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dissertation entitled

INFLUENCE OF ENVIRONMENTAL AND HOST FACTORS ON INFECTION OF ONION LEAVES AND SUBSEQUENT CONIDIAL FORMATION BY ALTERNARIA PORRI

presented by

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has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Botany & Plant Pathology

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Date 1/16/89

INFLUENCE OF ENVIRONMENTAL AND HOST FACTORS ON INFECTION OF ONION LEAVES AND SUBSEQUENT CONIDIAL FORMATION BY *ALTERNARIA PORRI*

By

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Botany and Plant Pathology

1989

ABSTRACT

INFLUENCE OF ENVIRONMENTAL AND HOST FACTORS ON INFECTION OF ONION LEAVES AND SUBSEQUENT CONIDIAL FORMATION BY *ALTERNARIA PORRI*

By

Kathryne L. Everts

Development of purple blotch (caused by *Alternaria porri* (Ellis) Cif.) on onion is dependent on many environmental and physiological factors. This research program was undertaken to study the influence of some of these factors on infection of onion and subsequent lesion and conidial formation by *A. porri*.

Several weather parameters (temperature, humidity, leaf wetness, rainfall, solar radiation) and *A. porri* conidial release were monitored in an onion plot at the M.S.U. Muck Experimental Farm were monitored in 1985 and in 1987. In both 1985 and 1987 the natural logarithm (ln) of numbers of conidia released during the current day (D-0) was significantly positively correlated with (1) average temperature during periods of low vapor pressure deficit (VPD) on the previous day (D-1), (2) the maximum hourly VPD on D-0, and (3) the ln of conidia released on D-1. A regression equation was developed to predict conidial release (R^2=0.66).

In studies conducted in controlled atmospheric conditions, germination, appressorial formation, formation

of infection hyphae, and lesion development increased as length of dew period increased. There was a significant positive correlation (r=0.78) between increasing length of dew period and increased lesion numbers at 23C.

Purple blotch lesions collected from onion leaves at the M.S.U. Muck Experimental Farm in 1985 were frequently colonized by *Stemphylium botryosum* Wallroth. Four isolates of *S. botryosum* were only weakly pathogenic on onion leaves; however, presence of *S. botryosum* on purple blotch lesions significantly increased lesion size.

Conidial size, germination, and infection rate of *A. porri* conidia were studied after dew periods of variable lengths. After 9, 12, or 16 hours of dew respectively, conidiophores bore conidia capable of germinating, forming flecks, or causing lesions.

This research increased our knowledge of various environmental and host factors which influence the progress of purple blotch on onion and will ultimately aid in planning more effective control measures, including timing of fungicidal sprays to coincide with periods of active sporulation and infection.

To

Mrs. Eda Hill

and

Mrs. Bealuh Ash

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to Dr. Mel Lacy, my major professor for his suggestions, advice, and sharing his computer expertise during the course of this project. I'd also like to thank the other members of my committee, Dr. Stuart Gage, Dr. Karen Klomparens and Dr. Don Ramsdell for their encouragement, suggestions and discussions on this project.

I extend thanks also to Dr. John Hart for graciously consenting to substitute on the committee when the thesis was in its final stages and offering many valuable suggestions.

Thanks also go to Mr. Richard Crum for his assistance in the technical aspects of this project. Several undergraduate students also contributed their help and along with many graduate students provided encouragment, moral support and discussions on science and life. Many thanks to them all.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Introduction

In comparison to all vegetable production in Michigan, dry onions ranked seventh in acreage planted in 1987 with a total of 8,000 acres. In addition, 350 acres were planted to green onions. There are 3 major foliar diseases of onion in Michigan caused by fungi: Botrytis leaf blight (*Botrytis squamosa*, Walker), downy mildew (*Peronospera destructor*, Berkeley) and purple blotch (*Alternaria porri*, (Ellis) Cif.). Extensive studies have been conducted on the epidemiology of Botrytis leaf blight and downy mildew in Michigan or under conditions similar to those in Michigan (James and Sutton, 1985; Lacy and Pontius, 1983; Meredith, 1966; Shoemaker and Lorbeer, 1977; Sutton and Hildebrand, 1985; Vincelli and Lorbeer, 1988). As a result, predictive models have been developed for these two onion diseases (Lacy and Pontius, 1983; Sutton and Hildebrand, 1985). However, purple blotch has not been extensively studied and more information is

necessary for development of control measures for this component of the onion foliar disease problem.

Host

The common onion (*Allium cepa* L.) is an important vegetable crop grown and consumed worldwide (Angell, 1929; Ellis, 1971; Jones and Mann, 1963). It probably originated and was first cultivated in present day Iran and Pakistan (Jones and Mann, 1963). Onion cultivation eventually spread to the rest of Europe and Asia and was brought to the Americas by early immigrants (Jones and Mann, 1963). Onions were cultivated in America as early as 1629 (Thompson, 1949). Today the common onion is widely cultivated throughout the United States. The major onion-growing regions are California, Texas, New York and Michigan (Jones and Mann, 1963; Ware and McCollum, 1975). Many other *Allium* spp. which are susceptible to purple blotch are grown commercially in the United States or are important vegetable crops in other areas of the world (Jones and Mann, 1963).

A. porri causes purple blotch on other *Allium* species such as leek (*A. porrum* L.), the Egyptian onion (*A. cepa* var. *bulbellifera*), Welsh or Japanese bunching onion (*A. fistulosum* L.), garlic (*A. sativum* L.), and the false shallot (*A. cepa* unnamed variety)(Angell, 1929; Ellis, 1971). Although garlic, leek and shallot are commercially grown in the United States, they are more commonly grown and consumed in Europe (Jones and Mann, 1963). The

Japanese bunching onion is widely cultivated in Asia and the Egyptian onion is primarily grown in home gardens (Jones and Mann, 1963; Ware and McCollum, 1975).

Symptoms

Purple blotch symptoms appeared six days after inoculation in experiments performed by Nolla (1927), and conidia formed as soon as five days after lesions first appeared. Twelve days later (16 days after inoculation) the leaves were partly or completely girdled. Lesions on leaves or seed stalks appeared first as sunken grey or white spots one to three days after infection. Three to six days after infection, lesions enlarged and became purple in color (Angell, 1929). The lesions were ovate and showed zonation in a concentric ring pattern. Lesions often girdled the leaf, blighting the leaf distal to the lesion. If numerous infections occurred on a single leaf, the leaf turned yellow and collapsed. As conidia began to form, the lesions became dark brown in color. Purple coloration of lesions has been associated with cool weather, while lesions often appear tan to white in hot weather (Dr. Marvin Miller, personal communication).

