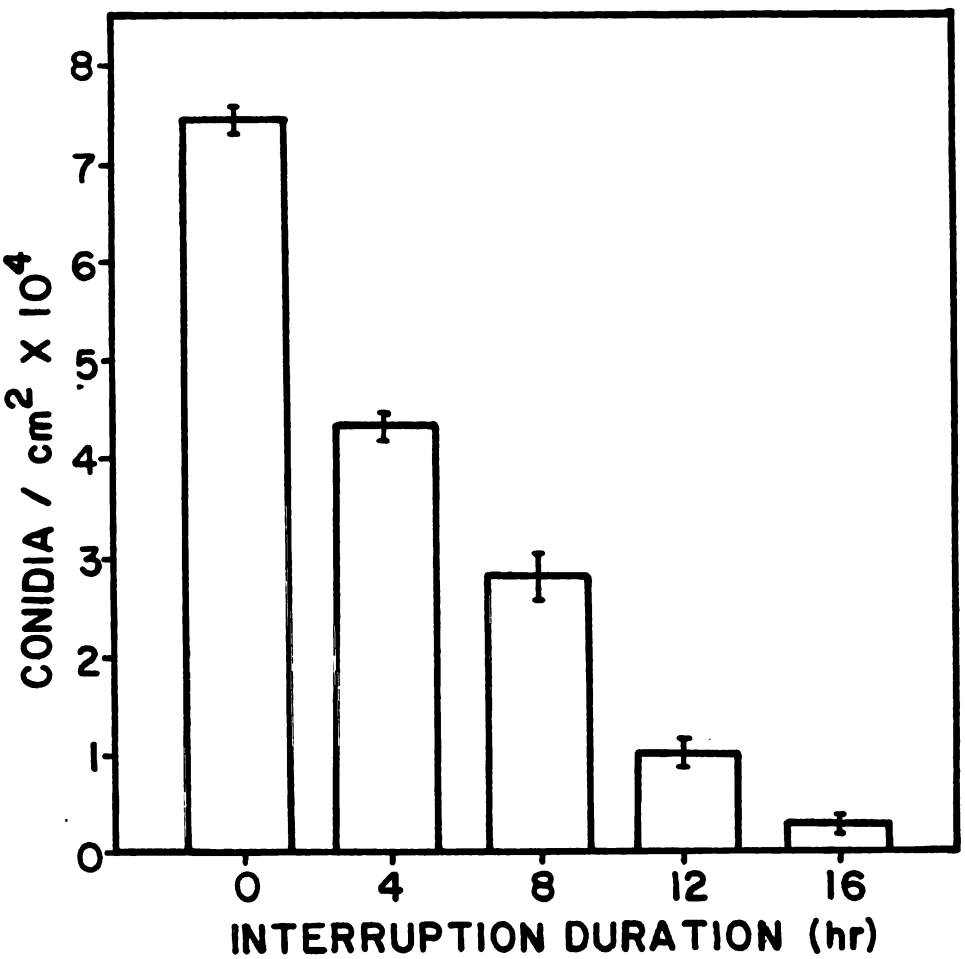

Figure 5.6. Influence of interruption duration within each 24 hour period on sporulation of B. squamosa from colonized leaf segments. Means and standard deviations were calculated from mean daily conidia/cm^2 leaf area and measurements were made over 5 days.

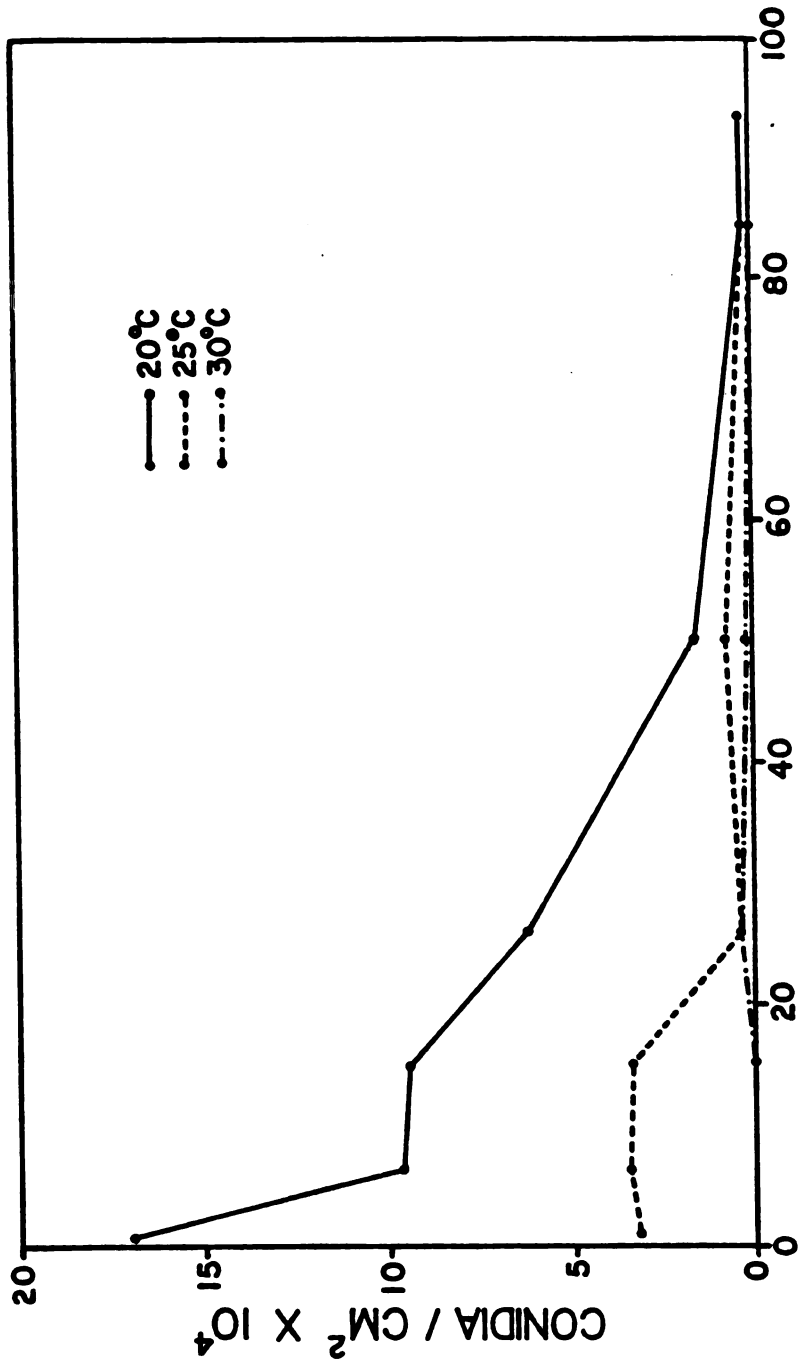

Figure 5.7. Sporulation of B. squamosa at 20, 25, or 30 C from colonized onion leaf segments placed on water agar adjusted to various water potentials with KCl.

would be necessary to supply moisture in senescing or necrotic leaf tissues for growth of B. squamosa. In addition, moist soil, especially in combination with a dense canopy of onion leaves to retain high humidities would be highly favorable for B. squamosa growing in leaves on or near the soil surface.

Sporulation of B. squamosa was evident within 2 days when dried, colonized leaves were incubated under moist conditions. Sporulation from leaves colonized for 3 days, dried, then incubated under moist conditions for two days was similar to sporulation following a 5 day period when leaves were inoculated and maintained under continually moist conditions, suggesting that a drying period did not cause any delay or reduction in sporulation. Field observations of B. squamosa indicated that large numbers of conidia are produced after 3 days of favorable environmental conditions (Lacy and Pontius, 1983). It is probable that much of this sporulation arose through previously colonized tissues.

Survival of B. squamosa in desiccated leaf tissue segments may have important implications in understanding leaf blight epidemiology. Previous studies have demonstrated that long term survival is by means of sclerotia (Ellerbrock and Lorbeer, 1977a; Walker, 1926) and conidial survival is short term (Ellerbrock and Lorbeer, 1977a). Conidia of B. squamosa were collected from overwintered cull onions by Ellerbrock and Lorbeer (1977b) and were observed by McDonald (1981). Survival of B. squamosa as mycelia within leaf tissues during a growing season may occur, as evidenced in this study, for at least a number of days.

Field observations of B. squamosa revealed that sporulation aften occurs as a large spore release, followed by smaller releases (Lacy and

In my study, when infected leaf segments were placed on water agar, abundant conidial production was observed after 2 or 3 days, followed by lesser production through 6 days.

Fluctuating wet-dry conditions greatly influenced the intensity of spore release. Amount of sporulation was related to duration of moisture period (or dry period). Thus, as observed under field conditions (Lacy and Paontius, 1983), continually moist conditions are most favorable for spore release, although fluctuating conditions with long moist periods will support lesser sporulation. Since conditions required for infection also require prolonged moist conditions, B. squamosa appears to be well adapted for conidial release under the environmental conditions most favorable for infection and continued leaf blight development.

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CHAPTER VI

FIELD OBSERVATIONS CONCERNING SPREAD OF BOTRYTIS LEAF BLIGHT

INTRODUCTION

Botrytis leaf blight epidemics have been associated with periods of prolonged moist conditions and moderate temperatures (Segall and Newhall, 1960; McLean, 1960; Small, 1970; Swanton, 1977). Spore production was promoted by leaf wetness periods >13 hours, 14-20 C temperatures, and leaf dieback, but was restricted by leaf wetness periods ≤12 hours and temperatures ≤12 C (Sutton et al., 1978). Large spore releases were preceded by a 2 to 3 day period of 12-20 C temperatures and vapor pressure deficits of 0-5 mb (air close to saturation) (Lacy and Pontius, 1983).

McDonald (1981) observed sporulation of B. squamosa under conditions of high relative humidity (RH) on necrotic leaves on moist soil. Small (1971) and Swanton (1977) also reported that sporulation of B. squamosa occurred only on necrotic or senescent tissues and that the latent period was shorter when necrotic tissues were present on plants. A diurnal periodicity in spore release was reported by Lorbeer (1966), Small (1970), Sutton et al. (1978), and Lacy and Pontius (1983).

Data relating spore release in the field to lesion numbers were published by Small (1970) and Sutton et al. (1978). Small (1970) reported that the logit of lesions per plant increased linearly with

the log of cumulative spores. He implied that a threshold value of 1,700 cumulative spores were needed before appreciable disease resulted.

The purpose of this study was to investigate the behavior of B. squamosa in an onion field plot at the M. S. U. Muck Farm, which would compliment aerobiology studies also being conducted at this location.

MATERIALS AND METHODS

Microscopic Examination of Lesions

Since lesions on onion can be induced by other fungi, such as Alternaria, lesions were examined for the presence of B. squamosa or other fungi. During August, 1980, onion leaves were sampled from an onion field plot at the M. S. U. Muck Farm. Fifty-five small and 59 large lesions were excised, stained in cotton blue in lactophenol, and examined under a light microscope for the presence of Botrytis and other fungi. Botrytis squamosa was identified by characteristics and morphology of the conidium, and to a lesser extent, infection hyphae.

Lesion Counts

The number of lesions on plants within a field plot at the M. S. U. Muck Farm was counted on 50 randomly selected plants on 7 occasions between August 11 and September 8, 1981. Conidial populations of B. squamosa in the air were monitored using a Burkard 7 day recording spore trap (Burkard Mfg. Co. Ltd., Rickmanswirth, Hertfordshire, England) (Lacy and Pontius, 1983).

Development of a Leaf Blight Epidemic

The progress of a leaf blight epidemic was followed at 6 sites along each of 38 rows in a field plot at the M. S. U. Muck Farm on August 11 and August 20. Plants at each site were rated visually using a scale of 0-5 where 0 represented no leaf spotting and 5 represented very severe leaf dieback; 1, 2, 3, or 4 represented trace, slight, moderate, and severe spotting and dieback, respectively.

RESULTS

Microscopic Examination of Lesions

In microscopic observations of Botrytis leaf blight lesions B. squamosa was observed within 22-42% of the lesions (Table 6.1). B. squamosa identification was based on infection hyphal diameter and conidial shape and size. Additional fungal genera associated with lesions included Alternaria and Stemphylium. These fungi were identified based on conidial morphology. Lesions containing infection hyphae with no associated spores were catagorized as unknown. Fungi were not observed in about half of the lesions examined.

Lesion Counts

Lesion numbers on August 11 were about 43 per plant (Table 6.2). Lesion numbers were not observed to increase between August 11 and August 31. Spore releases were very low during this period. Increases in lesion numbers closely followed large spore releases on September 2 and September 8 (Table 6.2).

Table 6.1. Percentage of various fungi observed within small and large lesions collected from onions grown at the M. S. U. Muck Farm during 1981.

Lesion type[a]	Total observed	% of lesions in which indicated fungi were observed				
		Botrytis squamosa	Alternaria spp.	Stemphylium spp.	Unidentified others	none
Small	55	22	18	4	51	5
Large	59	42	15	0	45	0

[a]Lesions were grouped as small (<2mm diameter) or large (≥2 mm diameter).

Table 6.2. Lesions per plant and cumulative spores trapped in an onion field plot at the M. S. U. Muck Farm during 1981.

Date	Lesions per plant	Cumulative spores trapped per 0.6 M^3 of air
August 11	43[a]	0.5×10^2
17	22	4.2×10^2
24	22	8.6×10^2
31	23	3.8×10^3
September 2	32	3.3×10^4
4	61	7.7×10^4
8	500	1.3×10^5

[a]Mean derived from 50 plants

Development of a Leaf Blight Epidemic

The progress of a leaf blight epidemic was monitored on August 11 (Figure 6.1) and August 20 (Figure 6.2) by means of visual ratings. On August 11 leaf spotting was observed throughout the plot, although it was most severe at the west end. On August 20, leaf blighting was more extensive. Greatest severity was observed in the west half of the plot and disease appeared to have increased uniformly across the remainder of the plot.

DISCUSSION

Examination of lesions from onions grown at the M. S. U. Muck Farm revealed that B. squamosa was the predominant organism associated with typical leaf blight lesions. This confirms reports of Hickman and Ashworth (1943), Viennot-Bourgin (1953), Page (1955), and Hancock and Lorbeer (1963) that B. squamosa is the predominant organism associated with leaf blight.

Although Alternaria porri has been associated with leaf spotting (Nolla, 1927; Skiles, 1953; Bock, 1964), A. porri was not observed in lesions collected at the Muck Farm during 1981. The Alternaria observed in lesions for the Muck Farm onions resembled A. tenuis and it was probably associated with dead leaf debris. Observations of dead onion leaves collected at the M. S. U. Muck Farm during 1980 and incubated in a moist chamber yielded abundant sporulation of A. tenuis.

The observation of many "sterile" lesions is also similar to observations of Yarwood (1938) and Hickman and Ashworth (1943). Some of the lesions may have been caused by other injuries (eg. chemical spray injuries). It is also possible that lesions were induced by

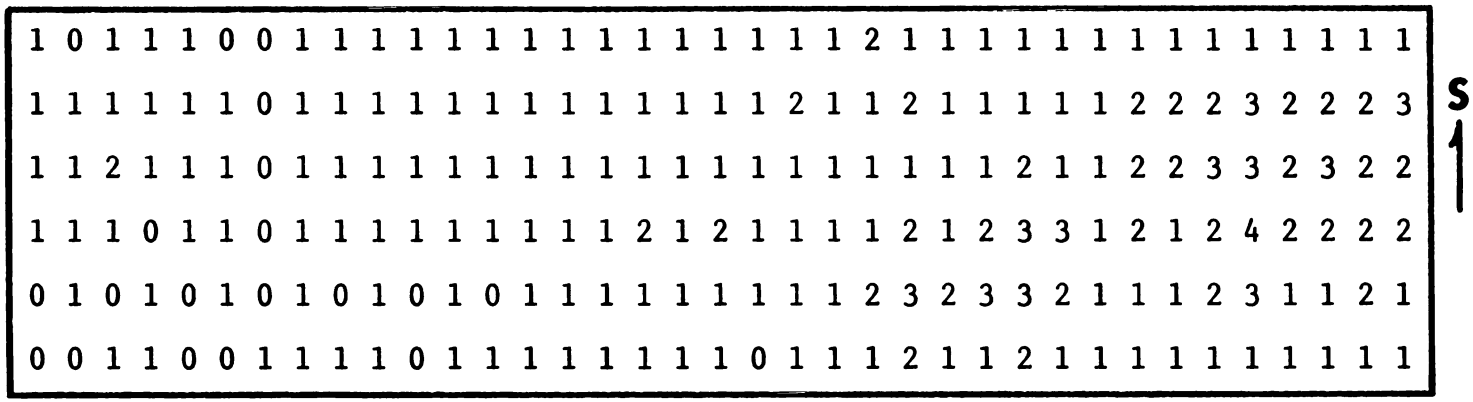

Figure 6.1. Severity of Botrytis leaf blight at 228 sites within a 50 X 200 foot field plot at the M.S.U. Muck Farm on August 11, 1980. Disease severity was rated using a visual rating scale of 0-5 where 0 = no lesions and 5 = severe blighting.

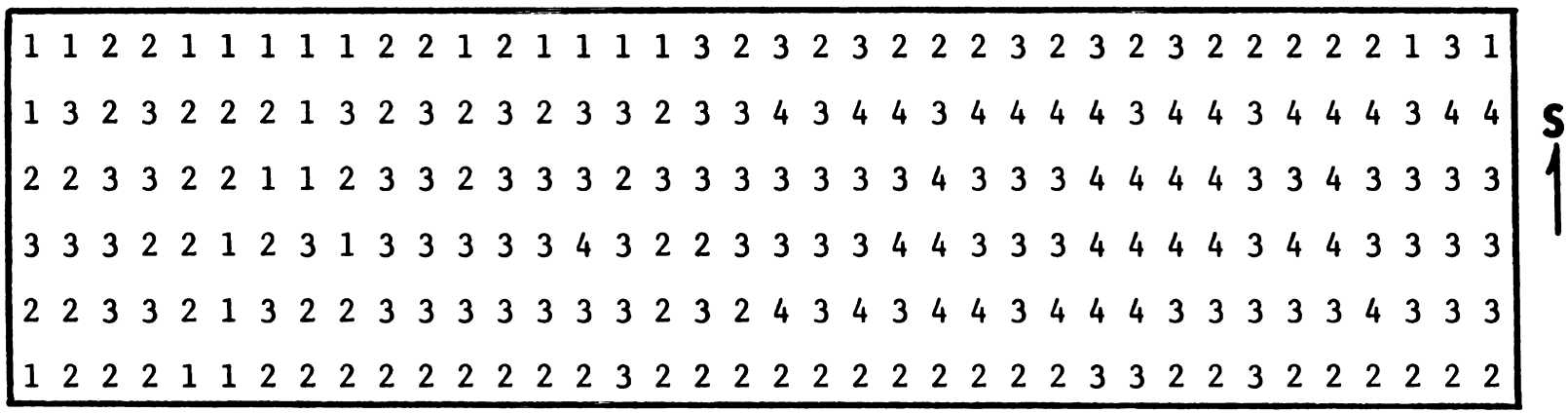

Figure 6.2. Severity of Botrytis leaf blight at 228 sites within a 50 X 200 foot field plot at the M.S.U. Muck Farm on August 20, 1980. Disease severity was rated using a visual rating scale of 0-5 where 0 = no lesions and 5 = severe blighting.

penetration of B. squamosa and then spores were dislodged from the leaf surface before infection hyphae became established.

Numbers of lesions per plant relative to cumulative spores suggests that large spore releases are required to induce detectable differences in lesion numbers. Similar observations were published by Swanton (1977) and Small (1970).

On August 11 lesions were present on the older foliage, but these leaves suffered natural senescence and death by August 17. Thus, reductions in lesions per plant were observed from August 11 through August 17. Although additional lesions probably occurred on August 24 and August 21, senescence of older leaves continued and therefore lesions per plant remained relatively constant. Lesions were observed on younger foliage on September 2, 4, and 8 when conditions were favorable for disease development.

The relationship between spore release, lesion numbers, leaf area and dead leaf area has received little attention, yet this information is essential for a complete understanding of leaf blight epidemiology, especially from a modeling standpoint. This is an area where future research is sorely needed.

A field plot at the M. S. U. Muck Farm revealed a fairly uniform progression of leaf blight development. The west end of the plot was infected first but the disease progressed uniformly across the remainder of the field. A uniform progression of leaf blight within a field plot was also observed by Small (1971). Thus, B. squamosa can apparently move rapidly through a field of onions. Under favorable conditions for disease the rapid spread could result in blighting in large areas of

onion fields. Explosive outbreaks of Botrytis leaf blight were reported by Page (1953), McLean and Sleeth (1959), and Small (1971).

Application of information gained through field and laboratory studies of B. squamosa are presented in Appendices A-D.

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CHAPTER VII

INFLUENCE OF TEMPERATURE AND WATER RELATIONS ON GROWTH OF BOTRYTIS ALLII

INTRODUCTION

Botrytis neck rot, caused by Botrytis allii Munn, is an important storage disease of onion. The fungus has been reported to infect bulbs prior to harvest through dead leaf tissues near the bulb neck or through wounds in living neck tissues resulting from mechanical harvesting (Munn, 1917; Walker, 1926). Succulent neck tissues are especially susceptible to infection after wounding (Kaufman and Lorbeer, 1967). The fungus is not believed to spread in significant amounts among bulbs in storage unless storage conditions are very moist (Munn, 1917; Walker, 1926).

Studies addressing measures of control have firmly established that artificial curing of onions with heated air significantly reduces neck rot incidence in storage (Walker, 1919; Newhall et al., 1959; Wallace and Hickman, 1955; Rosberg and Johnson, 1959; Vaughan et al., 1964; Harrow and Harris, 1969). Applications of fungicidal sprays or chemical desiccants (Kaufman and Lorbeer, 1967) have not been as successful as artificial curing in controlling neck rot.

Few studies of which I am aware have examined environmental influences on growth of B. allii. The optimal temperature for neck rot development was reported to be 20 C (Munn, 1917), but details of rates

of development at various temperatures were not recorded. Studies have also addressed the influence of moisture in neck rot development (Harrow and Harris, 1959; Walker, 1926), but none of these studies quantified fungal responses in terms of water potentials of the tissues in which B. allii was growing. Thus the influence of temperature and water potential on growth of B. allii on onion tissues is not clearly understood.

MATERIALS AND METHODS

Influence of Temperature on Growth of B. allii

Prior to temperature-water potential interaction studies the effect of temperature on growth of B. allii was examined. A 4-mm-diameter plug from the advancing margin of a 72-hour-old culture of B. allii was transferred to each of 4 replicate prune-lactose-yeast extract (PLY) agar plates. The plates were incubated at 5, 10, 15, 20, 25, 30, or 35 C. Radial growth was measured daily for 4 days then growth rates were calculated.

Influence of Temperature and Water Potential on Growth Rates on PLY Agar

Prune extract-lactose-yeast agar (PLY) as described by Talboys (1960) but with 20 grams of agar instead of 30 grams, was used as a basal medium. The osmotic potentials of the basal medium were adjusted using various concentrations of KCl, NaCl, sucrose, or polyethylene glycol (PEG) 8000. Measurements of water potentials were made using a Wescor dewpoint hygrometer (Wescor, Inc., Logan, Utah 84312). Four-mm-diameter plugs from the advancing margin of a 72-hour-old culture of B. allii were transferred to the center of PLY agar plates (1 plug/plate).

Four replicate plates per treatment were then incubated at 20, 25, or 30 C. Colony diameters were measured over 4 days and growth rates were calculated over days 2-4 since growth was linear during this time. For each osmoticum the experiment was repeated twice.

Influence of Temperature and Water Potential on Dry Weight in PLY Broth

Flasks containing 50 ml of PLY broth adjusted to various water potentials with KCl, sucrose, NaCl, or PEG 8000 were each inoculated with three 4-mm-diameter plugs from the advancing margin of 72-hour-old cultures of B. allii. Flasks were incubated in still culture at 20, 25, or 30 C for 4 days. Dry weights determined by collecting the contents of each flask on separate 7-cm-diameter preweighed glass microfiber filter papers (Whatman Ltd., England). Each filter was rinsed with 1000-1500 ml water to ensure complete removal of media and osmotica, then air dried in a forced air drier at 60 C until constant weight was obtained.

Influence of Temperature and Water Potential on Growth in Onion Leaves

Water potentials of sterilized (autoclaved for 85 minutes, 20 p.s.i.) green onion leaves were adjusted by positioning leaves over distilled water in sealed chambers, containing various concentrations of NaCl. Seven days were allowed for equilibration prior to inoculation of each leaf piece with a 4-mm-diameter plug from the advancing margin of a 3-day-old culture of B. allii. Radial growth on leaves was determined after 3 days incubation at 20, 25, or 30 C. Water potentials of the leaves were determined using a Wescor dewpoint hygrometer.

Influence of Inoculum Concentration on Lesion Development

The influence of inoculum concentration on lesion expansion was examined . A sterile 4-mm-diameter cork borer was used to remove a small plug of tissue from the outer 1-2 layer of healthy onion bulb scales. A 10 ul drop from a conidial suspension (10^1, 10^2, 10^3, 10^4, 10^5, or 10^6conidia per ml) was placed in each well, then the hole was covered with label tape to prevent excessive drying. Bulbs (5 per treatment) were incubated at 20 C. Lesion sizes were measured after 9 days.

Influence of Temperature on Rate of Disease Development

Onion bulbs were inoculated by placing 10 ul of a conidial suspension (10^3 conidia/ml) into a 5 mm wide by 3-4 mm deep well in each of 80 onion bulbs. Twenty control bulbs received water. Bulbs were incubated at 5, 10, 15, 20, or 25 C, and 5 replicate bulbs were sampled from each temperature after 1, 3, 6, and 9 weeks.

Effect of Prolonged High Temperature Exposure on Survival of B. allii

Curing practices for onion often involve placing the onions in heated air at 35-37 C for several days. The effect of prolonged high temperature exposures, coupled with desiccation of the tissues, on survival of B. allii was examined.

Onion leaves were inoculated with a 4-mm-diameter core from the advancing margin of a 72-hour-old culture of B. allii. After 3-4 days the leaves were removed and cut into 5-mm-long segments. The segments were air dried under a laminar flow hood for 2 hours, then were placed in sealed chambers over saturated NaCl solutions (25 segments per jar). After 3, 6, 9, or 12 days at 32 or 37 C, leaf pieces were placed on PLY. Plates were examined daily over 4 days for growth of B. allii.

In a separate experiment onion bulbs were inoculated by removing a 4-mm-diameter by 5 mm deep plug from the onion bulb and replacing it with a similar size plug from the advancing margin of a 72-hour-old B. allii colony on PLY agar. Bulbs were incubated at 20-25 C for 5 days. The perimeter of the infection zone was outlined using a felt tip pen, then bulbs were placed in an incubator at 32 or 37 C. After 3, 6, or 9 days 20 bulbs in each of 5 replicate sets were removed from each temperature treatment. Bulbs were incubated for an additional 10 days at 20-25 C then were examined for neck rot development.

In an additional experiment the influence of temperature on survival of B. allii in agar plugs was examined. Four-mm-diameter plugs from 7-day-old cultures of B. allii grown on PLY were placed on PLY (5 plugs per plate). The plates were placed in an incubator at 30, 40, 50, 60, or 70 C. After 24, 48, or 72 hours, 5 plates were removed and incubated at 20 C for 48-72 hours. The number of plugs yielding B. allii was recorded.

RESULTS

Influence of Temperature on Growth of B. allii

Growth of B. allii on PLY agar increased from 5 through 20 C, then declined through 35 C (Figure 7.1). At each temperature growth over days 2-4 was linear.

Influence of Temperature and Water Potential on Growth Rate on PLY Agar

Significant temperature-water potential interactions were observed for B. allii on agar adjusted to various water potentials with KCL, NaCl, sucrose, or PEG 8000 (Figure 7.2a-d). In the presence of KCL or sucrose at 20 or 25 C, growth was optimal at -5 to -10 bars, while

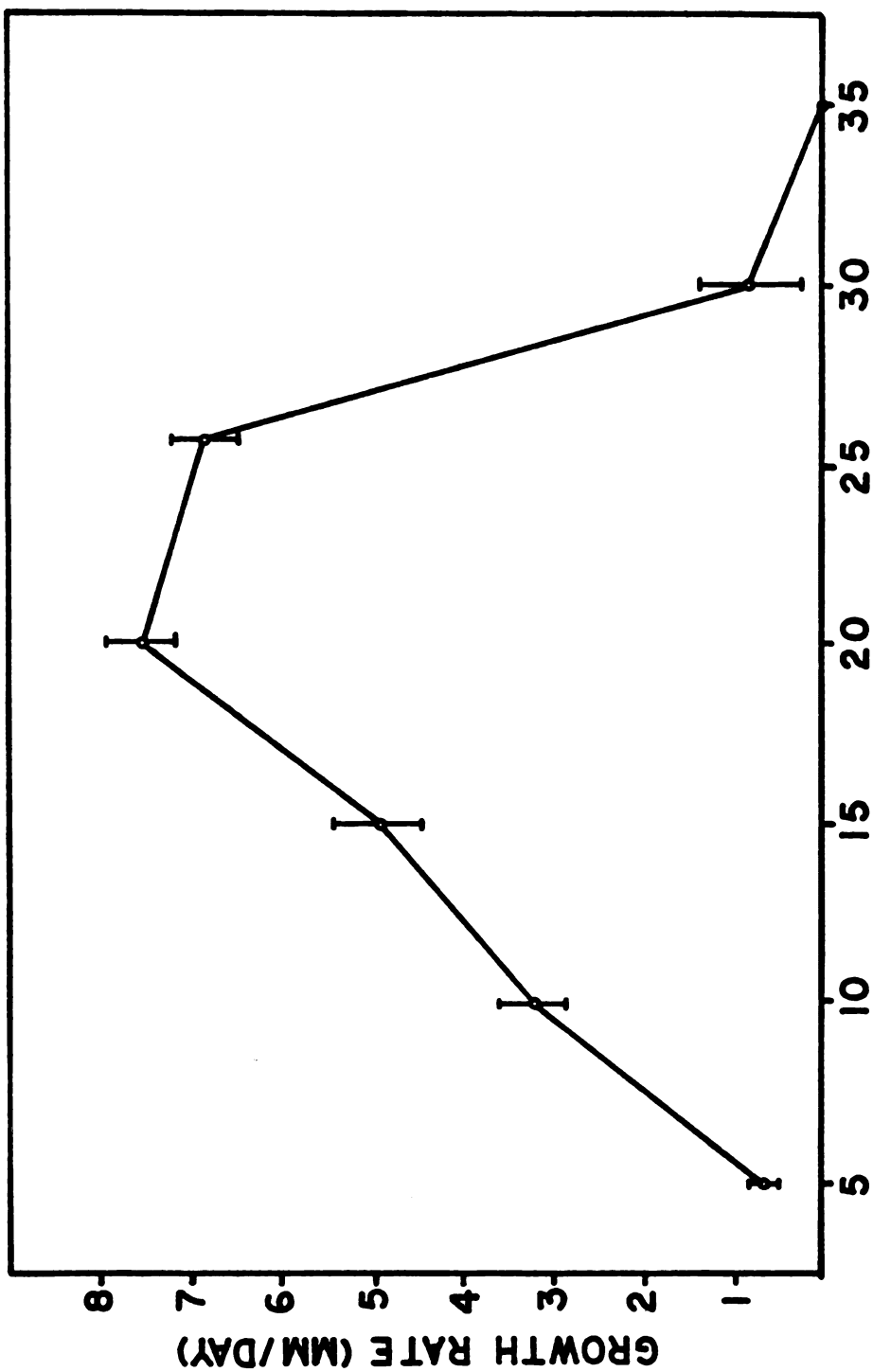

Figure 7.1. Growth of Botrytis allii on PLY agar at various temperatures.

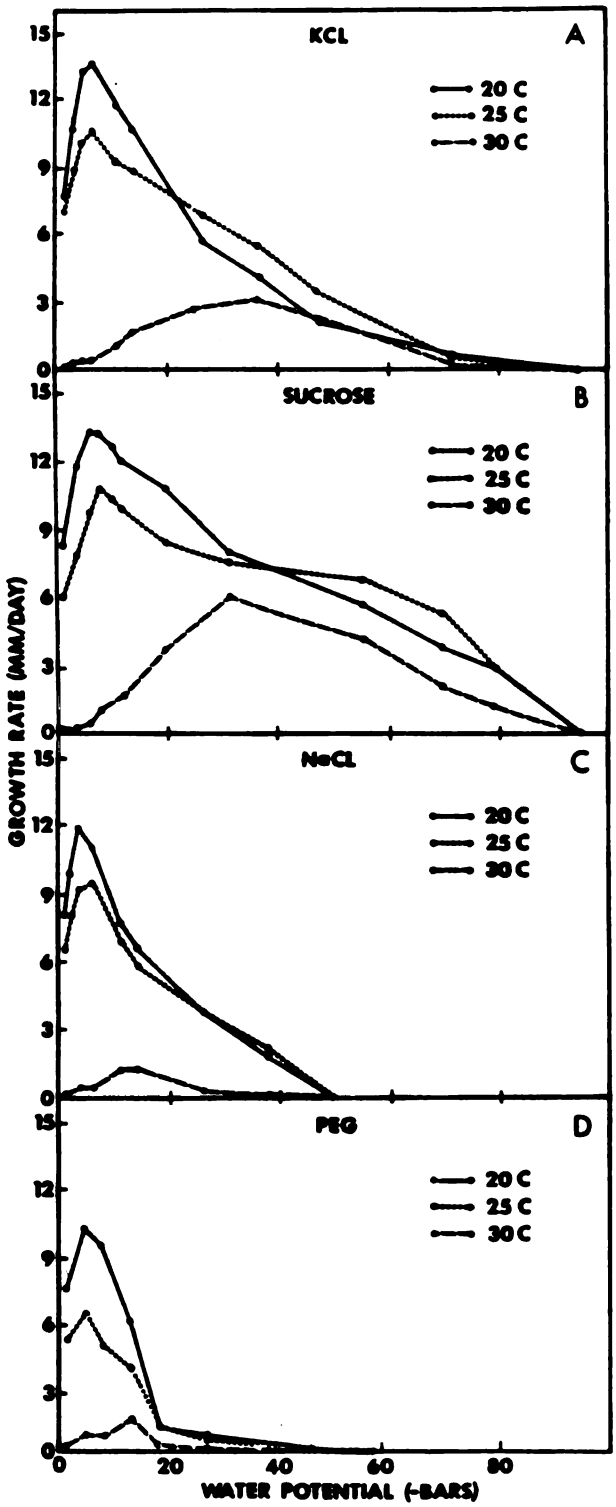

Figure 7.2. Growth of B. allii at 20, 25, and 30 C on PLY medium adjusted to various water potentials with KCl, NaCl, sucrose and PEG 8000.

at 30 C maximum growth occurred at -30 to -40 bars; growth was halted at -95 to -100 bars. In the presence of NaCl or PEG 8000 at 20 or 25 C, growth was optimal at -10 to -15 bars, while at 30 C growth rates were optimal at -10 to -15 bars; growth was halted at -50 to -60 bars. The optimal water potential for growth was observed to shift from higher to lower potentials with increasing temperature.

Influence of Temperature and Water Potential on Dry Weight in PLY Broth

Dry weights of B. allii grown in PLY broth at various water potentials adjusted with KCl, sucrose, NaCl, or PEG 8000 generally corresponded to growth rates on PLY medium. Greatest dry weights were produced at -5 to -10 bars (Figure 7.3a-d). In the presence of KCl or sucrose at 20 or 25 C growth ceased at -90 to -95 bars, while in the presence of NaCl or PEG 8000 at 20 or 25 C growth ceased at -30 to -45 bars.

Influence of Temperature and Water Potential on Growth in Onion Leaves

Growth of B. allii in onion leaves was similar at 20 and 25 C and very low at 30 C over a 3 day period (Figure 7.4). At 20 or 25 C growth decreased linearly with decreasing potentials from -30 to -95 bars. At 30 C B. allii failed to grow at potentials of 0 to -5 bars; some growth occurred between -5 and -55 bars and ceased near -75 bars.

Influence of Inoculum Concentration on Lesion Development

Lesion diameters on onion bulbs increased linearly with logarithmic increases in spore concentration. Mean lesion diameters on onion bulbs inoculated with 10 ul of conidial suspension (0, 10^2, 10^3, 10^4, 10^5, or 10^6 conidia per ml) were 0.0, 0.2, 0.43, 0.62, 0.92, and 1.17-cm-diameter, respectively, after 9 days (Figure 7.5).