Skiles (1953), who tested three species of *Alternaria* for pathogenicity on onions, found that lesions caused by *A. porri* could appear brown to purple in color. He also observed a few lesions caused by *A. alternata* (*A. tenuis*) or *A. tenuissima* infections. These lesions were similar in color and size to lesions caused by *A. porri*. Skiles

is the only researcher to my knowledge who has reported on the pathogenicity of *A. alternata* on onion.

Bock (1961) reported that two distinct lesion types occurred on onions exposed to *A. porri* spores: typical purple blotches, or smaller (0.5-2 mm^2) white irregular spots or flecks. He was unable to isolate the pathogen from the flecks.

Walker (1921) discovered that purple blotch occurred on white onion sets. Decay of the sets began as yellow or wine red spots, which turned dark brown or black. These lesions often appeared near the neck or wound sites. As the semi-watery decay moved through the scale, the tissue became sunken and bulb tissue shrank. Bulbs which were infected in the field rotted as early as 14 days after the bulbs were placed in storage (Bodine and Durrell, 1931). While rot could be very rapid, it could escape detection for periods of from 1 to 5 months (Skiles, 1953).

Lesions on leek were reported to be purple or white with purple centers, elliptical, and 1-4 cm long (Gladders, 1981). Large lesions had concentric rings as a result of alternating light and heavy areas of sporulation.

Nomenclature

The genus *Alternaria*, with the single species *A. alternata (A. tenuis)*, was described by C. G. Nees in 1817 (Elliot, 1917; Wiltshire, 1919). *A. alternata* was characterized as having catenulate conidia which were

obclavate, obpyriform, ovoid or ellipsoidal, often with a conical beak, with up to 8 transverse septa and several longitudinal septa (Figure 1.1a) (Ellis, 1971). Two years later Fries established *Macrosporium* (including the genus *Alternaria* which he did not recognize) as a genus distinct from *Cladosporium, Helminthosporium* and *Sporodesmium* because all species in the genus *Macrosporium* had muriform spores.

Elliot (1917) split the genus *Macrosporium.* He placed all species producing obclavate, cuneate, or ovate muriform spores produced in chains in the genus *Alternaria.* Species with globular, sarcinaeform, cubed or oblong spores without an apex or beak, which had previously been placed in the genus *Macrosporium,* were placed in the genus *Stemphylium.* The genus *Alternaria* included species which typically formed conidia singly. Conidial shape was correlated with catenulation, and all species tested formed spores in chains when placed under suitable conditions (Elliot, 1917).

In 1930 Wiltshire again examined the foundation species and scrutinized the distinction between *Alternaria* and *Macrosporium.* The two genera were separated primarily on whether the spores were borne in chains (*Alternaria*) or singly *(Macrosporium*). Because catenulation (formation of conidial in chains) can be greatly suppressed, and was variable, he questioned its validity as a generic criterion. Beak length of conidia was also variable,

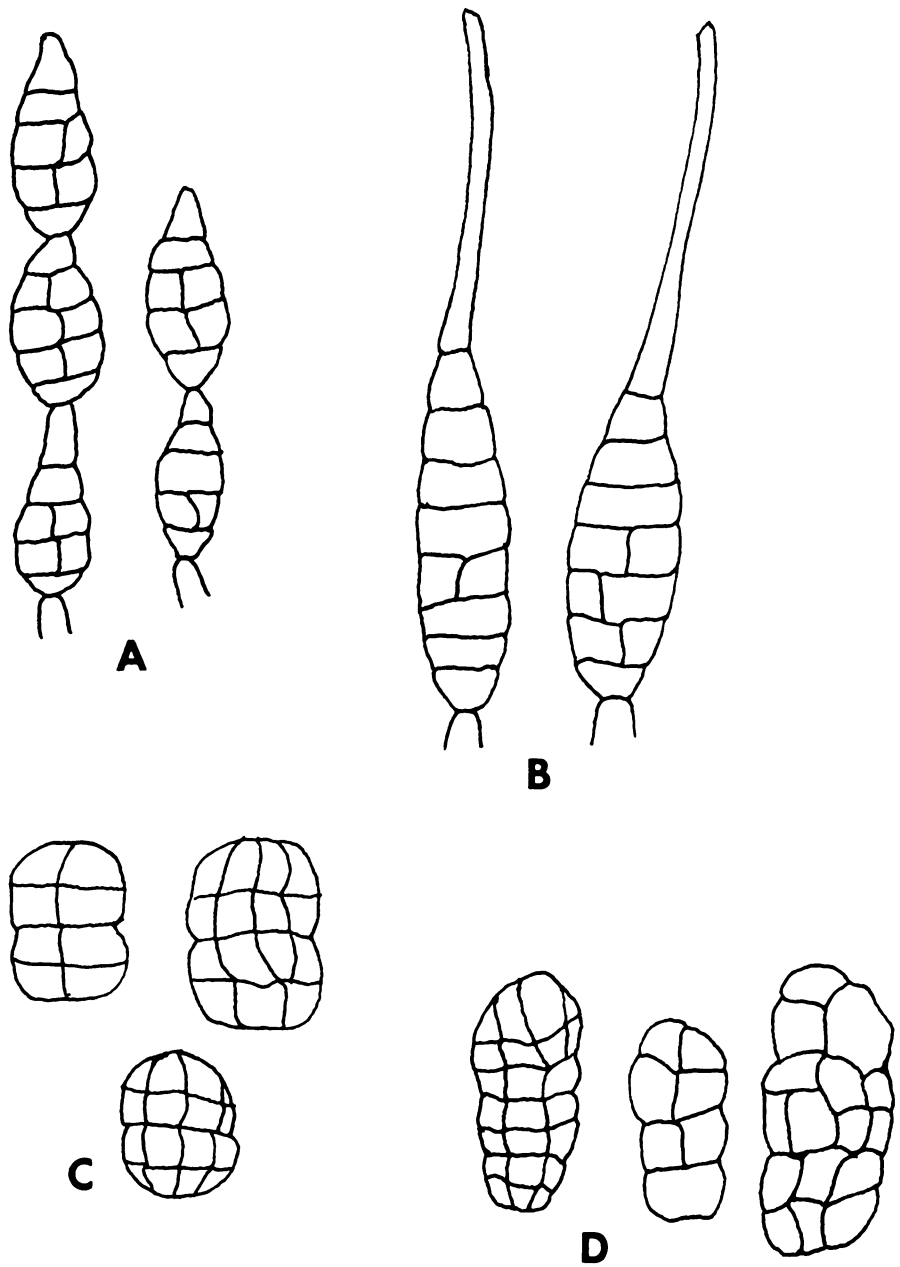


Figure 1.1. Conidial shape of (A.) Alternaria alternata, (B.) A. porri, (C.) Stemphylium botryosum, and (D.) S. vesicarium.

depending on the substrate on which the fungus grew, and was discarded as a generic trait. He concluded that the genus *Macrosporium* should be placed on the list of "nomina ambigua", and all species forming chains of shortly beaked conidia or rarely forming chains with long filiform beaks which had previously been placed in *Alternaria* or *Macrosporium* be consolidated in the genus *Alternaria*. Those species with sarcinaeforme conidia borne singly, without any beak, and usually constricted at a cross-wall, were placed in the genus *Thyrospora*. These genera have since been placed in the genus *Stemphylium* (Figure 1.1c and d) (Wiltshire, 1938).