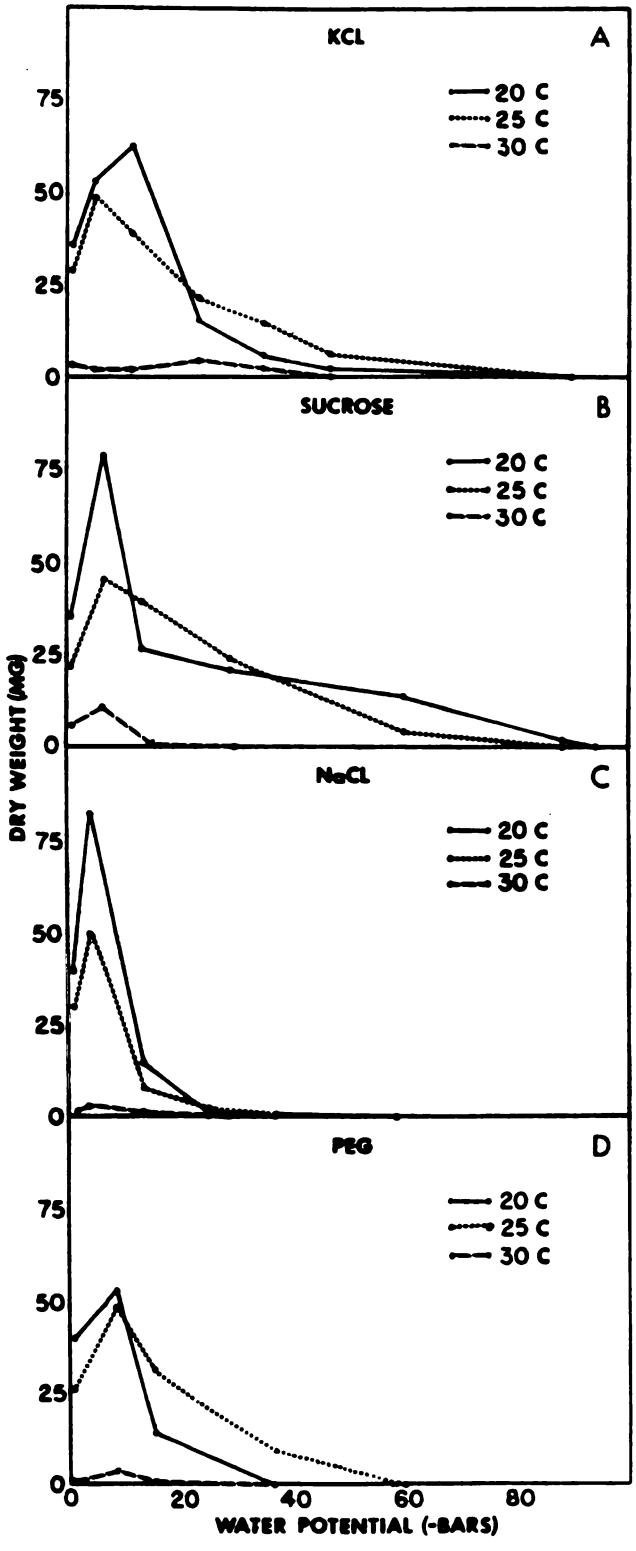

Figure 7.3. Dry weight of B. allii at 20, 25, and 30 C after 4 days in PLY broth, adjusted to various water potentials with KCl, NaCl, sucrose, and PEG 8000.

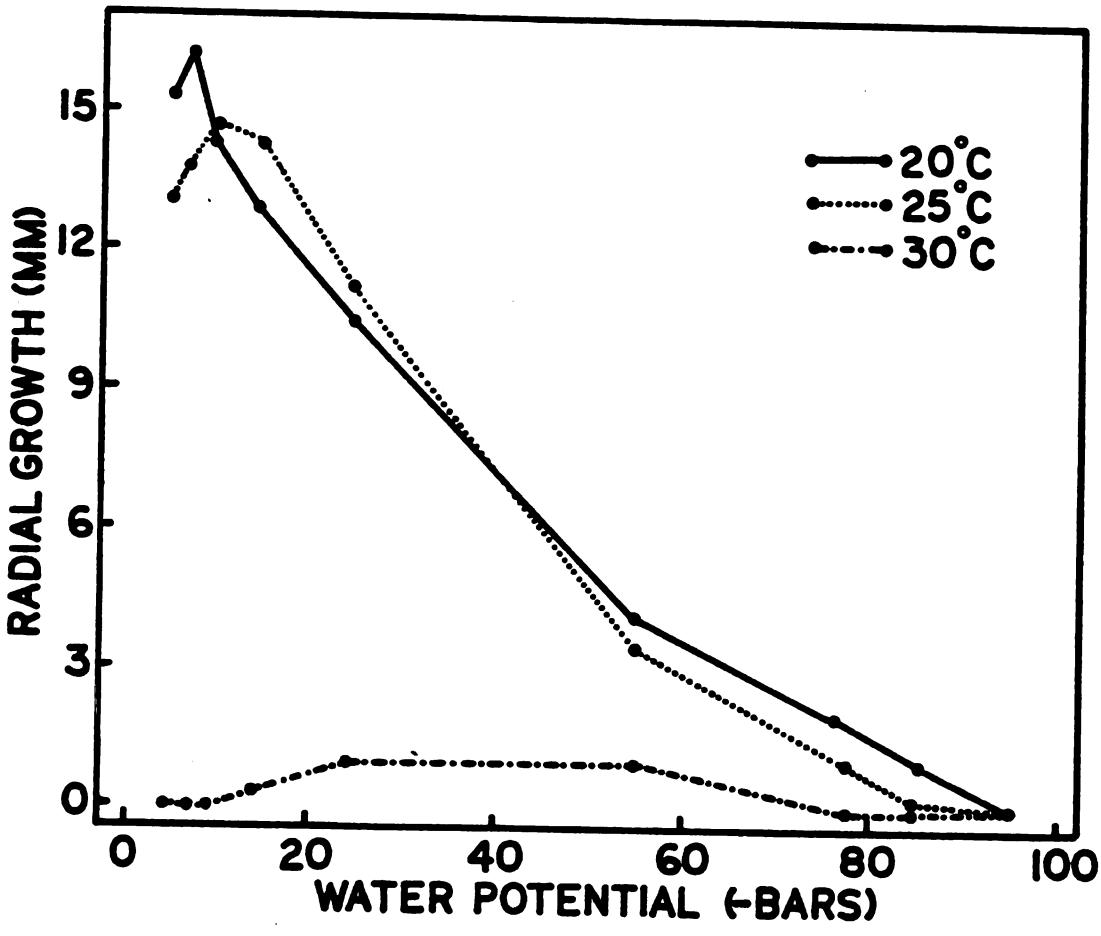

Figure 7.4. Radial growth of B. allii in onion leaves at 20,25, and 30 C at various water potentials.

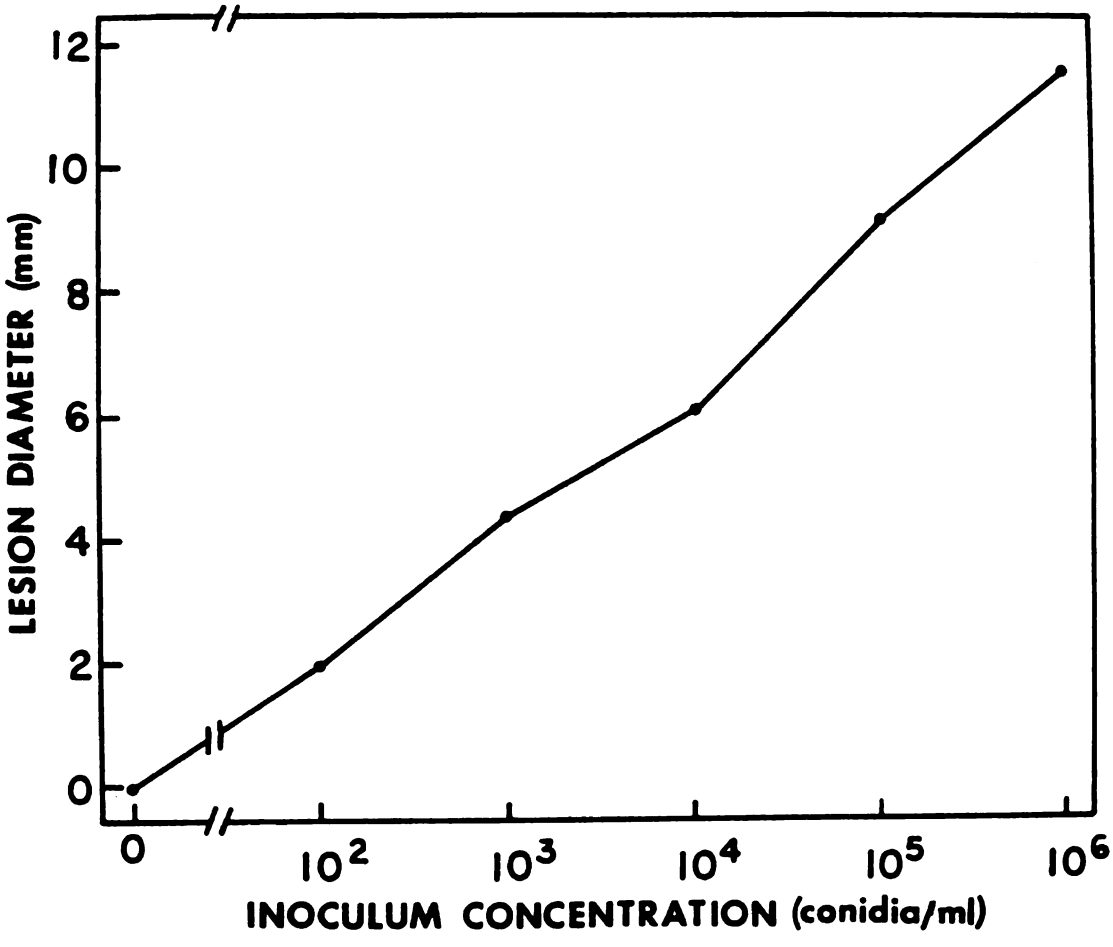

Figure 7.5. Lesion diameters in onion bulbs inoculated with various concentrations of B. allii conidia, after 9 days at 20 C.

Influence of Temperature on Rate of Disease Development

Growth of B. allii in onion bulbs increased linearly through time at each temperature (Figure 7.6). Most rapid development was observed at 20 C. Rate of lesion expansion was much greater within a bulb scale than between scales. After 3 weeks lesions were 4-8 cm in diameter, yet the fungus only penetrated 1-2 bulb scales deep. After 6 weeks, the lesions were 8-12 cm in diameter and sclae to scale penetrations were more evident near the neck of the bulbs. After 9 weeks many of the bulbs were completely decayed.

Effect of High Temperature on Survival of B. allii

Survival of B. allii in leaf segments was greater at 32 C than at 37 C (Figure 7.7). At 37 C viability of B. allii declined after 3 days and the fungus was not recovered after 6 days. At 32 C viability declined after 9 days. B. allii was not recovered from onion bulbs incubated at 37 C for 6, 9, or 15 days or from bulbs incubated at 32 C for 15 days. B. allii was recovered from about 5% of the bulbs incubated at 32 C for 3, 6, 9, or 12 days. At temperatures of 40 or 50 C viability of B. allii in agar plugs declined as the duration of exposures increased (Figure 7.8).

DISCUSSION

Results of these studies suggest that parameters important in the development of B. allii in onion tissues include temperature, initial inoculum concentration, water potential, and whether or not the infections are symptomless. Understanding the relationship between the parameters is epidemiologically important.

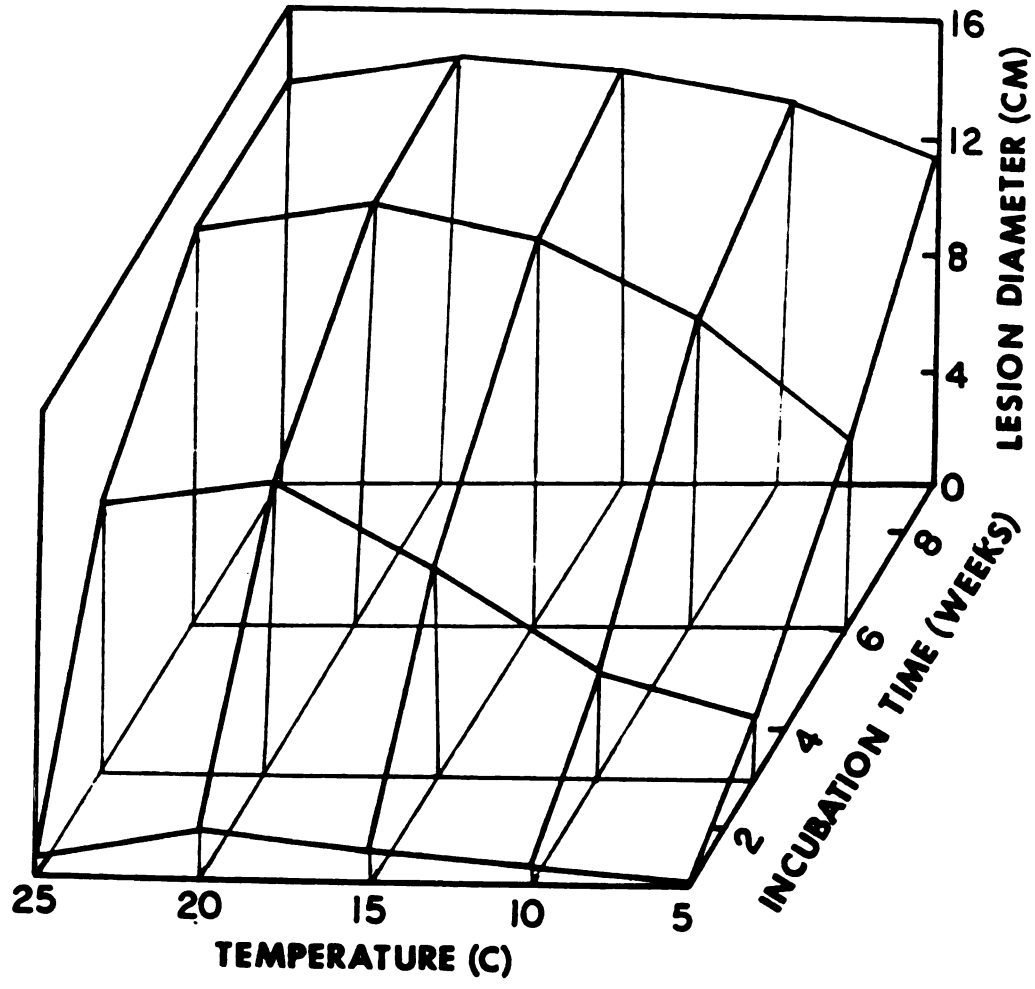

Figure 7.6. Influence of temperature and incubation period on lesion diameter in onion bulbs inoculated with B. allii.

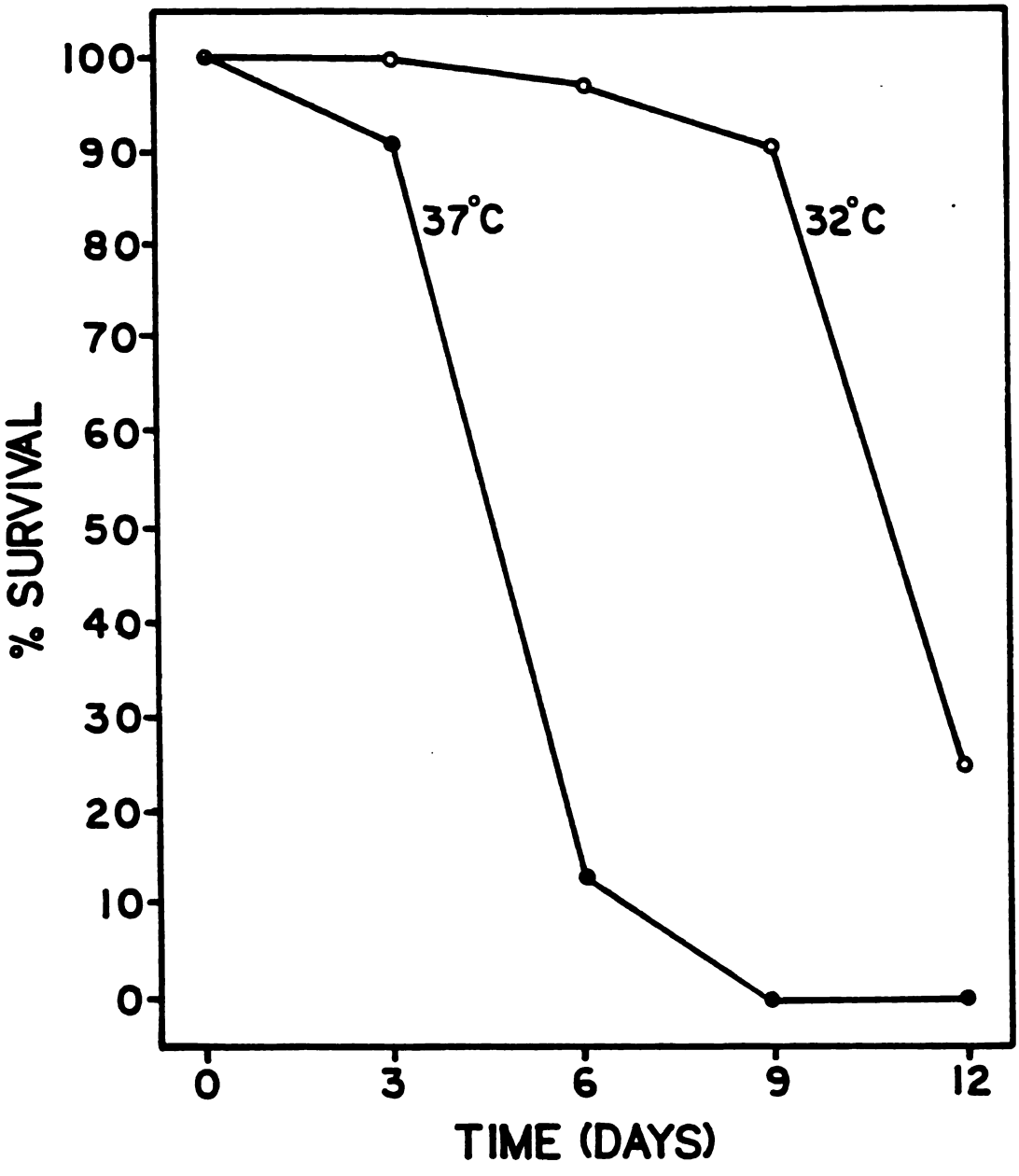

Figure 7.7. Survival of Botrytis allii in onion leaf segments after various incubation durations at 32 or 37 C.

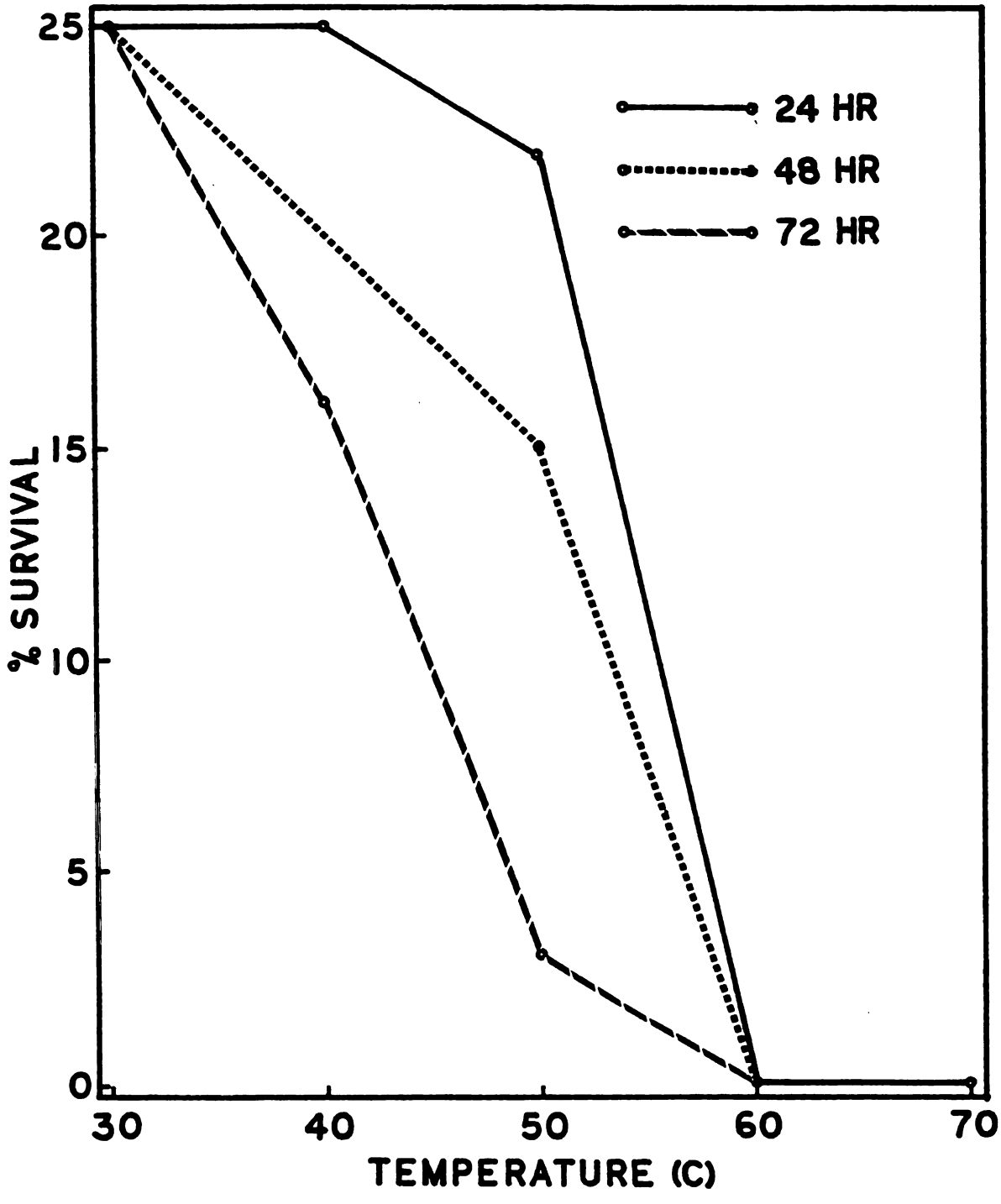

Figure 7.8. Survival of Botrytis allii in agar plugs at various temperatures after 24, 48, or 72 hours.

At temperatures of 5-20 C radial growth of B. allii increased with increasing temperature. At temperatures above 30 C, B. allii was restricted in its development within onion bulbs. Long term exposures to temperatures near 35 C have been shown to be lethal to B. allii (Harrow and Harris, 1969; Vaughan et al., 1964). This has been the basis of high temperature postharvest treatment to control neck rot in storage.

Improper curing of onions, or storage of onions in humid environments has been shown to be favorable for neck rot development (Harrow and Harris, 1969; Munn, 1917; Vaughan et al., 1964). Since water potentials of substrate will tend to equilibrate with surrounding water potentials of the air, the lower the atmospheric potential, the lower the substrate potential of dead onion tissues. Thus under humid storage conditions B. allii would be expected to continue its growth and development. B. allii was found to increase its growth rate at lower substrate water potentials as temperatures increased through 30 C. Similar trends were reported for Fusarium (Cook and Christen, 1976; Manandhar and Bruehl, 1973) and Verticillium (Cook and Christen, 1976). Cook and Christen (1976) suggested that this may play an adaptive role. Such an adaptation would be favorable for growth of B. allii under field conditions where warm day temperatures with high atmospheric potentials (high humidities) were followed by warm night temperatures and dew formation. At 20 C onion leaves equilibrated to atmospheric humidities below 95% probably would restrict the growth of B. allii.

The initial inoculum concentration applied to onion bulbs was found to determine the amount of lesion expansion. Thus, it is possible

that the rate of bulb decay in storage may reflect the degree of infection occurring prior to harvest or the amount of inoculum present at harvest.

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CHAPTER VIII

FIELD OBSERVATIONS CONCERNING *BOTRYTIS ALLII*

INTRODUCTION

Botrytis neck rot, caused by *Botrytis allii*, is a bulb decay disease which generally appears in bulbs in storage. Infections are believed to occur near to or during harvest, through dead leaves near bulbs, or through exposed succulent neck tissues when onions are topped (Munn, 1917; Walker, 1926). Wet conditions are believed to favor the spread and development of *B. allii* under field conditions (Munn, 1917; Maude and Presly, 1977a, 1977b). McKeen (1951) found that Spanish onion seedlings were predisposed to infection by *B. allii* at low soil temperatures.

Maude and Presly (1977a, 1977b) showed that bulbs grown from seed internally infected with *B. allii*, had more neck rot incidence in storage than bulbs from seed treated with benomyl. Neck rot was significantly reduced in storage by the use of benomyl (1 g/ kg seed) prior to planting. This control strategy has not been evaluated with onion seed produced in the United States. It may also be possible to reduce bulb rot from infected seedlings using similar treatments of U. S.- grown seed.

Tichelaar (1967) and Maude and Presly (1977a, 1977b) reported that *B. allii* conidia may spread the disease among field onions by infecting leaves asymptomatotically, then sporulating from dead leaf tips. If symptomless infections result from conidial inoculum in the field, then

fungicidal sprays might reduce such infections on leaves. This in turn could reduce neck rot incidence in storage. Reports by Kaufman et al. (1964) and Kaufman and Lorbeer (1967) revealed that fungicidal sprays applied at or near harvest reduced neck rot incidence in storage. Tichelaar (1967) felt that regular applications of fungicides may keep the inoculum density of B. allii low.

MATERIALS AND METHODS

Seed Treatments for Control of Neck Rot

Seed borne infections could imply that current control strategies for neck rot, which revolve around the harvest period should be reexamined. An experiment was initiated to examine the relationship between seed infestation and fungicides on neck rot incidence in storage. The experiment, outlined in Table 8.1 was designed as a randomized block containing 2 blocks and was conducted at the M. S. U. Muck Farm, Bath, MI, during the summer of 1980. Onion seeds were hand planted on May 9th. Onions were harvested on September 10th. At harvest, bulbs were collected in 10 foot sections of row in each treatment combination, counted and weighed. Bulbs were placed in a greenhouse until the necks were dry, then were stored in a cool (3.5 C) storage room. Bulbs were examined for neck rot in March, 1981.

Botrytis-infested seed remaining from the seed treatment experiment of 1980 were stored at 5 C. During August, 1980, 100 seeds for each treatment were surface-disinfested in 10% bleach for 1-3 minutes, rinsed in sterile water, then placed on PLY agar (25 seeds/plate). Plates were examined after 5-6 days for sporulating colonies of B. allii

During August, 1980, a minimum of 10 dead onion leaves were sampled from seven of the treatments. Leaves were placed in glass petri plates containing moist paper towels. After 3-5 days incubation at 20-25 C leaves were examined for conidiophores and conidia of B. allii, B. cinerea, or B. squamosa.

Effect of Inoculating Onion Leaves With B. allii Conidia on Neck Rot Incidence in Storage.

To asses the importance of foliar infection by B. allii conidia on neck rot incidence in storage, a field experiment was conducted during the summer of 1980, at the M. S. U. Muck Farm. The experiment contained 4 blocks and treatments included spraying either an aqueous suspension of B. allii conidia onto the foliage. Conidial inoculum was produced by growing B. allii on PLY agar at 20 C for 7-12 days. Conidia were suspended in water using Tween 20 (polyoxyethylene sorbitan monolaurate) (1 drop/100 ml water) and then sprayed onto onion foliage on August 13, 1980. At harvest, bulbs were collected in 10 foot sections of row from each treatment combination, then counted and weighed. Bulbs were placed on a greenhouse bench until the necks were dry, then were placed in cool (3.8 C) storage. Bulbs were examined for neck rot in March, 1981.

During 1981, an experiment was set up to examine the timing of inoculation of onion foliage with B. allii conidia on neck rot incidence in storage. Onions were inoculated 0, 4, 8, 12, or 16 weeks after planting. A conidial suspension was also applied to freshly cut necks of an additional group of onions after harvest. Bulbs were air dried in the greenhouse, then placed in cool storage. Bulbs were assessed for neck rot incidence in March, 1982.

During 1982, an experiment was set up at the M. S. U. Muck Farm in which late season fungicide applications were examined as a possible control of B. allii in storage. Onions were planted on May 4. Bravo or Benlate, at two rates, and a combination of the two chemicals, as listed in Table 8.5, were applied on a seven day schedule beginning in August. A conidial suspension of B. allii conidia were applied to onions on a similar schedule. Onions were harvested on September 15 and dried at the Muck Farm in storage racks for 1-2 weeks. Onion bulbs were stored at 3.5 C and examined for neck rot in March, 1983.

Incidence of B. allii in Onion Seed

During 1980, seed lots from Asgrow Seed Co, were examined for B. allii. Seeds were surface disinfected for 1-3 minutes in 0.5% NaClO + 1 drop/100 ml Tween 20, rinsed in sterile water then placed in PLY medium (25 seeds/plate). A total of 300 seeds per variety were examined.

During 1981 a sample of seed was obtained from Dr. James Zalewski (Ore Ida Foods, Inc.) in Oregon. One hundred seeds were placed in 10% bleach for 3, 6, or 9 minutes, rinsed in sterile water, then placed on PLY agar. Onion seed was also obtained from Dr. R. B. Maude in England. These seeds were surface disinfested in 0.5% NaClO for 1-3 minutes then placed on PLY agar.

RESULTS

Seed Treatments for Control of Neck Rot

No significant reductions in bulb weight were evident as a result of preplanting inoculations with B. allii conidia (Table 8.1). Neck

rot was not detected among bulbs in any of the treatments after 5 months storate (Table 8.1).

Table 8.1. Effect of fungicidal seed treatments on bulb weight and neck rot incidence (1980).

Treatment	Rate (g/kg seed)	Botrytis infested	Mean bulb weight (g)	Total # bulbs examined	% Neck rot
Benlate + Arasan	1 1	yes	352	110	0
Benlate + Arasan	1 1	no	320	114	0
Arasan	1	yes	320	107	0
Arasan	1	no	287	101	0
Benlate + Arasan	2 2	yes	364	100	0
Benlate + Arasan	2 2	no	324	88	0
Arasan	2	yes	295	102	0
Arasan	2	no	337	101	0
Nontreated	-	yes	353	106	0
Nontreated	-	no	373	101	0

Botrytis allii was recovered from non-fungicidal-treated and Arasan-treated seed from Asgrow Seed Co. stored at 5 C for 3 months. Recovery of *B. allii* was greater for artificially infested than for noninfested seed. *B. allii* was not recovered from seed treated with benomyl (Table 8.2).

Table 8.2. Effect of artificial infestation and fungicides on recovery of B. allii from onion seed (Asgrow Seed Co., cv. Spartan Banner) after three months storage at 5 C (1980).

Seed treatment	Rate (g/kg seed)	Infested with Botrytis[a]	Mean recovery from seed (%)
Benlate + Arasan	1 + 1	yes	0 ± 0
Benlate + Arasan	1 + 1	no	0 ± 0
Arasan	1	yes	18 ± 2
Arasan	1	no	1 ± 1
Arasan	2	no	1 ± 1
Nontreated	-	yes	23 ± 9
Nontreated	-	no	4 ± 3

[a]Twenty ml of a conidial suspension of B. allii (10^8 conidia/ml) were applied to 60 grams of onion seed, then seeds were air dried.

Botrytis allii, B. cinerea, and B. squamosa all were recovered from dead onion leaves collected from the 1980 neck rot trial. Botrytis leaf blight was present within the neck rot trial and accounts for the relatively high recovery of B. squamosa from the leaves (Table 8.3).

Effect of Foliar Application of B. allii on Neck Rot Incidence in Storage

Foliar inoculations of onion leaves with B. allii conidia two weeks prior to harvest during 1980 greatly enhanced the incidence of neck rot in storage. Fifty percent neck rot was observed in inoculated bulbs compared with 28% in control bulbs. Botrytis allii infections were not evident in either foliage or bulbs at harvest, or when bulbs

were initially placed in storage. Bulb weights at harvest (0.15 lb/bulb inoculated vs. 0.14 lb/bulb control) did not differ significantly as the result of conidial applications to the foliage two weeks prior to harvest.

Table 8.3. Recovery of Botrytis fungi from dead onion leaves collected from the seed treatment experiment at the M.S.U. Muck Farm (1980).

Seed treatment	Botrytis allii infested[a]	Recovery (%)		
		allii	cinerea	squamosa
Benlate + Arasan	yes	1	2	2
Benlate + Arasan	no	0	0	10
Arasan	yes	0	1	7
Arasan	no	2	1	6
Nontreated	yes	0	1	5
Nontreated	no	0	4	5

[a]Twenty ml of a conidial suspension of B. allii (10^8 conidia/ml) were applied to 60 grams of onion seed, then seeds were air dried.

Timing of conidial inoculation of foliage experiments suggested that B. allii infections in bulbs in storage were greatest after foliar inoculations near harvest (16 weeks after planting). Infections were also high after foliar inoculations, 4 weeks after planting. The greatest incidence of neck rot was observed in those plants inoculated on a fresh cut made in the neck after harvest (Table 8.4.).

Fungicidal spray applications on B. allii inoculated foliage prior to harvest did not significantly reduce the neck rot level in storage over nonsprayed controls (Table 8.5).

Table 8.4. Effect of timing of inoculation with B. allii conidia of foliage of field onions on neck rot incidence in storage.

Timing of inoculation (weeks after planting)	Mean bulb weight (grams)	% Neck rot
0	375	2.0
4	320	11.6
8	381	5.7
12	408	3.1
16	441	21.0
non inoculated	417	1.1
post inoculated[a]	-	81.2

[a]Conidia were applied after harvest to a fresh cut in the neck area.

Table 8.5. Effect of fungicides applied to onion foliage on neck rot incidence in storage

Fungicide	Lbs. AI/A	% Neck rot
Benlate	0.25	46.3 ± 7.9
Benlate	0.50	48.7 ± 13.6
Bravo	0.75	51.4 ± 10.3
Bravo	1.50	59.2 ± 2.5
Benlate + Bravo	0.25 0.75	43.2 ± 10.3
Control	-	53.4 ± 6.7

DISCUSSION

In 1977 Maude and Presly (1977b) reported that seeds infected with B. allii resulted in increased neck rot incidence in storage. In the seed treatment experiment of 1980 no neck rot developed in storage. The seed used by Maude and Presly (1977a, 1977b) was grown in Europe and was internally infected, while that used in the seed treatment experiment described above was surface-infested. Thus seedling infections as observed by Tichelaar (1967) and Maude and Presly (1977a, 1977b) may not have occurred in our trials where California-grown seed was used. A repeat of this experiment during 1981, which also included seed obtained from England, was lost due to heavy rain and flooding near harvest.

Examination of seed from Asgrow Seed Co., Kalamazoo, MI, and Desert Seed Co., Brooks, Oregon , yielded very low levels of B. allii. Maude (personal communication), who also examined American seed, stated that he found a very low incidence of B. allii in American seed. Thus, in the United States, seed borne infections may not be contributing significantly to neck rot incidence in storage. It is possible that greater incidences of seed borne B. allii may occur during periods of wet weather if fungicidal sprays are not effectively used.

The increase in neck rot following foliar applications of B. allii supports evidence from Maude and Presly and Tichelaar that B. allii infections can occur from an aerial deposition of conidia. There were gross symptoms of neck rot at harvest. This supports observations of Maude and Presly (1977a, 1977b) and Tichelaar (1967) that symptomless infections can occur in bulbs prior to harvest. The increased incidence of neck rot when conidia were applied at 4 and 16 weeks after planting

may have been due to favorable conditions for infection after inoculation. Additional studies concerning the aerial dessimination of B. allii and its ability to colonize onion tissues are needed.

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APPENDICES

APPENDIX A

SIMULATION MODELS OF BOTRYTIS LEAF BLIGHT OF ONION

PREFACE:

These models were completed in association with Systems Science 843 during spring term, 1983. The following individuals assisted in the preparation of the models: J. A. Biernbaum, M. Hoffhines, D. M. Timberlake, D. P. Welch, and R. O. Barr. A description of the model, written in manuscript format follows.

INTRODUCTION

Botrytis leaf blight is a leaf spotting and blighting disease of onion (Allium cepa L.), caused by the fungus Botrytis squamosa Walker. The disease is especially severe under prolonged moist conditions at temperatures of 15-24 C.

Presently, as many as 10-14 fungicidal sprays are applied annually to prevent Botrytis leaf blight epidemics. Since the disease is weather-dependent, an understanding of how weather conditions influence disease development could be applied in disease predictive systems. Using such systems, onion growers could judiciously apply fungicides only when needed, thus conferring substantial savings in production costs, and reducing the impact of pesticides on the environment.

Disease predictive models have been developed for sour cherry (9), apple scab (11), potato late blight (12,15), and tomato early blight (14,17). These models have increased knowledge of the biology of the pathogens, have proven to be effective in lowering disease control costs

and in increasing production of these important food crops, and have decreased the impact of pesticides on the environment. Similar systems could be derived for Botrytis leaf blight predictions in onion.

This report summarizes modeling efforts with the onion-Botrytis leaf blight system and defines area of future research needed for further development and refinement of the models.

MATERIALS AND METHODS

Description of the Botrytis Leaf Blight Prediction Model (BOLEB)

A Botrytis leaf blight prediction model (BOLEB) was constructed which uses inputs of ambient temperature and air humidity, expressed as vapor pressure deficit (VPD), to predict B. squamosa spore population dynamics. The model also provides estimates of lesion numbers and infected leaf area (blighted tissue). and simulates the influence of fungicidal sprays on blight epidemics.

There are four basic components of BOLEB. They are: a) spore release; b) lesion production; c)lesion expansion and tissue blighting; and d) fungicidal spray option.

a. The release of Spores. Lacy and Pontius (12) showed that parameters most closely associated with sporulation of B. squamosa were temperature and VPD. They expressed air humidity as vapor pressure deficit (VPD) (saturation vapor pressure - ambient vapor pressure) because of greater linearity between VPD and spore release at various temperatures than between relative humidity and spores released at various temperatures, resulting in better regression fits. A sporulation predictive index was constructed using 3-day averages of temperature and VPD, which estimated the probability of spore release on the following (4th consecutive) day.

The index value ranged from 0-1, where 1 represented the greatest probability of spore release. In field testing, the model has proven valuable in predicting large spore releases (unpublished data). Thus the model of Lacy and Pontius (12) represented an appropriate starting point in our modeling efforts. In fact, BOLEB uses their predictive index, which we have designated average index value (AIV).