Longitudinal and horizontal septation, length of beak on the distal end of the spore, and host specificity were found to be characteristics which differentiated species within the genus *Alternaria*. *Alternaria porri* can be distinguished from other species of *Alternaria* because it usually has acatenulate conidia, and the conidium body is ellipsoid and tapers to a beak on the distal end which is about the same length as the conidium proper (Figure 1.1b) (Ellis, 1971). Overall conidium length is usually 100-300 micrometers, and 15-20 micrometers thick at the broadest part. There are 8-12 transverse septae and 0 to several oblique septae (but usually few) in the body of the conidium.

Distribution

Ellis (1879) was the first to isolate *Alternaria* from lesions on leek leaves collected in New Jersey, and he identified it as *A. porri*. Thaxter (1898) reported a disease of onions in 1890 and identified the fungus as *Macrosporium* based on material collected in Connecticut and Maine. Purple blotch was subsequently found in Vermont, Ohio, Massachusetts and Puerto Rico in 1918, Cuba in 1920, and the Philippines in 1923 (Angell,1929; Nolla, 1927). Purple blotch was recorded on bulbs of white onions in Wisconsin and Illinois in 1921 (Walker, 1921) and on onion leaves in New York, Louisiana and Minnesota in 1923 (Angell, 1929). In 1944, purple blotch was reported in Tanzania and in 1949 in Kenya (Bock, 1964). The disease is now generally distributed throughout North America, Central and South America, Australia, New Zealand, Africa, Europe and Asia, including the U.S.S.R. (Ellis, 1971). Purple blotch on leek was first reported in Britain in 1981 (Gladders, 1981), although purple blotch on common onion had been previously reported (but not confirmed) in 1959. In addition to these reports of purple blotch on the leaves of onion plants, Chapman (1910) found that 7 of 10 seed samples tested were contaminated with *A. porri*, and one seed sample carried abundant conidia of the pathogen.

Yield Loss

During epidemics, high levels of damage to onion crops have occurred (Angell, 1929; Nolla, 1927; Skiles, 1953). Purple blotch caused yield loss primarily through loss of leaf tissue and reduced rate of bulb development. Since bulbs are graded and sold according to size, any reduction in bulb size results in loss of revenue to the grower.

Damage due to purple blotch is often very high in the southern and western onion growing regions of the United States, as well as on seed onion crops (Miller, 1983; Skiles, 1953; Angell, 1929). Skiles (1953) reported that purple blotch was a major limiting factor in onion production in the Arkansas Valley of Colorado. Losses ranged from 30 to 50 percent and had been as high as 100 percent. Seed onions in Louisiana sustained 30 to 50 percent losses due to this disease in one year (Angell, 1929). In Puerto Rico, in one year when disease was severe onion plantings were almost completely destroyed (Nolla, 1927).

The age of onions damaged by purple blotch influences the extent of yield loss which will occur (Miller, 1983). Removal of 25% of the leaves to simulate damage caused by *A. porri* up to 10 weeks before harvest did not result in significant yield reductions when compared to the controls. Yields of plants with 75 or 100% leaf loss was

reduced more if loss occurred 9 to 10 weeks prior to harvest rather than 1 to 2 weeks prior to harvest.

Early detection of disease and application of fungicides was critical in reducing foliar damage on onion by *A. porri* (Miller, 1982). When leaf damage was low (10-20%), sprays of mancozeb and chlorothalonil reduced damage to onions over untreated controls (Miller, 1984). However, once leaf damage level reached 20%, leaf damage subsequently increased at approximately the same rate whether the onions were sprayed or unsprayed. The increase in damage occurred in spite of the fact that new infections were reduced; lesions which were already established could girdle the leaf, resulting in death of all tissue distal to the lesion. Growth chamber studies (Miller, 1984) confirmed that four days after inoculation there were significant differences in damage between leaves with 1-3 lesions at the leaf bases and leaves with more than four lesions. These differences disappeared after 6 days.

History

Nolla (1927) reported a severe outbreak of a similar disease in Puerto Rico in 1924 and 1925, and suggested that it be named purple leaf spot. He proposed a new species for the pathogen (*Alternaria allii*). However, from his description of the symptoms, it seems probable that pathogen was *A. porri*. *A. allii* has never been recognized as a valid species.

Angell (1929) discussed incidence of an onion disease in Wisconsin and gave it the name "purple blotch" because he felt the name was more descriptive of the lesions than other names.

Toxin Production

Many *Alternaria* spp. have been shown to produce host specific toxins (Nishimura and Kohmoto, 1983). However all *Alternaria* spp. which produced host specific toxins were morphologically similar to *A. alternata* both in size and catenulation. Zinniol (3-methoxy-4-methyl-5(3-methyl-2-butenyl-oxy)-1,2 benzenedimethanol) is a non-specific toxin which was produced by many acatenulate , long-beaked *Alternaria* spp. including *A. porri* (Cotty and Mishagi, 1984). Several plants were assayed for sensitivity to zinniol and while onion plants were sensitive, they were less sensitive than pinto bean, broccoli, cotton, marigold, safflower, turnip and zinnia (Cotty and Mishagi, 1984).

ENVIRONMENTAL FACTORS AFFECTING GROWTH AND DEVELOPMENT OF *A. PORRI*

Germination

Germination rates of *A. porri* conidia were related to both the temperature and humidity on onion leaf surfaces. Angell (1929) found that germination took place in vitro in 45 minutes to 1.0 hour at 28 to 36C, 1.5 hours at 24C, 1.75 hours at 17.5C and 2.0 hours at 9C. On plants (in vivo), germination began within 3 hours (Angell, 1929).

Nolla (1927) observed that under field conditions germination occurred within 27-40 minutes at night and within 21-35 minutes in the morning. After 3 hours almost every cell in the conidia had germinated.