Quantity of spores trapped within an onion field/24 m^3 air/day/cm^2 infected leaf area per plant available to support sporulation (SPORREL) was estimated, using environmental conditions favorable for spore release, as determined by the AIV value, the infected leaf area (cm^2/plant) which was available to support sporulation, and potential spores corresponding to the infected leaf area which could be expected in 24 m^3 air sampled by a Burkard recording spore trap within a 24 hour period from a 50 X 200 foot onion field area (equation 1).

Equation 1. SPORREL = AIV * AILA * MAX

where: AIV = average index value

AILA = available infected leaf area in cm^2/plant

Max = 225

SPORREL estimates the quantity of spores trapped under various environmental conditions in a 24 hour period per 24 m^3 air sampled divided by the total leaf area available to support sporulation in cm^2 from a single plant. It is expressed in terms of a single plant for simplicity and convenience in model derivation and because of limitations in available data.

The value of MAX was derived using spore release and blighted leaf length data published by Swanton (18), and from equations of Bolgiano (5)

which related leaf length to surface area of onion leaves. MAX is a constant which represents the average number of conidia (225 conidia) per 24 m^3 air sampled/day/cm^2 of available infected leaf area of a single representative plant, under conditions highly favorable for blight development. It is also expressed in terms of a single plant, but also represents a field of onions at a given density. The relationship between planting density and conidial numbers possible is not known.

AIV is identical to the index value described by Lacy and Pontius (12). Its value ranges from 0-1, where 1 represents highly favorable conditions for sporulation. Under conditions less than optimal for sporulation, only a portion of possible conidia would be formed and released (1). Thus a remaining portion of infected leaf area (RILA) would support sporulation when favorable conditions resumed. Such cyclic sporulation in response to environmental conditions were reported by Alderman (1). The remaining infected leaf area (RILA) was calculated according to equation 2.

Equation 2. $$RILA = (1 - AIV) * AILA$$

The remaining infected leaf area available to support spore formation and release (RILA) was then added to newly produced infected leaf area (NILA) according to equation 3.

Equation 3. $$AILA = NILA + RILA$$

This AILA would then be recalculated and utilized in equation 1 each day, and represented infected leaf area which could support spore production on that day.

b. Lesion Production. Using the data of Sutton et al. (17), a relationship between conidial numbers under field conditions and resulting lesions was derived. From this relationship, linear regression models were formulated to estimate lesion numbers resulting from cumulative spores trapped. Reports of Alderman and Lacy (2) and Shoemaker and Lorbeer (16) established that lesions develop within 24 hours following arrival of the spores on the leaf surface under highly favorable conditions. BOLEB estimates lesion numbers on the day following spore release. Since Alderman and Lacy (2) and Ellerbrock and Lorbeer (8) reported poor survival of conidia after their release and impaction on leaves, under conditions not favorable for germination, a conidial survival component was not incorporated into lesion formation of subsequent days.

c. Infected Leaf Area. Usually lesions caused by B. squamosa reach a size of 1-2 X 3-5 mm, and most are restricted from developing further (2). Alderman and Lacy (2) reported that some lesions may continue to expand under favorable environmental conditions, and that the number of expanding lesions increases with increasing duration of favorable conditions (high AIV). Expanding lesions lead to blighting and leaf tissue death, and since sporulation of the causal fungus occurs only on dead or dying tissue, blighting caused by expanding lesions can greatly increase the area of leaf tissue on which the fungus can produce spores (AILA). Relationships between numbers of expanding lesions, infected leaf area, and spores released were estimated using spore release data from the Michigan State Univeristy Muck Farm during 1981. Equations were derived which take the following discrete time form (equation 4.)

Equation 4. $ELES_{(t+3)} = a * LES_{(t)}$

where ELES = expanding lesions contributing to blighted leaf area, t = current day, a = a constant which is the slope parameter, and LES = the current day's lesions. B. squamosa developing in expanding lesions creates infected leaf area, but this area is not utilized in the model for 3 days. On the fourth day the value of ELES is redefined as newly infected leaf area (NILA) and is utilized in equation 3. The 3 day delay is equivalent to the latent period and is consistent with observations of Lacy and Pontius (12) who found that three days of favorable conditions preceded large spore releases from blighted tissue.

The proportion of lesions which will become expanding lesions depends on the favorability of environmental conditions. Conditions favorable for formation of expanding lesions are also favorable for spore production (1). Thus, environmental conditions leading to a high AIV would yield a greater proportion of expanding lesions. Several linear regression models are used to calculte ELES in BOLEB, and the particular model used in a given day depends on the AIV values

The value of ELES is the current day's contribution of lesions on the plant and is calculated using the spore release value of the preceding day and linear regression models. Several regression equations are used which take into account the reduction in healthy leaf area as lesions accumulate on the leaves.

d. Spray Component. An optional spray component was added to examine the influence of simulated fungicidal sprays on simulated blight epidemics. Assumptions incorporated in the spray option wer that i) sprays would

remain effective for 7 days; ii) sprays would reduce lesion numbers by 75%; and that iii) sprays would reduce spores released by 25%.

A seven day interval between sprays was chosen as a fairly conservative estimate of the length of protection provided. The 75% reduction in lesions per plant resulting from a fungicidal spray was derived from 8 years of unpublished field data by J. W. Lorbeer (Cornell Univeristy) who quantified reductions in lesions per leaf in fungicide-sprayed vs. unsprayed plots. The 25% reduction in spores released as a result of fungicide sprays was derived from a 1981 field data set from the Bolthouse farm, Sheridan, MI.

The fungicide spray option was triggered when the AIV was greater than 0.5 and when a spray was not applied within the previous 7 days. The 0.5 AIV "trigger" value is arbitrary, based on field observation and experience (7), and may be altered up or down to make the model more or less conservative.

Onion Growth Model

As a further refinement of the onion blight model, blighted leaf area predicted by the blight model was used in an onion growth model to simulate yield losses due to reduction in leaf area by blighting. Our goal in development of an onion growth model was to design a simple, yet biologically realistic model which would imitate yield reductions recorded in earlier artificial defoliation studies (3,9). In particular, Hawthorn (9) and Baker and Wilcox (3) found that yield reductions were most acute at the time of bulb initiation, or approximately five weeks prior to harvesting. We felt that destruction of phtosynthetically active tissues by B. squamosa would result in an effect similar to that achieved when removal of such tissues was done in defoliation studies.

The onion model treats the onion plant as consisting of two components: i) the leaf tissues per plant in square centimeters, and ii) the bulb in grams dry weight. Each component was modeled as a discrete system with the following form:

$$GLSA_{(t+1)} = GLSA_{(t)} + f(DEGDAY)$$

$$BLBWT_{(t+1)} = BLBWT_{(t)} + g(GLSA)$$

where GLSA = green leaf surface area per plant in cm^2; BLBWT = bulb dry weight in grams; t = current day; and DEGDAY = cumulative degree days. The f and g functions relate growth of leaves and bulb to cumulative degree days and green leaf surface area, respectively, and are the best fit linear regression coefficients based on data generated using an onion model constructed by Bolgiano (5). Degree days are calculated using a base of 5.6 C which is the lower growth limit implied for onion (5,6). Diurnal temperature curves were approximated as sine curves from maximum and minimum temperatures (4).

The experiment of Baker and Wilcox (3) was simulated using our onion model and Baker and Wilcox's data derived from actual defoliation studies. Baker and Wilcox (3) found 30, 45, and 60% reductions in yield after removal of 30, 60, or 90% of the leaf area respectively. Our model accurately mimicked the effects of artificial defoliation on yield in the latter portion of the growing season (Figure A.1) so we felt confident that defoliation caused by B. squamosa would be similar to results obtained in artificial defoliation experiments.

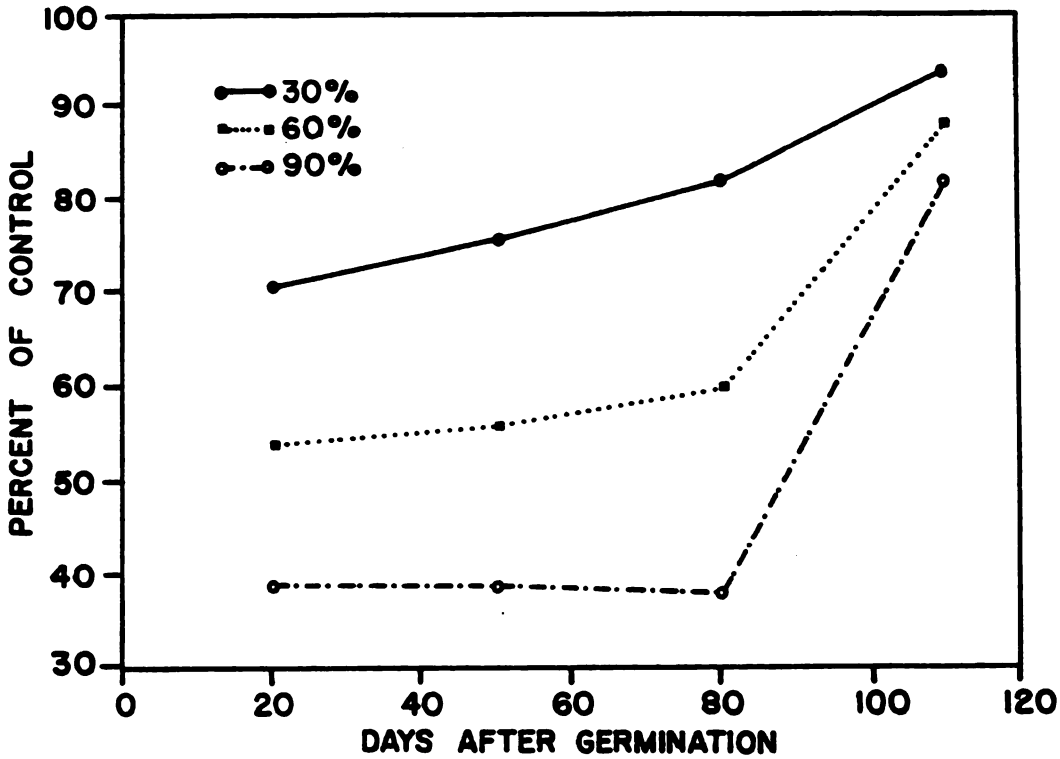

Figure A.1. Simulation of yield as percent of control after defoliation of 30, 60, or 90% of the leaf tissue on various days after germination of onion.

Model Runs

Model runs were conducted using BOLEB, and ONLEG (BOLEB joined to the onion growth model). The initial inputs of BOLEB were i) the initial infected leaf area, which could be estimated from the number of lesions per plant, and the area per lesion, and ii) the average daily temperatures in Farenheit and the average VPD, which were converted to AIV. Temperature and relative humidity (converted to VPD) were collected at the M. S. U. Muck Farm, Bath, MI, during 1978 and 1981 and at the Bolthouse farm, Sheridan, MI, during 1981 and 1982.

The initial inputs of ONLEG, in addition to those of BOLEB were i) the day after seed germination that one lesion per leaf was first observed, ii) the duration of model simulation run, in terms of either degree days of days; and iii) frequency of output. Output included day, degree day, green leaf surface area, and bulb dry weight.

RESULTS AND DISCUSSION

Leaf Blight Model (BOLEB)

Simulations were run using data from the Muck farm during 1978 and 1981 (Figure A.2a,b) and from the Bolthouse farm, 1981 and 1982 (Figures A.2c,d). Weather conditions at the M. S. U. Muck Farm during 1978 were very unfavorable for leaf blight development. On only two occasions during the season did AIV values rise above 0.5 (the condition where fungicidal sprays were advised). By the end of the season 22,000 cumulative spores/ 24 m^3 air/ day were trapped using a Burkard recording spore trap (Figure A.2a), compared with 274,000 during 1981 (Figure A.2b). No sprays were applied to these plots in either year. During 1981, conditions were favorable for leaf blight epidemics. Numbers of

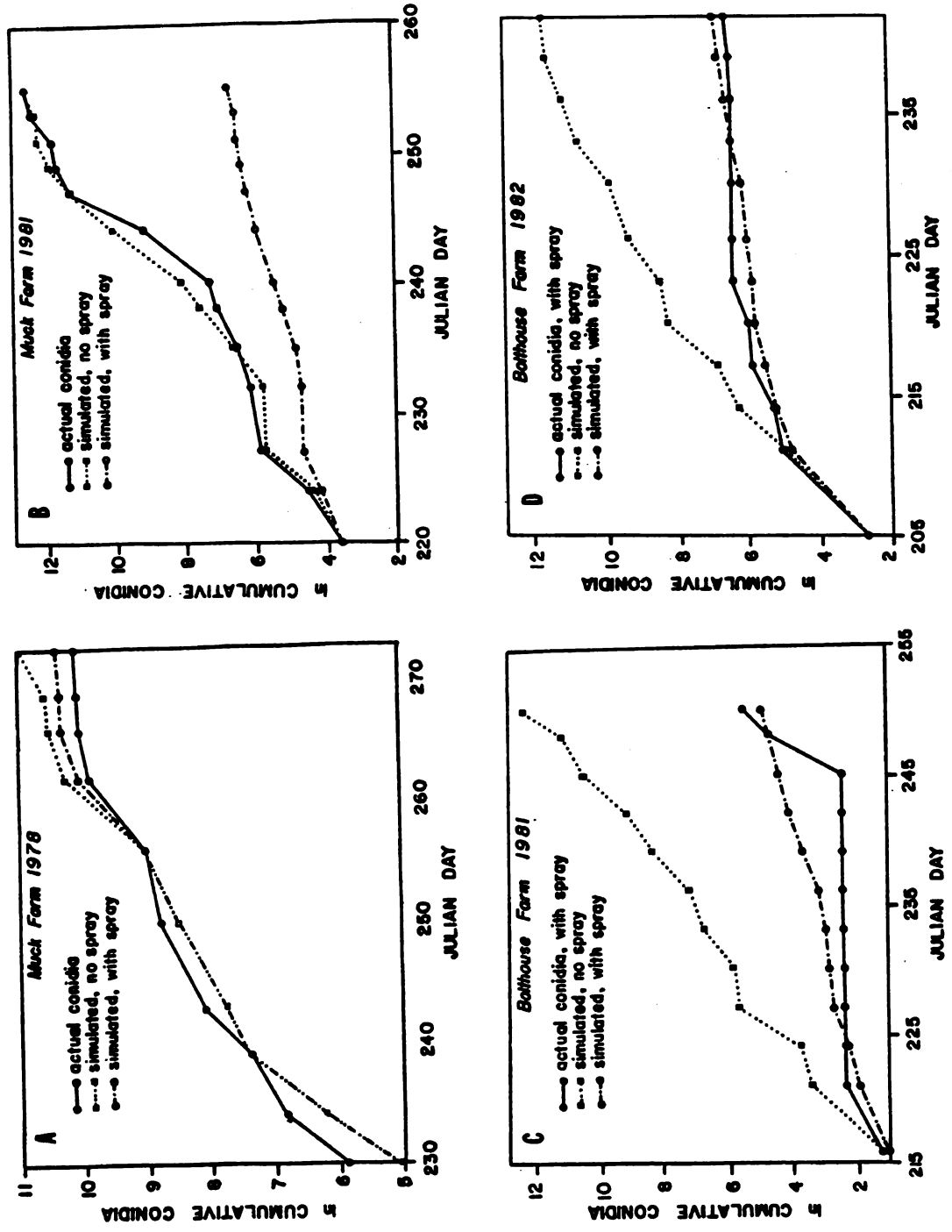

Figure A.2a-d. Actual cumulative conidia / m^3 air sampled, and simulated conidia sampled with and without the spray option

spores predicted by the model using different weather data sets closely followed spores actually trapped in the field under the same conditions, whether sprayed or unsprayed (Figures A.2 b,c). The predicted spores trapped when plots were sprayed using the AIV trigger of 0.5 were greatly reduced compared to numbers of spores predicted by the model if plots had been unsprayed (Figures A.2c,d).

At the Bolthouse farm all plots were sprayed with fungicidal sprays when the AIV exceeded 0.5. Both 1981 and 1982 were favorable for blight development. The importance of fungicidal sprays in reducing disease was apparent (Figures A.2a-d). Nonetheless, the number of sprays needed to control leaf blight was lowered. In years where the AIV was above 0.5 on only two occasions during the season (such as in 1978), few, if any, pesticide applications would be needed.

Onion Leaf Blight Growth Model (ONLEG)

ONLEG simulation runs were completed using data sets from the M.S.U. Muck Farm, 1981 (Figure A.3a,b) and Bolthouse farm, 1981, and 1982 (Figure A.3 c,d). The first date of spore release for the Muck Farm, 1981, was August 8 or eighty days after seed germination. Although spores were detected several days earlier at the Bolthouse farm during 1981 and 1982, the first day in the model run was considered to be the same (day 80) so that onions would have the same leaf area for comparison purposes. The simulations were terminated after the accumulation of 1800 degree days, which corresponded to 117 days after germination or approximately September 17. Each simulation was also run using the fungicide spray option.

An additional simulation was run to determine the effect of initial degree of infection of final leaf area on yield. The estimation of

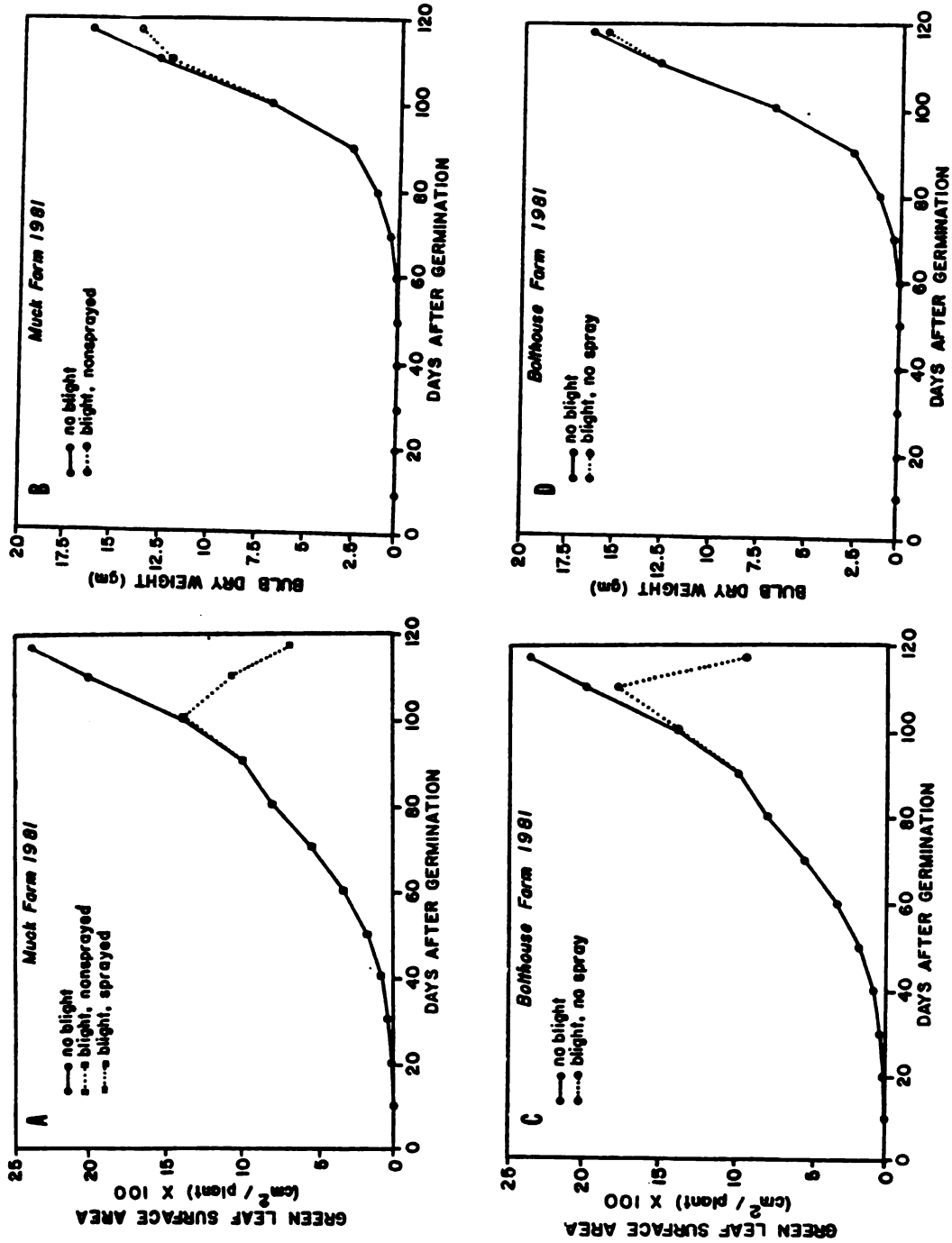

Figure A.3a-d. Green leaf surface area and bulb weight simulated by ONLEG with and without the spray option.

initial infected leaf area from the actual M.S.U. Muck Farm 1981 data set (0.39 cm^2) was replaced with 0.59 cm^2 and the model was run again using the AIV values derived from the same data set. The increase in initial infected leaf area represented a greater degree of infection early in the season.

The predicted leaf surface area of a healthy, noninfected onion plant from ONLEG after 1800 degree days was 2378 cm^2 (Figure A.3e) Running the model with the AIV's calculated from the Muck Farm 1981 weather data, without simulated fungicide sprays, the final predicted leaf area was 676 cm^2 per plant (Figure A.3a). The majority of this predicted decrease in leaf surface area occurred in the final two weeks of growth and resulted in an 18% reduction in predicted bulb weight (Figure A.3b). When the simulation was run with simulated fungicidal sprays, there was no significant loss of leaf area (Figure A.3a) or decrease in bulb weight (Figure A.3b). Similar results in predicted infected leaf area were obtained using simulated sprays with the actual Bolthouse farm weather data set (Figures A.3c,d).

Using the daily AIV's calculated from the Bolthouse farm 1981 weather data the final predicted leaf surface area without the spray option was 930 cm^2 per plant. However, the majority of this reduction occurred in the last week of the season (Figure A.3c) and the predicted reduction in bulb weight was only 7% (Figure A.3d). A comparison of the time of occurrence of leaf blight and the effects on yield indicates that the projected yield was more sensitive to early reductions in leaf area. This is further supported by a comparison of the Bolthouse farm 1981 and 1982 data (Figures A.2c,d). Although the final reduction in leaf area at the end of the season was less in 1982 (Figure A.3e) than in 1981,

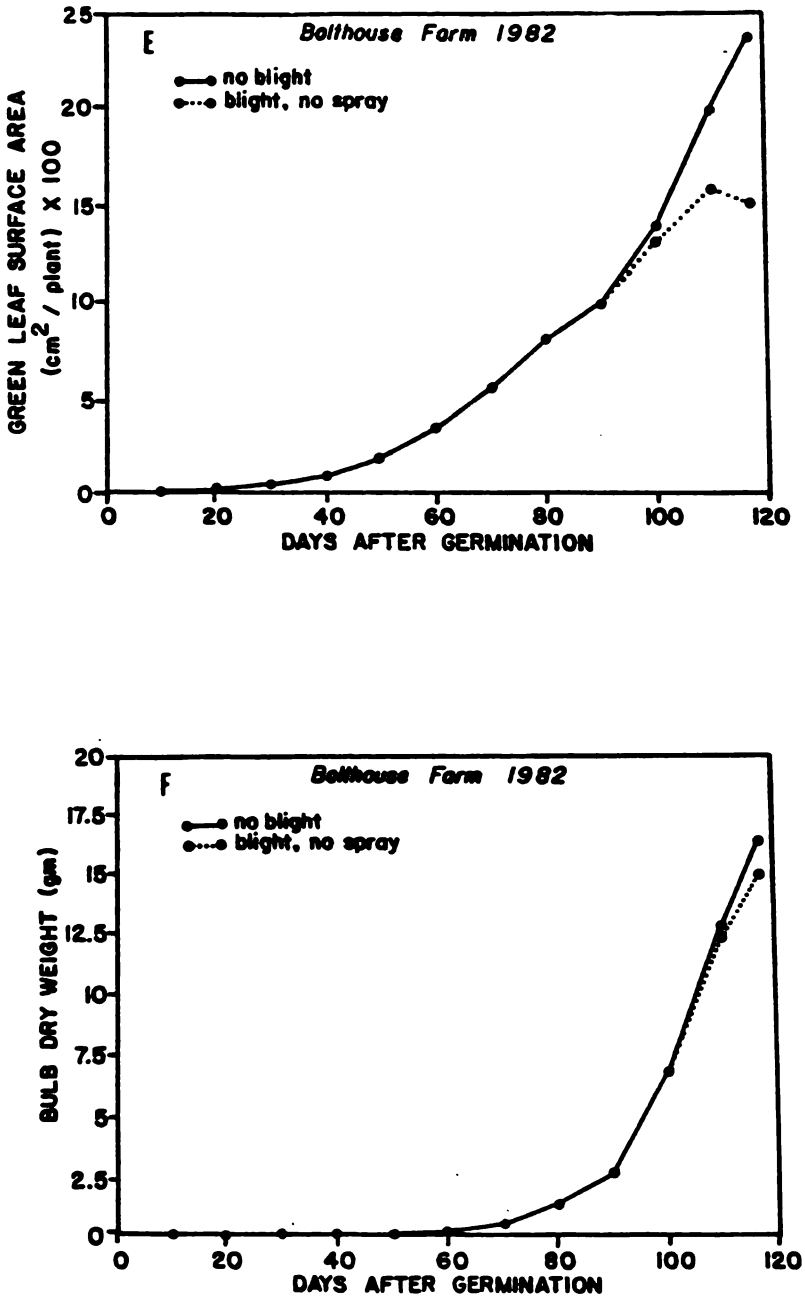

Figure A.3e-f. Green leaf surface area and bulb weight simulated by ONLEG with and without the spray option.

the reduction in yield (Figure A.3f) was slightly larger in 1982. This was due to the occurrence of some blighting earlier in the season.

In an attempt to mimic conditions of earlier seasonal development of leaf blight, a simulation was run where the initial infected leaf area was increased by 50%. In this simulation, almost the entire leaf area was blighted by the end of the season (Figure A.4a) and the bulb weight reduction went from 18-21% (Figure A.4b). A more accurate simulation of early season blight development would be to assume the first occurrence of spores at 65 days after germination instead of 80 days after germination. This was not attempted since actual AIV's for these earlier days were not available.

As expected, the outputs of spore number, lesion number, and blighted leaf area from ONLEG were identical to outputs from the blight subroutine run separately with the same AIV values. The sum of the green leaf surface area and the blighted infected leaf area was approximately equal to the green leaf surface area of the onion model run without entering the blight subroutine.

There are no accurate records of the actual percent of the leaf surface area blighted for the data sets of data tested. In the case of the Bolthouse farm, this was not possible, since all field areas were sprayed, and the spore release prediction model was based on data from unsprayed plots. Observations of Lacy and Pontius (7) indicated that severe blighting occurred at the Muck farm during 1981, and we observed very little blighting at the Bolthouse farm during 1981 and 1982. However, the predicted yield reductions fall within the range reported by Baker and Wilcox (2) for leaf tissue removal.

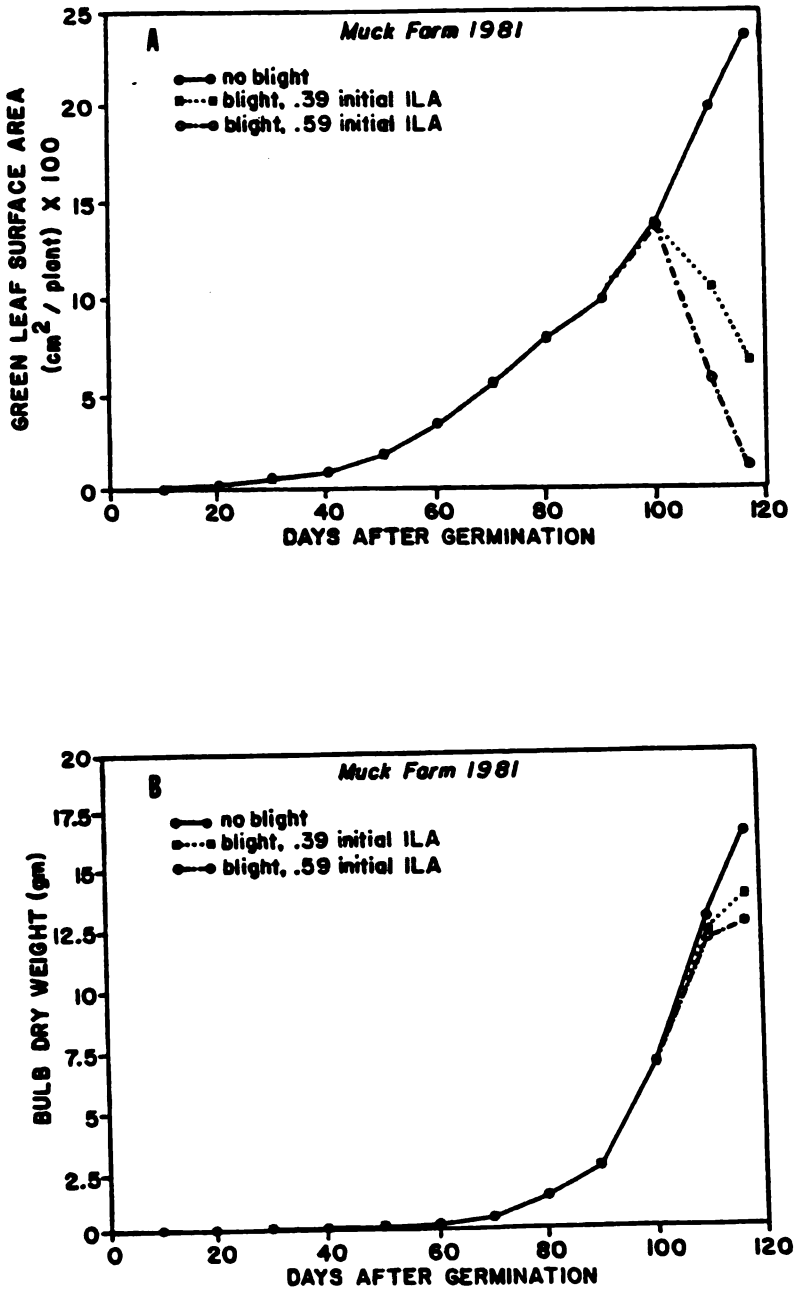

Figure A.4a-b. Simulated green leaf surface area and bulb weight in response to various levels of infestation by B. squamosa early in the season.

The timing of epidemics and degree of initial infected leaf area had a significant effect on the projected yield. These models may be helpful in testing the implications of the timing and severity of epidemics, the importance of amount of initial infections, and the potential impact of spray applications.

Areas of Future Research

The following areas have been outlined as needing further data to confirm and strengthen the present model and as direction for future expansion of the model:

1) The collection of more onion growth data relating environmental conditions to leaf surface area and bulb growth.
2) More field data concerning the interrelationships between environmental conditions and spores, lesions, blighting, and the release of spores from blighted tissue.
3) Data describing the effectiveness of fungicidal sprays on reduction of lesion numbers, spore numbers, spore viability, and blighted tissue.
4) Data describing the effects of lesion numbers and leaf blighting on yields so that economic thresholds for optimization of spray reductions can be estimated.
5) Eventual application of the model to field situations so that onion yields can be optimized.

The programs for ONLEG and BOLEB are included in appendicies B-D.