Rate of germ tube growth increased with temperature, and although amount of germination was not inhibited by sunlight, germ tube growth was reduced. Germination of a majority of spores occurred within 24 hours and more than one germ tube was produced (Fahim and El-Shehedi, 1966). These germ tubes grew variable distances, up to six times conidial length.

The optimal temperature for hyphal growth (as measured on artificial media) was 22-30C, and dropped sharply at 30-34C (Angell, 1929).

Infection

Angell (1929) initially reported that *A. porri* penetrated only through stomata. Walker (1952) and Nolla (1927) reported that penetration occurred through stomata and wounds. Subsequently Fahim and El-Shehedi (1966) found that *A. porri* penetrated onion leaves directly, as well as through stomata or wounds. Once penetration had occurred, hyphae filled the substomatal chamber (in the case of stomatal penetration) or ramified intercellularly and intracellularly (Angell, 1929). This work was generally confirmed by Bock (1964) who showed that appressoria had begun forming within 2 hours after the onset of dew. Optimum temperature for appressoria

formation was 20-25C (Bock, 1964). Appressoria formed either over stomata and penetration occurred through stomata, or direct penetration occurred at some distance from stomata.

Lesion Formation

Lesions formed at 16-30C, and optimum temperature for lesion formation was 20-25C (Bock, 1964). When environmental conditions were not favorable for development of purple blotch lesions, only small white flecks developed. These flecks were often sterile (Bock, 1964). Since these lesions looked atypical, and the pathogen could not be isolated from them, detection of *A. porri* was difficult. More white flecks than typical lesions developed if plants got 6 or fewer hours of high humidity. If plants were exposed to 8 or more hours of high humidity fewer white flecks developed. The incidence of sterile white flecks was highest at low relative humidities, and the proportion of purple lesions to flecks increased as relative humidities rose (Bock, 1964).

The amount of damage caused by purple blotch lesions on onion depended on age of leaf tissue. Miller (1983) found the least amount of damage on the youngest leaves examined and more damage on older leaves. Damage increased as leaf age increased. An individual leaf became more susceptible to damage by *A. porri* as it aged, and emerging young leaves were more susceptible as they emerged closer to bulb maturity.

Sporulation

A. porri does not sporulate well under artificial conditions (Nolla, 1927; Angell, 1929; Skiles, 1953). Sporulation has been induced by wounding mycelia (Angell, 1929). Skiles (1953) increased sporulation by macerating the mycelial mat and then exposing it to direct sunlight for 4 days. Exposure to direct sunlight followed by a 2 day dark period resulted in abundant sporulation on potato agar and moderate sporulation on onion and onion leaf agar (Fahim, 1966). Although maximum sporulation occurred on plates exposed to sunlight for 120 minutes, little sporulation occurred even in these plates. (An average of 26 spores formed per petri dish.) Fahim (1966) also found that infected excised leaves sporulated only if exposed to sunlight.

Light-induced sporulation occurs in many classes of fungi (Turain, 1974). Sporulation in *A. porri* was influenced by light, possibly mediated through photo inactivation of flavins, as in *Alternaria solani* (Lukens, 1963). In several species of *Alternaria* light stimulated the production of conidiophores and inhibited conidium formation (Carlile, 1965; Leach, 1967). Ultraviolet (UV) light greatly enhanced the conidiophore development of *A. porri* on artificial media (Fahim, 1966). Very few conidia developed when hyphae were exposed to 1-50 minutes of UV light. The number of conidia which developed increased as UV light exposure increased to 12 hours.

A. porri conidial morphology and size were shown to vary even on the same lesion (Nolla, 1927). Large and small conidia formed on one lesion could infect and form new lesions which in turn gave rise to both large and small conidia (Nolla, 1929). Older conidia often had branching beaks (Nolla, 1929).

The optimum temperature for spore production on potato agar was 25C (Fahim, 1966). More spores formed on potato agar disks incubated at 90% relative humidity than on disks incubated at 100, 98, 95, 93, 81, 73, 52, 22 or 0% relative humidity. In contrast to earlier reports, wounding did not influence sporulation in Fahims' (1966) studies.

Conidial Survival

Nolla (1927) showed that spore survival was affected by ambient humidity at 78F (25.6C). Spores stored under dry conditions germinated for up to 18 days, and spores stored at high humidity (near saturation) conditions germinated for up to 27 days.

Conidial Release

Early investigators recognized the relationship between weather and disease development. Nolla (1927) reported that epiphytotics of purple blotch followed rainy weather, and Angell (1929) reported that light showers which kept the air saturated were particularly favorable for infection.

The influence of weather on conidial formation and release in unspecified *Alternaria* spp.has also been studied. A volumetric spore trap sampled air above an open field in Rothamsted Experimental Station, Harpenden, England between June and September, 1953 (Sreeramulu, 1959). *Alternaria* spp. had well defined diurnal periodicities. The lowest number of conidia was caught at 0600 hours and the highest at 1400 hours. No attempt was made to separate trapped conidia into species; however the number of beaked *Alternaria* conidia caught peaked approximately two hours earlier than conidia without beaks. During peak hours, most *Alternaria* spores were beaked and about 700 conidia/cubic meter of air were observed.

Low numbers of *Alternaria* conidia were observed between the third week of June and the end of July, whereas high numbers of *Alternaria* conidia were recorded during all of August and the numbers of conidia gradually decreased in September (Sreeramulu, 1959).

A. porri conidia in the air above an onion field in Nebraska had a characteristic and well defined periodicity (Meredith, 1965). Peak concentrations occurred between 8am and 2pm when wind velocity and temperature were rising or high and relative humidity was decreasing or low.

Meredith (1965) also found that if wind speed was relatively high (16-21 m.p.h.), conidia were detected in the air at night. In calm weather, only mature conidia

were found. In stormy weather, mature and immature conidia were also found. Spraying with fungicides or insecticides released many conidia into the air, probably because it caused air turbulence.

Workers in Texas (Miller, 1975) found a significant positive correlation (P=0.6461) between 11 or more hours of leaf wetness and trapping 5 or more *A. porri* spores (the volume of air sampled was not specified) the following day. They also found a significant positive correlation (P= 0.5100) between the occurrence of 14 or more hours of relative humidity above 90% and trapping 5 or more spores the following day. Numbers of purple blotch lesions increased within one week following the increase of *A. porri* spores trapped. Plots sprayed with mancozeb only when there were 12 or more hours of leaf wetness did not differ in disease level from plots sprayed weekly (Miller, 1975).

Survival

A. porri survived over the winter in soil on cull onions and onion debris which remained in the field (Bodine and Durrell, 1931). Angell (1929) placed sporulating cultures of *A. porri* outside his laboratory window in Madison, Wisconsin for the months of December through March. In March conidia were removed from conidiophores and transferred to potato-dextrose agar; 100% germinated. Cultures were incubated at 34C for three months or one year and conidia were still viable.