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APPENDIX B

ONION MODEL

```
      PROGRAM ODRIVE(INPUT,OUTPUT)
*
      IMPLICIT REAL (A-Z)
      INTEGER REPLY,DAY,LIMTYPE,INTERVL,FREQOUT,DYSPGRM,FARMER
      LOGICAL GERMFLG,LIMIT,BLGHT,INTRPT
      LOGICAL HEREPR(6)
*
*   DEFINE COMMON BLOCKS
*
      COMMON/BLOCK0/GLSA,DEGRDAY,OLDDEG,BULBWT,
     +              MAXBULB,MAXGLSA,GERMFLG
      COMMON/BLOCK1/INTGLSA,INITBWT,GERMDAY,DAY,DYSPGRM,FARMER
     +              ,BLGHT
      COMMON/BLOCK2/HEREPR
      COMMON/BLOCK3/DEGDAYC(6),DEGLIM(6)
      COMMON/TEMP/MINTEMP,MAXTEMP
*
*   SET INITIAL CONDITIONS
*
1     CALL SIMINIT
*
*********************************************************************
*********************************************************************
*
*               START OF SIMULATION
*
      PRINT *,'  '
      PRINT *,'DO YOU WANT TO USE THE BLIGHT PORTION OF SIMULATION?'
      READ (*,'(A10)')REPLY
      IF ((REPLY .EQ. 1HY).OR.(REPLY .EQ. 3HYES)) THEN
       BLGHT=.TRUE.
       CALL BEGBLYT
2      PRINT *,'  '
       PRINT *,'WHICH DAY AFTER GERMINATION FOR FIRST '
       PRINT *,'SPORE RELEASE?'
       READ *,FARMER
       IF (FARMER .LT. 0.) GOTO 2
      ELSE
       BLGHT=.FALSE.
      ENDIF
      PRINT *,'  '
      PRINT *,'WILL YOU BE CHANGING GLSA DURING THE COURSE OF A SEASON?'
      READ (*,'(A10)')REPLY
```

```
      IF ((REPLY .EQ. 1HY).OR.(REPLY .EQ. 3HYES)) THEN
       INTRPT=.TRUE.
       PRINT *,' '
       PRINT *,'HOW FREQUENTLY DO YOU WISH TO BE PROMPTED?'
       READ *,INTERVL
       IF (INTERVL .LT. 1) THEN
        INTERVL=10
       ENDIF
      ELSE
       INTRPT=.FALSE.
      ENDIF
3     PRINT *,' '
      PRINT *,'HOW DO YOU WISH TO INDICATE THE END OF A GROWING DEASON?'
      PRINT *,' '
      PRINT *,'    TYPE: "1" FOR DAY LIMIT, OR'
      PRINT *,'          "2" FOR DEGREE-DAY LIMIT'
      READ *,LIMTYPE
      IF ((LIMTYPE .NE. 1) .AND. (LIMTYPE .NE. 2)) GOTO 3
      IF (LIMTYPE .EQ. 1) THEN
       PRINT *,' '
       PRINT *,'LAST DAY OF RUN?'
       READ *,ENDPNT
      ELSEIF (LIMTYPE .EQ. 2) THEN
       PRINT *,' '
       PRINT *,'LAST DEGREE-DAY OF RUN?'
       READ *,ENDPNT
      ENDIF
      PRINT *,' '
      PRINT *,'FREQUENCY OF OUTPUT?'
      READ *,FREQOUT
      IF (FREQOUT .LT. 1) FREQOUT=10
4     CONTINUE
      CALL DAYGRTH
      IF (BLGHT) THEN
       IF (DYSPGRM .GE. FARMER) THEN
        CALL BLIGHT(INLFAR)
        GLSA=GLSA-INLFAR
        IF (GLSA .LT. 0.) GLSA = 1.0
       ENDIF
      ENDIF
      IF(INTRPT) THEN
       IF (MOD(DAY,INTERVL) .EQ. 0) THEN
        PRINT *,' '
        PRINT *,'PRESENT GREEN LEAF AREA IS...',GLSA
        PRINT *,' '
        PRINT *,'DO YOU WANT TO CHANGE THIS?'
        READ (*,'(A10)')REPLY
        IF ((REPLY .EQ. 1HY).OR.(REPLY .EQ. 3HYES)) THEN
         PRINT *,' '
         PRINT *,'WHAT IS THE NEW VALUE FOR GLSA?'
         READ *,GLSA
        ENDIF
       ENDIF
      ENDIF
```

```
      IF (MOD(DAY,FREQOUT).EQ. 0) THEN
       PRINT *,' '
       PRINT *,' STATS:  DAY        : ',DAY
       PRINT *,'         DEGREE-DAY: ',DEGRDAY
       PRINT *,'         GLSA       : ',GLSA
       PRINT *,'         BULB WT    : ',BULBWT
      ENDIF
      IF (LIMTYPE .EQ. 1) THEN
       IF (DAY.LT.ENDPNT) GOTO 4
      ELSE
       IF (DEGRDAY.LT.ENDPNT) GOTO 4
      ENDIF
      IF (MOD(DAY,FREQOUT) .NE. 0) THEN
       PRINT *,' '
       PRINT *,' STATS:  DAY        : ',DAY
       PRINT *,'         DEGREE-DAY: ',DEGRDAY
       PRINT *,'         GLSA       : ',GLSA
       PRINT *,'         BULB WT    : ',BULBWT
      ENDIF
      PRINT *, ' '
      PRINT *,'DO YOU WANT TO START AGAIN?'
      READ (*,(A10)')REPLY
      IF ((REPLY .EQ. 1HY).OR.(REPLY .EQ. 3HYES)) THEN
       GOTO 1
      ELSEIF ((REPLY .NE. 1HY).AND.(REPLY .NE. 3HYES)) THEN
       PRINT *,' '
       PRINT *,'ARE YOU SURE?'
       READ (*,'(A10)')REPLY
       IF ((REPLY .NE. 1HY).AND.(REPLY .NE. 3HYES)) THEN
        PRINT *,' '
        PRINT *,'OK, ONCE AGAIN, DO YOU WANT TO START ANEW?'
        READ (*,'(A10)')REPLY
        IF ((REPLY .EQ. 1HY).OR.(REPLY .EQ. 3HYES)) GOTO 1
       ENDIF
      ENDIF
      END
*******************************************************************************
*******************************************************************************
      SUBROUTINE SIMINIT
*
      IMPLICIT REAL (A-Z)
      INTEGER REPLY,DAY,LIMTYPE,INTERVL,FREQOUT,DYSPGRM,FARMER,I
      LOGICAL GERMFLG,LIMIT,BLGHT,INTRPT
      LOGICAL HEREPR(6)
*
*   DEFINE COMMON BLOCKS
*
      COMMON/BLOCK0/GLSA,DEGRDAY,OLDDEG,BULBWT,
     +              MAXBULB,MAXGLSA,GERMFLG
      COMMON/BLOCK1/INTGLSA,INITBWT,GERMDAY,DAY,DYSPGRM,FARMER
     +              ,BLGHT
      COMMON/BLOCK2/HEREPR
      COMMON/BLOCK3/FEGDAY(6),DEGLIM(6)
```

```
      COMMON/TEMP/MINTEMP,MAXTEMP
*
*    SET INITIAL CONDITIONS
*
      DO 10.I=1,6
       HEREPR(I) = .FALSE.
      DEGDAY(I) = 0.
10    CONTINUE
      DEGLIM(1) = 310.
      DEGLIM(2) = 280.
      DEGLIM(3) = 170.
      DEGLIM(4) = 280.
      DEGLIM(5) = 270.
      DEGLIM(6) = 280.
      GERMFLG   = .FALSE.
      GLSA      = 0.
      DAY       = 0
      DEGRDAY   = 100
      OLDDEG    = 0.
      GERMDAY   = 116.
      INTGLSA   = 1.17
      INITBWT   = 0.0001
      MAXGLSA   = 2400.
      MAXBULB   = 24.
      DYSPGRM   = 0
                                  DEFAULT SETS DEGREE-DAYS FOR RUN W/O
*                                 WEATHER FILE
      CALL DEFAULT
      RETURN
      END
*******************************************************************************
*******************************************************************************
*
      SUBROUTINE DAYGRTH
*
      IMPLICIT REAL (A-Z)
      INTEGER DAY,I,REPLY,DYSPGRM,FARMER
      LOGICAL GERMFLG,BLGHT
      COMMON/BLOCK0/GLSA,DEGRDAY,OLDDEG,BULBWT,
     +              MAXBULB,MAXGLSA,GERMFLG
      COMMON/BLOCK1/INTGLSA,INITBWT,GERMDAY,DAY,DYSPGRM.FARMER
     +              ,BLGHT
      COMMON/DEFLT/DEF(120)
*
      DAY = DAY+1
      OLDDEG = DEGRDAY
*
*     DEGRDAY=DEGRDAY+ DGRDY(DUM)      FOR USE WITH WEATHER FILE
*
      DEGRDAY=DEF(DAY)
      IF ( DEGRDAY .LT. GERMDAY) THEN
       GERMFLG = .FALSE.
      ELSEIF ((DEGRDAY .GE. GERMDAY) .AND. (.NOT. GERMFLG)) THEN
```

```
       GERMFLG = .TRUE.
       GLSA = INTGLSA
       BULBWT = INITBWT
       IF (BLGHT) DYSPGRM = DYSPGRM + 1
      ELSE
       IF (BLGHT) DYSPGRM = DYSPGRM + 1
       CALL NEWGRTH
      ENDIF
      RETURN
      END
*******************************************************************************
*******************************************************************************
*
      SUBROUTINE ATEMP
*
*       ATEMP READS FROM A DATA FILE THE DAILY MAX AND MIN TEMPERATURES
*       IN DEGREES F. AND CONVERTS THEM INTO DEGREES C.
*
      IMPLICIT REAL (A-Z)
      COMMON/TEMP/TMIN,TMAX
      READ(1,50)TMAX,TMIN
50    FORMAT(8X,2F6.0)
      TMAX=(TMAX-32.)*5./9.
      TMIN=(TMIN-32.)*5./9.
      RETURN
      END
*******************************************************************************
*******************************************************************************
*
      SUBROUTINE NEWGRTH
*
*   'NEWGRTH' UPDATES THE GLSA AND BULB WEIGHT FOR THE NEW DEGREE DAY
*
*     DEGRDAY - TOTAL DEGREE-DAYS THROUGH PRESENT DAY
*     OLDDEG  - TOTAL DEGREE-DAYS THROUGH YESTERDAY
*     GLSA    - GREEN LEAF SURFACE AREA(G.L.S.A.)THROUGH PRESENT DAY
*     NEWGLSA - G.L.S.A. THROUGH THE NEW DAY(= GLSA AT END OF THIS
*               SUBROUTINE)
*
      IMPLICIT REAL (A-Z)
      INTEGER I,K
      LOGICAL GERMFLG,HEREPR(6)
      COMMON/BLOCK0/GLSA,DEGRDAY,OLDDEG,BULBWT,
     +              MAXBULB,MAXGLSA,GERMFLG
      COMMON/BLOCK2/HEREPR
      COMMON/BLOCK3/DEGDAY(6),DEGLIM(6)
*
*   GREEN LEAF SURFACE AREA RELATED TO DEGREE-DAYS BY THE FOLLOWING
*   SET OF EQUATIONS:
*
*        116 <= DEGRDAY  < 225
*
*          LOG(BASE 10)GLSA = -.5667+.00551*DEGRDAY  R=.99
*
```

```
*         225 <  = DEGRDAY < 340
*
*           LOG(BASE 10)GLSA = -.3434+.00456*DEGRDAY R=.99
*
*         340 < = DEGRDAY < 520
*
*           LOG(BASE 10)GLSA = .2573+.00285*DEGRDAY R=.99
*
*         520 < = DEGRDAY < 760
*
*           LOG(BASE 10)GLSA = .6771+.00201*DEGRDAY R=.96
*
*         760 < = DEGRDAY < 1160
*
*           LOG(BASE 10)GLSA = .9853+.001588*DEGRDAY R=.99
*
*         1160 < = DEGRDAY < 1428
*
*            GLSA = -1123+1.489*DEGRDAY R=.99
*
*         1428 < = DEGRDAY <1800
*
*           LOG(BASE 10)GLSA = 1.477+.001057*DEGRDAY  R=.99
*
       IF (GLSA.LT.MAXGLSA) THEN
        IF (DEGRDAY .LT. 225.) THEN
         NEWGLSA=10**(.00551*(DEGRDAY-OLDDEG)+ALOG10(GLSA))
        ELSE IF ((DEGRDAY .GE. 225.) .AND. (DEGRDAY.LT. 340.)) THEN
         NEWGLSA=10**(.00456*(DEGRDAY-OLDDEG)+ALOG10(GLSA))
        ELSE IF ((DEGRDAY .GE. 340.) .AND. (DEGRDAY .LT. 520.)) THEN
         NEWGLSA=10**(.00285*(DEGRDAY-OLDDEG)+ALOG10(GLSA))
        ELSE IF ((DEGRDAY .GE. 520.) .AND. (DEGRDAY .LT. 760.)) THEN
         NEWGLSA=10**(.00201*(DEGRDAY-OLDDEG)+ALOG10(GLSA))
        ELSE IF ((DEGRDAY .GE. 760.) .AND. (DEGRDAY .LT. 1160.)) THEN
         NEWGLSA=10**(.001588*(DEGRDAY-OLDDEG)+ALOG10(GLSA))
        ELSE IF ((DEGRDAY .GE. 1160.) .AND. (DEGRDAY .LT. 1428.)) THEN
         NEWGLSA=1.489*(DEGRDAY-OLDDEG)+GLSA
        ELSE IF ((DEGRDAY .GE. 1428.) .AND. (DEGRDAY .LT. 1800.)) THEN
         NEWGLSA=10**(.001058*(DEGRDAY-OLDDEG)+ALOG10(GLSA))
        ELSE
*
* SENESCENCE CURRENTLY IS A NO-GROWTH SITUATION
*
         IF (GLSA .GE. 20.) THEN
          NEWGLSA=GLSA
          GLSA=NEWGLSA-20.
         ELSE
          NEWGLSA=GLSA+.00001
          GLSA=.000001
          ENDIF
         ENDIF
        ELSE
         IF (GLSA .GE. 20.) THEN
```

```
          NEWGLSA=GLSA
          GLSA=NEWGLSA-20.
         ESLE
          NEWGLSA=GLSA+.00001
          GLSA=.000001
         ENDIF
        ENDIF
       ELSE
        IF (GLSA .GE. 20.) THEN
         NEWGLSA=GLSA
         GLSA=NEWGLSA-20
        ELSE
         NEWGLSA=GLSA+.00001
         GLSA=.000001
        ENDIF
       ENDIF
*
*   BULB WEIGHT RELATED TO GLSA BY THE FOLLOWING SET OF EQUATIONS:
*
*        1 < = GLSA < 31
*
*          BWT=-.0000278+.00008387*GLSA  R=.99
*
*        31 < = GLSA < 117
*
*          LOG(BASE 10)BWT=-2.8234+.01136*GLSA  R=.99
*
*        117 < = GLSA < 240
*
*          LOG(BASE 10)BWT=-1.9973+.004433*GLSA  R=.99
*
*        240 < = GLSA < 609
*
*          LOG(BASE 10)BWT=-1.6214+.002843*GLSA  R=.99
*
*        609 < = GLSA <1015
*
*          LOG(BASE 10)BWT=-.8066+.001602*GLSA  R=.99
*
*        1015 < = GLSA <1879
*
*          BWT=-4.310+.010437*GLSA  R=.99
*
*        1879 <= GLSA
*
*          BWT=-1.26629+.008710  R=.99
*
      IF (BULBWT.LT.MAXBULB) THEN
*
*       BULB GROWTH RATES ARE, AS EXPLAINED ABOVE, CALCULTED AS FUNCTIONS
*   OF G.L.S.A. HOWEVER, THE IMPORTANCE OF DEGREE-DAYS IS RECOGNIZED IN
*   TWO WAYS. FIRST, A BULB CAN ONLY SPEND A LIMITED NUMBER OF DEGREE-
*   DAYS WITH A PARTICULAR GROWTH RATE. THIS INTERVAL WAS CALCULTED AS
*   THE NUMBER OF DEGREE-DAYS THAT A MODEL PLANT. I.E. A PLANT GROWN
```

```
*    WITH N.B.'S MODEL, ACCUMULATED DURING THE GROWTH INTERVALS USED
*    BELOW(FOR THE LIMITS USED HERE, SEE SUBROUTINE 'SIMINIT'), SECONDLY,
*    NO PLANT MAY REVERT TO AN EARLIER GROWTH RATE.  THAT IS, ONCE A
*    PLANT HAS, AT SOME POINT IN ITS HISTORY, ATTAINED A PARTICULAR
*    GROWTH RATE IT IS NO LONGER CAPABLE OF RETURNING TO A PREVIOUS REGIME.
*
      IF ((GLSA.GE.1.) .AND. (GLSA.LT. 31.) .AND. (.NOT.HEREPR(1))) THEN
       DEGDAY(1) = DEGDAY-OLDDEG+DEGDAY(1)
       IF (DEGDAY(1).GT.DEGLIM(1)) HEREPR(1) = .TRUE.
       BULBWT=BULBWT+(NEWGLSA-GLSA)*.00008387
      ESLEIF ((GLSA.LT.117.) .AND. (.NOT.HEREPR(2))) THEN
       HEREPR(1) = .TRUE
       DEGDAY(2) = DEGDAY-OLDDEG+DEGDAY(2)
       IF (DEGDAY(2).GT.DEGLIM(2)) HEREPR(2) = .TRUE.
       BULBWT =10**(.010111*(NEWGLSA-GLSA)+ALOG10(BULBWT))
      ELSEIF ((GLSA.LT.240.) .AND. (.NOT.HEREPR(3))) THEN
       HEREPR(2) = .TRUE.
       DEGDAY(3) = DEGRDAY-OLDDEG+DEGDAY(3)
       IF (DEGDAY(3).GT.DEGLIM(3)) HEREPR(3) = .TRUE.
       BULBWT=10**(.004433*(NEWGLSA-GLSA)+ALOG10(BULBWT))
      ELSEIF ((GLSA.LT.609.) .AND. (.NOT.HEREPR(4))) THEN
       HEREPR(3) = .TRUE.
       DEGDAY(4) = DEGRDAY-OLDDEG+DEGDAY(4)
       IF (DEGDAY(4).GT.DEGLIM(4)) HEREPR(4) = .TRUE.
       BULBWT=10**(.002843*(NEWGLSA-GLSA)+ALOG10(BULBWT))
      ELSEIF ((GLSA.LT.1015.) .AND. (.NOT.HEREPR(5))) THEN
       HEREPR(4)= .TRUE.
       DEGDAY(5) = DEGRDAY-OLDDEG+DEGDAY(5)
       IF (DEGDAY(5).GT.DEGLIM(5)) HEREPR(5) = .TRUE.
       BULBWT=10**(.001602*(NEWGLSA-GLSA)+ALOG10(BULBWT))
      ELSEIF ((GLSA.LT.1879.) .AND. (.NOT.HEREPR(6))) THEN
       HEREPR(5) = .TRUE.
       DEGDAY(6) = DEGRDAY-OLDDEG+DEGDAY(6)
       IF (DEGDAY(6).GT.DEGLIM(6)) HEREPR(6) = .TRUE.
       BULBWT=BULBWT+(NEWGLSA-GLSA)*.010437
      ELSE
*
*    GLSA >= 1879 EITHER NOW OR AT SOME TIME IN ITS PAST
*
       HEREPR(6) = .TRUE.
       BULBWT=BULBWT+(NEWGLSA-GLSA)*.00871
      ENDIF
      ENDIF
      GLSA=NEWGLSA
      RETURN
      END
**********************************************************************
**********************************************************************
*
      FUNCTION DGRDY(JJ)
*
*       DGRDY RETURNS THE TOTAL NUMBER OF DEGREE DAYS FOR A GIVEN DAY.
*
```

```
*        ENTRY:TMAX, MAXIMUM TEMP FOR THE DAY, AND,
*              TMIN, MINIMUM TEMP FOR THE DAY IN COMMON BLOCK
*
*        EXIT :DGRDYS CALCULATED
*
      IMPLICIT REAL (A-Z)
      COMMON/TEMP/TMIN,TMAX
      DATA TPI/6.283185308/,HPI/1.570796327/,BASE/5.6/
      IF (TMAX.GT.BASE) GOTO 10
      DGRDY=.00001
      RETURN
      CONTINUE
      Z=TMAX-TMIN
      XM=TMAX+TMIN
      IF (TMIN.LT.BASE) GOTO 20
      DGRDY=XM/2.-BASE
      IF (DGRDY.GT.0.) GOTO 30
      DGRDY=.00001
30    RETURN
20    CONTINUE
      TBASE=BASE*2.
      A=ASIN((TBASE-XM/Z)
      DGRDY=(2.*COS(A)-(TBASE-XM)*(HPI-A))/TPI
      IF (DGRDY.GT.0.) GOTO 40
      DGRDY=.00001
40    RETURN
      END
**************************************************************************
**************************************************************************
      SUBROUTINE DEFAULT
*
      IMPLICIT REAL (A-Z)
      COMMON/DEFLT/DEF(120)
      DEF(1)=116.
      DEF(2)=131.
      DEF(3)=147.
      DEF(4)=161.
      DEF(5)=177.
      DEF(6)=188.
      DEF(7)=198.
      DEF(8)=211.
      DEF(9)=225.
      DEF(10)=230.
      DEF(11)=240.
      DEF(12)=252.
      DEF(13)=270.
      DEF(14)=282.
      DEF(15)=297.
      DEF(16)=312.
      DEF(17)=325.
      DEF(18)=341.
      DEF(19)=354.
      DEF(20)=367.
```

```
DEF(21)=379.
DEF(22)=393.
DEF(23)=408.
DEF(24)=428.
DEF(25)=450.
DEF(26)=463.
DEF(27)=474.
DEF(28)=490.
DEF(29)=507.
DEF(30)=520.
DEF(31)=537.
DEF(32)=551.
DEF(33)=564.
DEF(34)=580.
DEF(35)=593.
DEF(36)=602.
DEF(37)=612.
DEF(38)=626.
DEF(39)=646.
DEF(40)=662.
DEF(41)=676.
DEF(42)=693.
DEF(43)=709.
DEF(44)=727.
DEF(45)=744.
DEF(46)=762.
DEF(47)=781.
DEF(48)=802.
DEF(49)=825.
DEF(50)=839.
DEF(51)=856.
DEF(52)=877.
DEF(53)=897.
DEF(54)=910.
DEF(56)=924.
DEF(57)=958.
DEF(58)=977.
DEF(59)=995.
DEF(60)=1012.
DEF(61)=1025.
DEF(62)=1036.
DEF(63)=1047.
DEF(64)=1062.
DEF(65)=1079.
DEF(66)=1096.
DEF(67)=1109.
DEF(68)=1119.
DEF(69)=1132.
DEF(70)=1144.
DEF(71)=1157.
DEF(72)=1173.
DEF(74)=1207.
DEF(75)=1225.
```

```
DEF(76)=1240.
DEF(77)=1257.
DEF(78)=1274.
DEF(79)=1289.
DEF(80)=1305.
DEF(81)=1322.
DEF(82)=1336.
DEF(83)=1349.
DEF(84)=1366.
DEF(85)=1381.
DEF(86)=1397.
DEF(87)=1409.
DEF(88)=1414.
DEF(89)=1420.
DEF(90)=1428.
DEF(91)=1440.
DEF(92)=1454.
DEF(93)=1467.
DEF(94)=1480.
DEF(95)=1494.
DEF(96)=1506.
DEF(97)=1520.
DEF(98)=1535.
DEF(99)=1553.
DEF(100)=1570.
DEF(101)=1587.
DEF(102)=1602.
DEF(103)=1620.
DEF(104)=1635.
DEF(105)=1651.
DEF(106)=1665.
DEF(107)=1679.
DEF(108)=1693.
DEF(109)=1705.
DEF(110)=1716.
DEF(111)=1722.
DEF(112)=1732.
DEF(113)=1747.
DEF(114)=1760.
DEF(115)=1774.
DEF(116)=1788.
DEF(118)=1812.
DEF(119)=1824.
DEF(120)=1836.
RETURN
END
```