Mycelia also remained viable after four months incubation in the Wisconsin winter.

Growth in Pure Culture

A. porri hyphal growth was good on oatmeal agar, Cooks II agar, and Czapeks agar; fair on nutrient agar, 3% saccharose agar, 3% dextrose agar, 3% lactose agar, 3% dextrine and 3% maltose agar (Nolla, 1927). Mycelial growth was good, and a few conidia formed, on cornmeal agar, Thaxters' potato dextrose agar, potato saccharose agar, and potato lactose agar. Vegetative growth on sterilized onion scales and leaves was very good. Amount of light present did not influence mycelial growth (Nolla, 1927). Water soluble pigments were produced by *A. porri* when it was grown on most media, but no pigment was produced on sugar free media (Angell, 1929).

CONTROL OF PURPLE BLOTCH ON ONION

Resistant Varieties

Angell (1929) reported that white, yellow, red and brown cultivars of onion were equally susceptible to purple blotch. Later Bock (1964) conducted field trials to determine resistance of onion varieties to *A. porri* and found large differences in susceptibility. Bombay Red was highly susceptible; white creole was fairly resistant; white Mexican and burgundy red were resistant and red creole (both hybrid and open pollinated) and yellow creole were very resistant. All six resistant varieties also

were known to be resistant to neck rot (*Botrytis allii* Munn).

Leaf extracts were tested to determine if they influenced germ tube growth (Bock, 1964). Twenty-five grams of leaf tissue were ground in distilled water (dH_2O) and a series of dilutions were made. There were no differences in *A. porri* germination rate when conidia were placed on media containing extracts of resistant and susceptible varieties. However, all extracts increased germination rate over that in distilled water. There were also no differences in germination rate of spores placed on resistant or susceptible leaf pieces.

Resistance of onion leaves to infection was reduced by prolonged incubation at high humidity or wounding with carborundum (Bock, 1964). Resistance was associated with cuticle thickness and differences in susceptibility between varieties did not vary with environment. (Stomatal number per unit area leaf was similar in both susceptible and resistant varieties.)

Chemical Efficacy

Bordeaux spray reduced purple blotch damage from 40-50% to practically no damage when sprayed with Kayso sticker (1 lb/100 gallons of spray)(Bodine and Durrell, 1931). In addition to reducing disease in the field, sprays also reduced the amount of bulb and neck infection which occurred prior to harvest.

Bock (1964) tested the effect of nine fungicides which were sprayed weekly for 10 weeks on yield. Bulbs were graded to determine how fungicide treatment affected bulb size. Mancozeb (1 lb/acre) and dicloran (1 lb/acre) significantly increased overall yield (tons/acre). Captan (1lb/acre), thiram (2 lb/acre), zineb (1 lb/acre), maneb (1 lb/acre), mancozeb (1 lb/acre) and dicloran (1 lb/acre) significantly increased the tons/acre which fell into the largest bulb size grade (Grade 1). Unfortunately, Bock did not report if these rates were the amount of active ingredient or formulation used per acre. The most significant ($p=0.001$) increase in yield occurred in onions treated with mancozeb and dicloran.

Mancozeb and dicloran sprays applied at 10-day intervals were more effective for purple blotch than sprays applied at 14-day intervals or longer. High (100 to 150 gallons) volume and low (25-30 gallon/acre) volume applications of mancozeb and dicloran were equally effective.

Schwartz (1988) significantly reduced the visual symptoms of purple blotch using mancozeb, metalaxyl + mancozeb (Ridomil MZ58), and iprodione over unsprayed controls. When leaf damage level was 10 to 20%, chlorotholonil and mancozeb sprayed on onions delayed an increase in foliage damage by *A. porri* by 7-10 days and 3-5 days respectively over untreated controls (Miller, 1982). Metomeclan and iprodione sprayed at 7 day

intervals resulted in better control of purple blotch and higher yields than mancozeb and chlorothalonil.

Cultural Control

Several cultural practices such as crop rotation and proper curing and storage can make conditions less favorable for disease development. One to two year crop rotation reduces the amount of overwintering inoculum to which the onion crop is exposed (Bodine and Durrell, 1931). To decrease bulb rot and discoloration in storage, bulbs should be thoroughly cured in the field, and diseased bulbs should be culled (Bodine and Durrell, 1931). Storage at 33-36F (.56 to 2.22C) in low humidity was recommended. Little or no decay occurred at 4C, a moderate amount at 14C, and decay was very rapid at 22C and above (Angell, 1929).

Forecasting and Timing of Fungicide Applications

One ongoing research project in Texas has been the development of a purple blotch disease prediction system based on length of onion leaf wetness periods (O'Higgins, 1988; Naegely, 1985). The predictive system was based on the observation that outbreaks of purple blotch increased when onion leaves were wet for at least 10 hours in a 24 hour period. Temperature may not have been an important factor in disease development in South Texas due to the occurrence of warm temperatures during the growing season which were conducive for disease to occur development.

Biological Control

Fokkema and Lorbeer (1974) examined components of the onion phyllosphere mycoflora for antagonism to *A. porri*. Application of *Aureobasidium pullulans* and *Sporobolomyces roseus* reduced the number of *A. porri* infections by approximately 50%. *A. porri* was more sensitive to antagonism by *A. pullulans* than *Botrytis squamosa* Walker or *Botrytis cinerea* Pers. were. Antagonism was highly variable in different experiments and unrelated to carbohydrate and amino acid concentrations or pH of the leachate on leaves. The saprophytes caused a slight reduction in germination, but not enough to account for the decreased infections. The saprophytes did not influence the number of germ tubes developing per conidium. However, superficial development of germ tubes on leaf surfaces was greatly reduced.

Biological control of *A. porri* by utilizing antagonistic yeasts *A. pullulans* and *S. roseus* has been proposed (Fokkema and Lorbeer, 1974). However, waxy onion leaves provide suboptimum conditions for saprophytic colonization by yeasts which were antagonistic to *A. porri* and maintenance of high populations would be difficult.

Summary

The effect of environment on conidial formation, release, and infection of onion by *A. porri* in a temperate climate such as Michigan has not been extensively studied. Many questions remain on the influence of environmental

factors on these processes. A major portion of this thesis is devoted to examining these factors and using the information to develop an empirical model to predict sporulation of *A. porri* under onion growing conditions. The project can be broken down into two major areas (Figure 1.2.). Examination of lesion formation in an onion plot and in controlled environmental condition will be discussed in chapter III. The study of conidial formation and release was studied at the M.S.U. Muck Experimental Farm (Chapter II) and under controlled environmental conditions (Chapter IV).

Figure 1.2. Project organization.

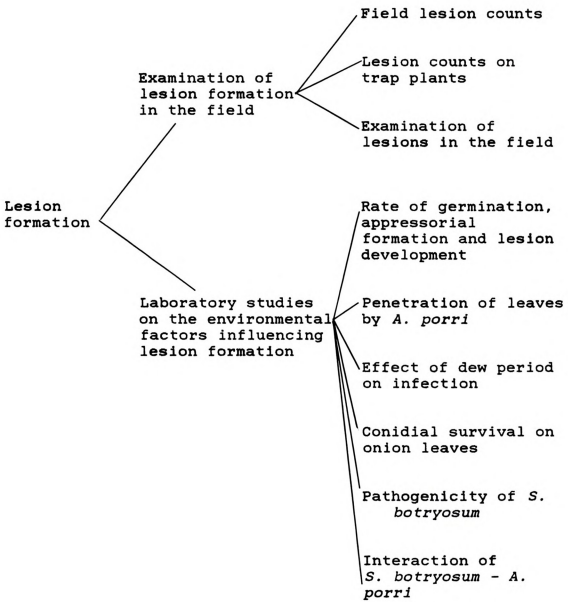
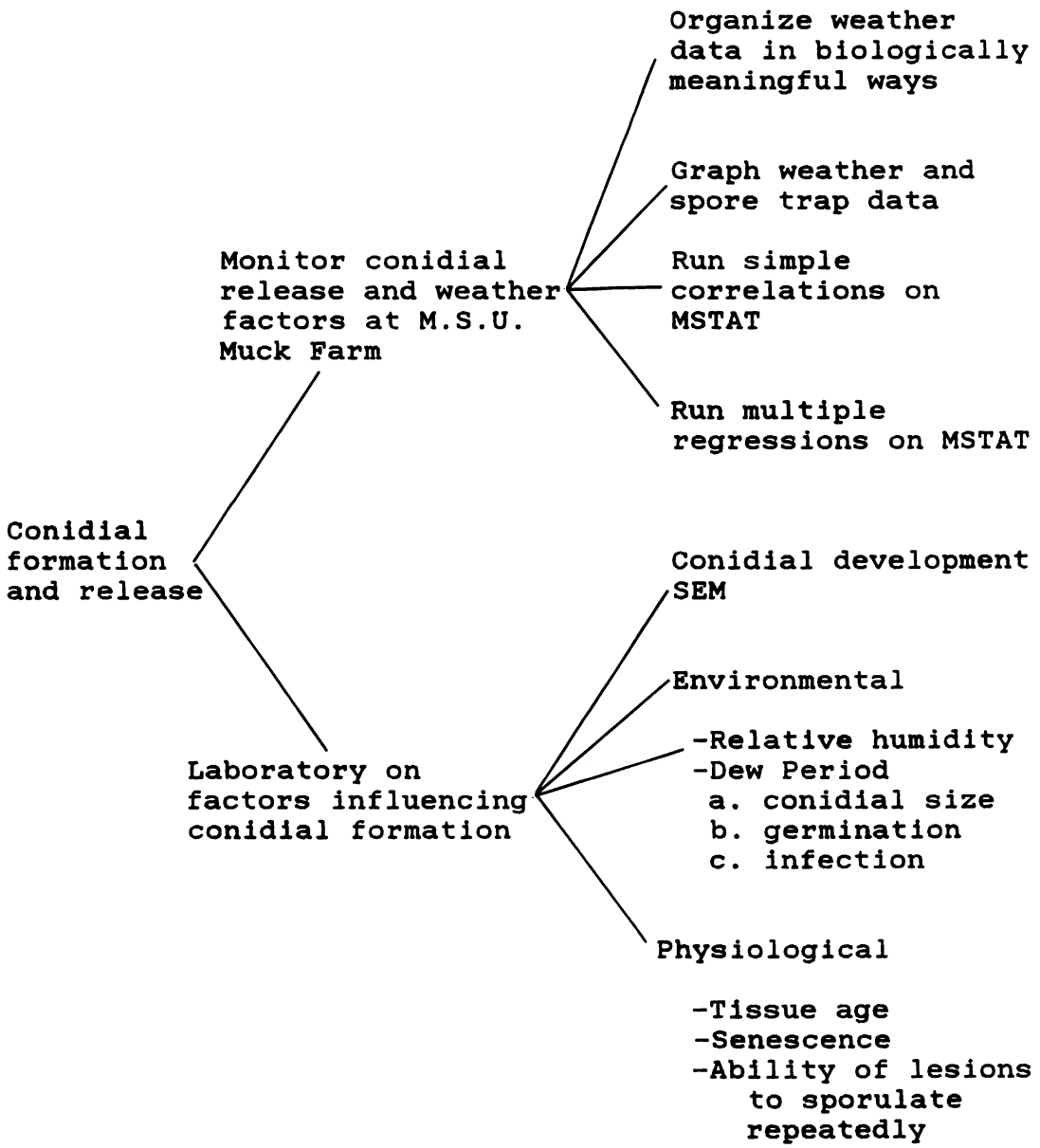


Figure 1.2(cont'd.).



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CHAPTER II

OCCURRENCE OF *ALTERNARIA PORRI* CONIDIA IN THE AIR AND OF PURPLE BLOTCH ON ONION IN THE FIELD

INTRODUCTION

Infection, lesion development and sporulation during the progress of a disease are dependent on many environmental and physiological factors. Understanding these influences can aid in more effective control measures, including timing of fungicide sprays to coincide with periods of active sporulation and infection.

Purple blotch infection is favored by humid weather where air remains near saturation continuously for a 24 hour period (Angell, 1929). High relative humidity (above 80%) under field conditions favors development of expanding lesions (Bock, 1964) while low relative humidity (20-80%) for prolonged periods results in the development of white flecks only (Bock, 1964). Optimum temperatures for infection range from 21 to 30C, and the resistance of an onion plant to purple blotch can be overcome by

incubation for prolonged periods at high relative humidities (Bock, 1964).

The physiological age of the plant also influences infection and lesion expansion. In previous investigations lesion formation was more likely to occur just before and subsequent to bulbing than earlier in plant development (Nolla, 1927). Individual onion leaves were more susceptible to infection as they aged, younger leaves had lower levels of damage and more mature leaves were more heavily damaged (Miller, 1983).