APPENDIX C

SUBROUTINE BEGBLYT

```
      SUBROUTINE BEGBLYT
*
*
*
**************************************************************************
* THIS SUBROUTINE CALCULATES : NUMBER OF SPORES / 24 M**3 / DAY
*                            : CUMULATIVE SPORES
*                            : LESIONS / PLANT
*                            : CUMULATIVE LESIONS / PLANT
*                            : TOTAL INFECTED LEAF AREA IN CM**2
*                            : INFECTED LEAF AREA CM**2 / DAY
* VARIABLES INCLUDE:
*      AILA    = ADJUSTED INFECTED LEAF AREA
*      AIV     = AVERAGE INDEX VALUE OCER LAST THREE DAYS.
*      AIVS    = TABLE OF AVERAGE INDEX VALUES
*      AIVX    = X COORDINATE OF AIV TABLE
*      AIVY    = Y COORDINATE OF AIV TABLE
*      ALNNES  = NATURAL LOG OF LESION NUMBERS
*      ALNSPOR = NATURAL LOG OF SPORE NUMBERS
*      ATEMP   = THREE DAY AVERAGE  OF AVERAGE TEMPERATURES
*      AVETEMP = AVERAGE TEMPERATURE OVER THREE DAY PERIOD.
*      AVPD    = THREE DAY AVERAGE OF VAPOR PRESSURE DEFICITS
*      DLTALES = LESIONS ATTRIBUTED TO CURRENT DAY
*      ELES    = NUMBER OF EXPANDING LESIONS (BLIGHT) / DAY
*      HIGHTMP = HIGH TEMPERATURE FOR THE DAY IN CENTIGRADE.
*      ILA     = INFECTED LEAF AREA IN CM**2 / PLANT
*      LES     = TOTAL NUMBER OF LESIONS / PLANT
*      LESSUM  = SUM OF DLTALES
*      LOWTEMP = LOW TEMPERATURE FOR THE DAY IN CENTIGRADE.
*      NTOTILA = NATURAL LOG OF TOTILA INFECTED LEAF AREA
*      RILA    = REMAINING INFECTED LEAF AREA WHICH HAS POTENTIAL TO
*                   RELEASE SPORES
*      RH      = RELATIVE HUMIDITY.
*      SEVEN   = COUNTER FOR DETERMINING WHEN A SPRAY IS NO LONGER
*                   EFFECTIVE
*      SPORREL = SPORES RELEASED
*      SPORSUM = SUM OF SPORES RELEASED
*      TEMPDTA = ARRAY OF TEMPERATURES AND RELATIVE HUMIDITIES
*      TILA    = ARRAY OF TOTAL INFECTED LEAF AREAS OVER 4 DAY PERIOD.
*      TOTILA  = TOTILA INFECTED LEAF AREA
*      VPD     = VAPOR PRESSURE DEFICIT.
*      VPDFCT  = SUBROUTINE THAT CALCULATES VAPOR PRESSURE DEFICITS
```

```
*
*      FUTURE IS USED TO COMPUTE (TODAY + 3) POSITION FOR THE
*                REVOLVING FOUR ELEMENT ARRAY INFECTED LEAF AREA.
*      LASTVAL IS USED TO COMPUTE YESTERDAYS INFECTED LEAF AREA).
**********************************************************************
**********************************************************************
      IMPLICIT REAL (A-Z)
      COMMON/TEMPS/HIGHTMP,LOWTMP,RH
      DIMENSION TILA(4),INDXVAL(3),TEMPDTA(3,2)
      INTEGER AIVS(32,17),TEMPLOC
      INTEGER TODAY,LASTVAL,FUTURE,SEVEN,IVCNT,INDX,J,ANS,AIVX,AIVY
      RILA=0.0
      TOTILA=0.0
      ELES=0.0
      DLTALES=0.0
      YESTLES=0.0
      LES=0.0
      SPORSUM=0.0
      SEVEN=0
      LESSUM=0.0
      TODAY=-1
      INDX=0
      SRCMILA=0.0
      SPORREL=0.0
      TEMPLOC=0
      MAX=225
**********************************************************************
*** READ IN TABLE OF AIV VALUES                                    ***
**********************************************************************
      DO 50 I=1,32
        READ(1,'(1714)')(AIVS(I,J),J=1,17)
   50 CONTINUE
**********************************************************************
*** READ IN INITIAL INFECTED LEAF AREA                             ***
**********************************************************************
      PRINT*,'INITIAL INFECTED LEAF AREA IS  '
      READ*,TILA(1)
      ILA=TILA(1)
**********************************************************************
*** ZERO OUT THE REST OF THE INFECTED LEAF AREA ARRAY              ***
**********************************************************************
      DO 75 J=2,4
        TILA(J)=0.0
   75 CONTINUE
**********************************************************************
*** INITIALIZE TEMPDTA ARRAY TO THE SAME FIRST VALUE OF AVETEMP AND VPD
**********************************************************************
      AVETMP=(HIGHTMP+LOWTMP)/2
      CALL VPDFCT(AVETMP,RH,VPD)
      DO 85 J=1,3
        TEMPDTA(J,1)=AVETMP
        TEMPDTA(J,2)=VPD
   85 CONTINUE
      RETURN
```

```
*
      ENTRY BLIGHT (DUMMY)
*** CALCULATE AVERAGE TEMPERATURE FOR THE DAY ***
      AVETMP=(HIGHTMP+LOWTMP)/2
*** CALCULATE VAPOR PRESSURE DEFICIT ***
      CALL VPDFCT(AVETMP,RH,VPD)
      TEMPLOC=TEMPLOC+1
      IF (TEMPLOC.EQ.4) TEMPLOC=1
      TEMPDTA(TEMPLOC,1)=AVETMP
      TEMPDTA(TEMPLOC,2)=VPD
*************************************************************************
*** CALCULATE SPORULATION INDEX VALUE                                 ***
*************************************************************************
      ATEMP=0.0
      AVPD=0.0
      DO J=1,3
        ATEMP=ATEMP+TEMPDTA(J,1)
        AVPD=AVPD+TEMPDTA(J,2)
   10 CONTINUE
      ATEMP=ATEMP/3
      AVPD=AVPD/3
      AIVX=NINT(AVPD*4.0)+1
      AIVY=NINT(AVETMP)-7
      AIV=AIVS(AIVX,AIVY)
*************************************************************************
*** ROTATE THE INFECTED LEAF AREA ARRAY                               ***
*************************************************************************
      TODAY=MOD(TODAY+1,5)
      IF (TODAY.EQ.0) TODAY=1
      LASTVAL=MOD(TODAY-2,4) + 1
      FUTURE=LASTVAL
*************************************************************************
*** ASSIGN TODAYS PREDICTED INFECTED LEAF AREA                        ***
*************************************************************************
      TILA(FUTURE)=ELES
*************************************************************************
*** CALCULATE TOTAL INFECTED LEAF AREA                                ***
*************************************************************************
      IF (ILA.LT.0.0) ILA=0.0
      TOTILA=TOTILA + ILA
      IF (TOTILA.LE.0.0) THEN
        NTOTILA=0.0
      ELSE
        NTOTILA=ALOG (TOTILA)
      ENDIF
      PRINT*, 'TOTILA IS****' , TOTILA, '****', NTOTILA
*************************************************************************
*** END TOTAL INFECTED LEAF AREA CALCULATION                          ***
*************************************************************************
*** CALCULATE THE ADJUSTED INFECTED LEAF AREA, SPORES RELEASED, AND    *
*** REMAINING INFECTED LEAF AREA WHICH HAS POTENTIAL TO RELEASE SPORES *
*************************************************************************
      YESTLES=LES
      AILA=ILA+RILA
```

```
      SPORREL=AIV*AILA*MAX
      RILA=(1-AIV)*AILA
**********************************************************************
*** END SPORE RELEASE CALCULATIONS                                 ***
**********************************************************************
*** CALCULATE CURENT DAYS LESIONS                                  ***
**********************************************************************
      IF ((SPORSUM.GE.0).AND.(SPORSUM.LT.21000)) THEN
        LES=(0.75*SPORSUM/10)
      ELSEIF ((SPORSUM.GE.21000).AND.(SPORSUM.LT. 45000)) THEN
        LES=(0.286*SPORSUM/10)+996
      ELSEIF (SPORSUM.GE. 45000) THEN
        LES=(.08*SPORSUM/10)+1900
      ELSE
      PRINT*,'***NEGATIVE SPORE RELEASE***'
      ENDIF
      DLTALES=LES-YESTLES
**********************************************************************
*** END LESION CALCULATION                                         ***
**********************************************************************
*** SPRAY OPTION                                                   ***
**********************************************************************
      IF (SEVEN.GT.0) THEN
        SEVEN=SEVEN-1
        SPORREL = SPORREL*.75
        DLTALES=DLTALES*.25
      ELSE
        IF (AIV.GE..5) THEN
          PRINT*,'***THERE IS A CHANCE OF A SPORE RELEASE***'
          PRINT*,'DO YOU WANT TO SPRAY****'
          READ(*,'(A10)')ANS
          IF((ANS.EQ.1HY).OR.(ANS.EQ.3HYES)) THEN
            DLTALES=DLTALES*.25
            SPORREL=SPORREL*.75
            SEVEN=7
          ENDIF
        ENDIF
      ENDIF
**********************************************************************
*** END SPRAY OPTION                                               ***
**********************************************************************
*** CALCULATION OF TOTAL LESIONS                                   ***
**********************************************************************
      LESSUM=LESSUM+DLTALES
      IF (LESSUM.LE.0.0) THEN
        ALNLES=0.0
      ELSE
        ALNLES=ALOG (LESSUM)
      ENDIF
      PRINT*, 'LESIONS ARE...',LESSUM,'.....',ALNLES
**********************************************************************
*** END TOTAL LESION CALCULATION                                   ***
**********************************************************************
```

```
*** CALCULATE FUTURE INFECTED LEAF AREA (EXPANDING LESIONS)          ***
*************************************************************************
      IF (DLTALES.LT.0.0) DLTALES=0.0
      IF ((AIV.GE.0.0).AND.(AIV.LT..70)) THEN
        ELES=.2*DLTALES
      ELSEIF ((AIV.GE..70).AND.(AIV.LT..85)) THEN
        ELES=.30*DLTALES
      ELSEIF ((AIV.GE..85).AND.(AIV.LT..95)) THEN
        ELES=.5*DLTALES
        ELSEIF (AIV.GE..95) THEN
        ELES=.55*DLTALES
      ENDIF
*************************************************************************
*** END EXPANDING LESION CALCULATION                                  ***
*************************************************************************
      SPORSUM=SPORSUM+SPORREL
      ALNSPOR=ALOG (SPORSUM)
      PRINT*, 'SPORES RELEASED ARE...',SPORSUM,'.....',ALNSPOR
      IF (LASTVAL.EQ.0) LASTVAL=4
      ILA=TILA(TODAY)
      PRINT*,'         ***************                '
      PRINT*,' '
      DUMMY=ILA
*************************************************************************
*** BOTTOM OF DAILY LOOP                                              ***
*************************************************************************
      RETURN
      END
```

```
      SUBROUTINE VPDFCT(TEMPC,RH,EDFCT)
*
* THIS SUBROUTINE WILL CALCULATE THE VAPOR PRESSURE DEFICIT
* FROM CENTIGRADE TEMPERATURE AND RELATIVE HUMIDITY BY
* FIRST FINDING THE SATURATION AND AMBIENT VAPOR PRESSURES
*
*SATVP MATEMATICALLY ESTIMATES THE SATURATION V.P.
*
      CALL SATVP(TEMPC,ESAT)
      EAMB=(RH*ESAT/100)
      EDFCT=ESAT-EAMB
      RETURN
      END
*
      SUBROUTINE SATVP(T,E)
      A0=6.107799961
      A1=4.436518521E-01
      A2=1.428945805E-02
      A3=2.650648471E-04
      A4=3.031240396E-06
      A5=2.034080948E-08
      A6=6.136820929E-11
*
      E=A0+T*(A1+T*(A2+T*(A3+T*(A4+T*(A5+A6*T)))))
      RETURN
      END
```

APPENDIX D

PROGRAM BLIGHT (BOLEB)

```
      PROGRAM BLIGHT
************************************************************************
************************************************************************
* THIS PROGRAM CALCULATES    : NUMBER OF SPORES / 24 M**3 / DAY        *
*                            : CUMULATIVE SPORES                       *
*                            : LESIONS / PLANT                         *
*                            : CUMULATIVE LESIONS / PLANT              *
*                            : TOTAL INFECTED LEAF AREA IN CM**2       *
*                            : INFECTED LEAF AREA CM**2 / DAY          *
* VARIABLES INCLUDE:                                                   *
*      AILA    = ADJUSTED INFECTED LEAF AREA                           *
*      AIV     = AVERAGE INDEX VALUE OVER LAST THREE DAYS.             *
*      ALNLES  = NATURAL LOG OF LESION NUMBERS                         *
*      ALNSPOR = NATURAL LOG OF SPORE NUMBERS                          *
*      DLTALES = LESIONS ATTRIBUTED TO CURRENT DAY                     *
*      ELES    = NUMBER OF EXPANDING LESIONS (BLIGHT) / DAY            *
*      ILA     = INFECTED LEAF AREA IN CM**2 / PLANT                   *
*      LES     = TOTAL NUMBER OF LESIONS / PLANT                       *
*      LESSUM  = SUM OF DLTALES                                        *
*      NTOTILA = NATURAL LOG OF TOTILA INFECTED LEAF AREA              *
*      RILA    = REMAINING INFECTED LEAF AREA WHICH HAS POTENTIAL TO   *
*                   RELEASE SPORES                                     *
*      SEVEN   = COUNTER FOR DETERMINING WHEN A SPRAY IS NO LONGER     *
*                   EFFECTIVE                                          *
*      SPORREL = SPORES RELEASED                                       *
*      SPORSUM = SUM OF SPORES RELEASED                                *
*      TILA    = ARRAY OF TOTAL INFECTED LEAF AREAS OVER 4 DAY PREIOD. *
*      TOTILA  = TOTILA INFECTED LEAF AREA                             *
*                                                                      *
*      FUTURE IS USED TO COMPUTE (TODAY + 3) POSITION FOR THE          *
*                REVOLVING FOUR ELEMENT ARRAY INFECTED LEAF AREA.      *
*      LASTVAL IS USED TO COMPUTE (YESTERDAYS INFECTED LEAF AREA).     *
************************************************************************
      IMPLICIT REAL (A-Z)
      DIMENSION TILA(4)
      INTEGER TODAY,LASTVAL,FUTURE,SEVEN,J,ANS
      RILA=0.0
      TOTILA=0.0
      ELES=0.0
      DLTALES=0.0
      YESTLES=0.0
      LES=0.0
```

```
      SPORSUM=0.0
      SEVEN=0
      LESSUM=0.0
      TODAY=-1
      MAX=225
*** READ IN INITIAL INFECTED LEAF AREA***
      PRINT*,'INITIAL INFECTED LEAF AREA IS '
      READ*,TILA(1)
       ILA=TILA(1)
*** ZERO OUT THE REST OF THE INFECTED LEAF AREA ARRAY ***
      DO 75 J=2,4
        TILA(J)=0.0
   75 CONTINUE
*************************************************************************
*** TOP OF DAILY LOOP                                                  ***
*** INPUT THE AVERAGE INDEX VALUE                                      ***
   10 PRINT*,'WHAT IS AIV? '
      READ*,AIV
*** ROTATE THE INFECTED LEAF AREA ARRAY ***
      TODAY=MOD(TODAY+1,5)
      IF (TODAY.EQ.0) TODAY=1
      LASTVAL=MOD(TODAY-2,4) + 1
      IF (LASTVAL.EQ.0) LASTVAL=4
*** ASSIGN TODAYS PREDICTED INFECTED LEAF AREA ***
      ILA=TILA(TODAY)
*************************************************************************
*** CALCULATE TOTAL INFECTED LEAF AREA ***
      IF (ILA.LT.0.0) ILA=0.0
      TOTILA=TOTILA + ILA
      IF (TOTILA.LE.0.0) THEN
        NTOTILA=0.0
      ELSE
        NTOTILA=ALOG (TOTILA)
      ENDIF
      PRINT*, 'TOTILA IS*****' ,TOTILA, '*****', NTOTILA
*** END TOTAL INFECTED LEAF AREA CALCULATION ***
*************************************************************************
*** CALCULATE THE ADJUSTED INFECTED LEAF AREA, SPORES RELEASED, AND   **
*** REMAINING INFECTED LEAF AREA WHICH HAS POTENTIAL TO RELEASE SPORES *
      AILA=ILA+RILA
      SPORREL=AIV*AILA*MAX
      RILA=(1-AIV)*AILA
*** END SPORE RELEASE CALCULATION ***
*************************************************************************
*** CALCULATE CURRENT DAYS LESIONS ***
      YESTLES=LES
      IF ((SPORSUM.GE.0).AND.(SPORSUM.LT.21000)) THEN
        LES=(0.75*SPORSUM/10)
      ELSEIF ((SPORSUM.GE.21000).AND.(SPORSUM.LT. 45000)) THEN
        LES=(0.286*SPORSUM/10)+996
      ELSEIF (SPORSUM.GE. 45000) THEN
        LES=(.08*SPORSUM/10)+1900
      ELSE
```

```
      PRINT*,'***NEGATIVE SPORE RELEASE***'
      ENDIF
      DLTALES=LES-YESTLES
*** END LESION CALCULATION ***
*****************************************************************************
*** SPRAY OPTION ***
      IF (SEVEN.GT.0) THEN
        SEVEN=SEVEN-1
        SPORREL = SPORREL*.75
        DLTALES=DLTALES*.25
      ELSE
        IF (AIV.GE..5) THEN
          PRINT*,'***THERE IS A CHANCE OF A SPORE RELEASE***'
          PRINT*,'DO YOU WANT TO SPRAY****'
          READ(*,'(A10)')ANS
          IF((ANS.EQ.1HY).OR.(ANS.EQ.3HYES)) THEN
            DLTALES=DLTALES*.25
            SPORREL=SPORREL*.75
            SEVEN=7
          ENDIF
        ENDIF
      ENDIF
*** END SPRAY OPTION ***
*****************************************************************************
*** CALCULATION OF TOTAL LESIONS ***
      LESSUM=LESSUM+DLTALES
      IF (LESSUM.LE.0.0) THEN
        ALNLES=0.0
      ELSE
        ALNLES=ALOG (LESSUM)
      ENDIF
      PRINT*, 'LESIONS ARE...',LESSUM,'.....',ALNLES
*** END TOTAL LESION CALCULATION ***
      SPORSUM=SPORSUM+SPORREL
      ALNSPOR=ALOG (SPORSUM)
      PRINT*, 'SPORES RELEASED ARE...',SPORSUM,'.....',ALNSPOR
*****************************************************************************
*** CALCULATE FUTURE INFECTED LEAF AREA (EXPENDING LESIONS) ***
      IF (DLTALES.LT.0.0) DLTALES=0.0
      IF ((AIV.GE.0.0).AND.(AIV.LT..70)) THEN
        ELES=.2*DLTALES
      ELSEIF ((AIV.GE..70).AND.(AIV.LT..85)) THEN
        ELES=.30*DLTALES
      ELSEIF ((AIV.GE..85).AND.(AIV.LT..95)) THEN
        ELES=.5*DLTALES
        ELSEIF (AIV.GE..95) THEN
        ELES=.55*DLTALES
      ENDIF
      FUTURE=LASTVAL
      TILA(FUTURE)=ELES
*** END EXPANDING LESION CALCULATION ***
*****************************************************************************
```

```
      PRINT*,'          ****************          '
      PRINT*,' '
      GOTO 10
*** BOTTOM OF DAILY LOOP ***
*************************************************************************
      END
```