The first event in spread of disease is the formation and release of conidia or other spores into the environment, where they can be transported by air currents to susceptible hosts. The influence of environment on conidial formation of several other *Alternaria* spp. has been studied (Hirst, 1953; Rotem, 1963; Bashi and Rotem, 1975a, 1975b, 1975c and 1976). Hirst (1953) found a well defined periodicity in the presence of conidia of *Alternaria* spp. in the air (no attempt was made to separate the species). When conidia were trapped in a plot of mangolds (a variety of beet used as cattle feed), then grass and flax (linseed), then potatoes, lowest conidia concentrations occurred in the early morning when wind velocities were low and relative humidities were high. Maximum concentrations occurred in the afternoon when wind velocities were high and relative humidities were low. Wind velocity, direction, temperature,

humidity, sunshine, rainfall and dew also influenced the diurnal periodicity of *Alternaria* conidia (Hirst, 1953). Air microflora in the semi-arid region of Israel was examined during periods when early blight was observed on potato and tomato plants (Rotem, 1963). The microflora was primarily composed of *A. solani* and *A. alternata (= A. tenuis)*. Conidial numbers were lowest at night began increasing at 0900 hours, peaked at 1100 hours, and subsequently decreased sharply. Sixty percent of *A. solani* and 70% of *A. alternata* conidia were trapped each day between 1000 and 1400 hours. This coincided with a decrease in relative humidity and an increase in wind velocity. Daily wind velocity and daily conidial release were positively correlated (Rotem, 1963). While dew was the moisture source for conidial formation, conidia were released after dry or partially dry nights leading Rotem to hypothesize that conidia forming on dewy nights may remain on their conidiophores and be released one or more days later.

Relatively little has been reported about conidial formation by *A. porri*. Meredith (1966) trapped *A. porri* conidia over an onion plot in Nebraska to determine periodicity and influence of weather on conidia release. Peak concentrations of *A. porri* conidial occurred between 0800 and 1400 hour, which corresponded to times when wind velocity and temperatures were rising or high, and relative humidities were decreasing or low. The fewest

conidia were trapped daily between the hours of 2000 and 0600. Wind gusts occurring at night increased numbers of conidia caught during the nighttime hours. When plots were sprayed with pesticides the resulting air turbulence greatly increased conidial concentrations in the air. Meredith (1966) also noticed that during calm weather only mature *A. porri* conidia were caught; however, in stormy weather, mature and immature conidia, mycelial fragments, and conidiophores were also trapped. Rainfall greatly decreased numbers of *A. porri* conidia trapped while rain was falling; however, conidial release increased following rain and irrigation. Decreasing humidity seemed to weaken the conidial attachment (Meredith, 1966), but did not cause active conidial release.

There are several predictive models for the timing of fungicide sprays for two other important foliar diseases of onion: Botrytis leaf blight (Lacy and Pontius, 1983; James and Sutton, 1985; Shoemaker and Lorbeer, 1977; Vincelli and Lorbeer, 1988) and downy mildew (Sutton and Hildebrand, 1985). However, until recently there has not been a predictive model for the timing of fungicide sprays for purple blotch on onion (O'Higgins, 1988).

In a study to determine the relationships between environmental conditions and conidial production by *A. porri* and purple blotch incidence, Miller (1975) found a significant positive correlation between the occurrence of at least 11 hours of leaf wetness and the trapping of 5 or

more *A. porri* conidia in an unspecified volume of air during the following day. He also found a significant positive correlation between the occurrence of 14 or more hours of relative humidity above 90% and trapping of 5 or more *A. porri* conidia the following day. In Texas, leaf wetness duration was recorded at four locations and this information alone was used to predict significant releases of *A. porri* conidia (O'Higgins, 1988). This is the only model I know that does not employ temperature to help drive the predictive model.

This study examined the progress of purple blotch disease in the field and the relationship of environmental factors with release of *A. porri* conidia. Although laboratory and field studies had identified high relative humidity, high temperatures, prolonged dew periods, and wind as factors which favor conidial formation, the relationship between the magnitude and duration of these weather parameters and the magnitude of conidia which form as a result was not known. Likewise a relationship between these same weather parameters and physiologically aging plants and lesion formation was known to exist but had not been quantitatively described.

MATERIALS AND METHODS

Presence of *Alternaria porri* and other conidia on lesions from the field

The progress of purple blotch of onion at the M.S.U. Experimental Muck Farm was visually monitored each week

from July 12 to August 30, 1985. On each sample date 25 plants were brought to the lab, and expanding type lesions were identified, excised, mounted on glass slides, stained with 0.01% cotton blue in 85% lacto-phenol, and examined under the microscope. The types of spores present on the lesions were identified and recorded based on their morphology.

Disease progression in the field

Numbers of purple blotch lesions present on onions in an unsprayed plot were monitored at the M.S.U. Muck Farm from July 10 to August 15, 1986 and from July 23 to August 13, 1987. In 1986, numbers of lesions present on 10 plants each in five randomly selected rows, and in 1987 numbers of lesions present on nine foot sections of six randomly selected rows were counted weekly. Only lesions on non-senescent leaves were counted. Lesion counts were discontinued in 1986 and 1987 when most of the onion leaves had fallen over and were senescent.

Occurrence of lesions on trap plants

Ten trap plants also were placed in the field at weekly intervals in 1987 so that fresh, noninfected plants would be in the field each week. These plants were grown from dormant bulbs in a disease-free environment in the greenhouse for 5 weeks, placed in a non-sprayed onion plot at the Muck Farm for one week, then returned to the mist chamber in the greenhouse for 24 hours to enable any spores produced and deposited during that week to infect

and form lesions. Lesions were counted three days later so that numbers of lesions produced on trap plants in a given week could be correlated with numbers of spores produced and released into the atmosphere (as measured by a spore trap in the same plot) that same week.

Effect of weather conditions on conidial formation and release

Weather conditions were monitored hourly at the M.S.U. Muck farm during 1985 and 1987. Temperature and humidity were monitored using a Weathermeasure (P.O. Box 41257, Sacramento, CA, 95841) model H311 or Weathertronics (2777 Del Monte St., West Sacramento, CA, 95691) model 5021 hygrothermograph, rainfall using a Weathermeasure model P501 remote recording rain gauge, leaf wetness using a Dewitt leaf wetness meter, intensity of direct and indirect solar radiation using a Weathermeasure model 3010-01 mechanical pyranograph, and wind speed and direction using a Weathermeasure model W123 recording 3-cup anemometer and airfoil vane. Numbers of conidia present hourly were counted after being caught in a Burkard (Rickmansworth, Herts, England) seven day recording volumetric spore trap. After conidia were counted, the data were transformed by adding one to each daily total (so that no values of zero were present in the data) and taking the natural log [ln (Y+1)] of each number. Natural log transformations were necessary because variances in the data were high (Steel and Torrie,

1980). Simple correlations between the weather data and daily conidial releases between 0700 the previous day (D-1) and 0600 the current day (D-0) were determined using the MSTAT statistical package (Dept. of Crop and Soil Sciences, Michigan State University). Weather variables which most influenced conidial formation and release were identified and multiple regressions between the weather data and daily conidial releases between 0700 on D-1 and 0600 on D-0 were also run. The procedures used in development of this model are summarized (Figure 2.1.).

RESULTS

Presence of *Alternaria porri* and other conidia on lesions from the field

The first lesion associated with *Alternaria porri* conidia appeared on July 26 (Table 2.1). This lesion had only *A. porri* spores associated with it. However, all lesions examined on August 2, 7, 21 and 30 on which *A. porri* was actively sporulating also supported sporulation of one or more spores of the following fungi: short-tailed *Alternaria, Stemphylium,* or *Peronospora* spp. Few typical purple blotch lesions were actively sporulating on any sample date. Four percent of lesions examined had *A. porri* sporulating on July 26, 12% on August 2, 4% on August 7, 8% on August 21 and 24% on August 30.

Though *Stemphylium* and short-tailed *Alternaria* spores were present on purple blotch lesions on almost all sample

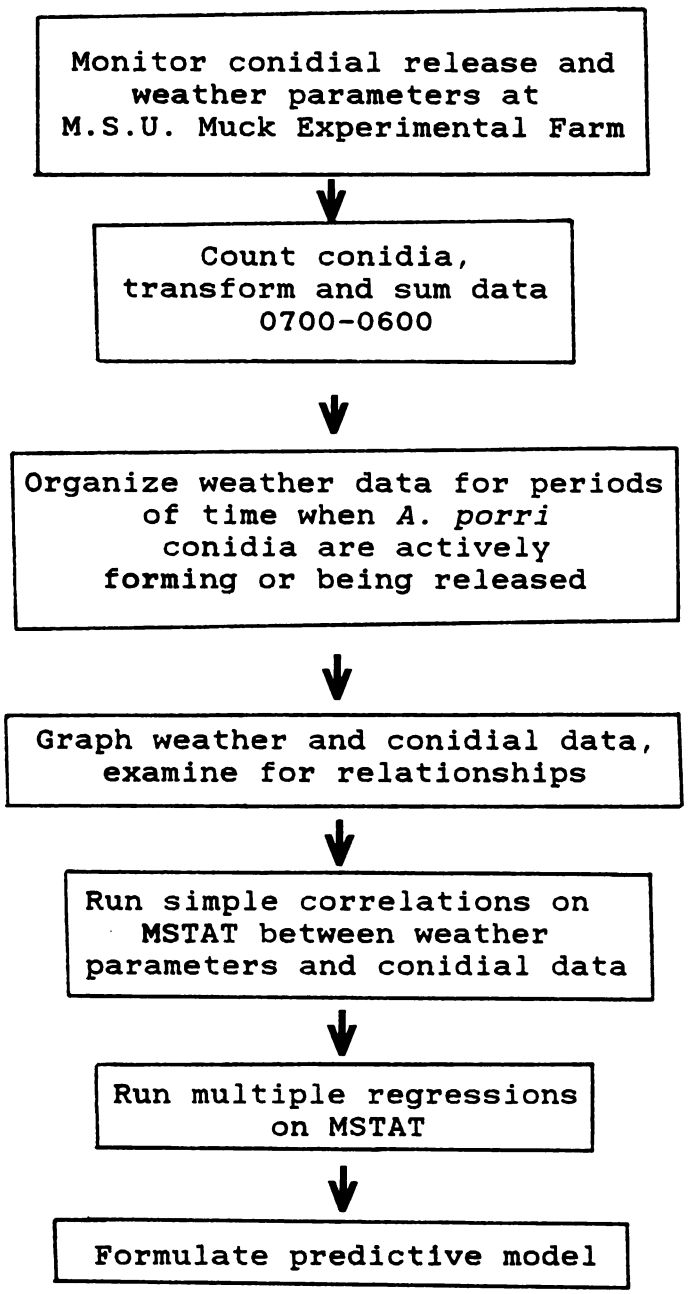

Figure 2.1. Flow chart of the steps used in the development of an empirical conidial formation and release prediction model for *A. porri* on onion.

dates, *Stemphylium* was more prevalent at later sample dates.

Most lesions on which *A. porri* was observed were elliptical lesions with alternating dark and light concentric rings ranging in color from purple, red brown, light brown to brown. Lesions ranged in size from 0.75 x 0.3cm to 4.0cm x 1.5cm. *A. porri* infection did not appear to be related to tip die back ("tip burn") and conidia were only detected twice on leaves with approximately 5cm tip die back.

Table 2.1. Number of lesions examined on which various fungi were observed on onion plants collected from the M.S.U. Muck Farm during July and August, 1985.

	No. of lesions/25 examined on which fungi were observed		
Date	*Alternaria porri*	*Stemphylium* spp.	*Alternaria* spp.(catenulate)
July 12	0	1	1
19	0	1	15
26	1	2	7
August 2	3	7	11
7	1	21	0
21	2	22	2
30	6	25	8

Disease progression in the field

Lesions appeared sooner (July 23) in 1987 than in 1986 (July 31) and onions in all rows had lesions on every observation date in 1987 (Table 2.2.). As in 1986, older

susceptible leaves became senescent, lesions coalesced, and leaves died so there appeared, at times, to be a decrease in lesion numbers when lesions on dead leaves (counted the previous but not the current week) outnumbered new lesions.

Table 2.2. Average numbers of lesions/plant present on onions monitored at the M.S.U. Muck Farm in 1986 and 1987.

Date		1986	1987
July	10	0[a]	-
	17	0	-
	23	0	0.28[b] ± 0.05[c]
	31	0.50 ± 0.12	1.48 ± 0.05
August	7	0.38 ± 0.16	0.94 ± 0.06
	13	0.56 ± 0.24	-
	15	-	1.26 ± 0.10

[a] Mean of 5 sample rows.
[b] Mean of 6 sample rows.
[c] Standard error.

Occurrence of lesions on trap plants

Lesion formation on trap plants placed in the field varied over the 6 sample periods in 1987 (Figure 2.2.). Larger numbers of lesions present did not generally coincide with weeks when large spore releases occurred. However, there were some dates when large spore releases preceded a high infection rate. The increase in lesions on trap plants removed from the field on September 1 (day 243) probably resulted from large spore releases between the afternoon of August 30 (day 241) through September 1.

Figure 2.2. Lesions per trap plant and the ln of the number of conidia trapped daily during the time the plants were in an onion field plot at the M.S.U. Muck Farm in 1987.

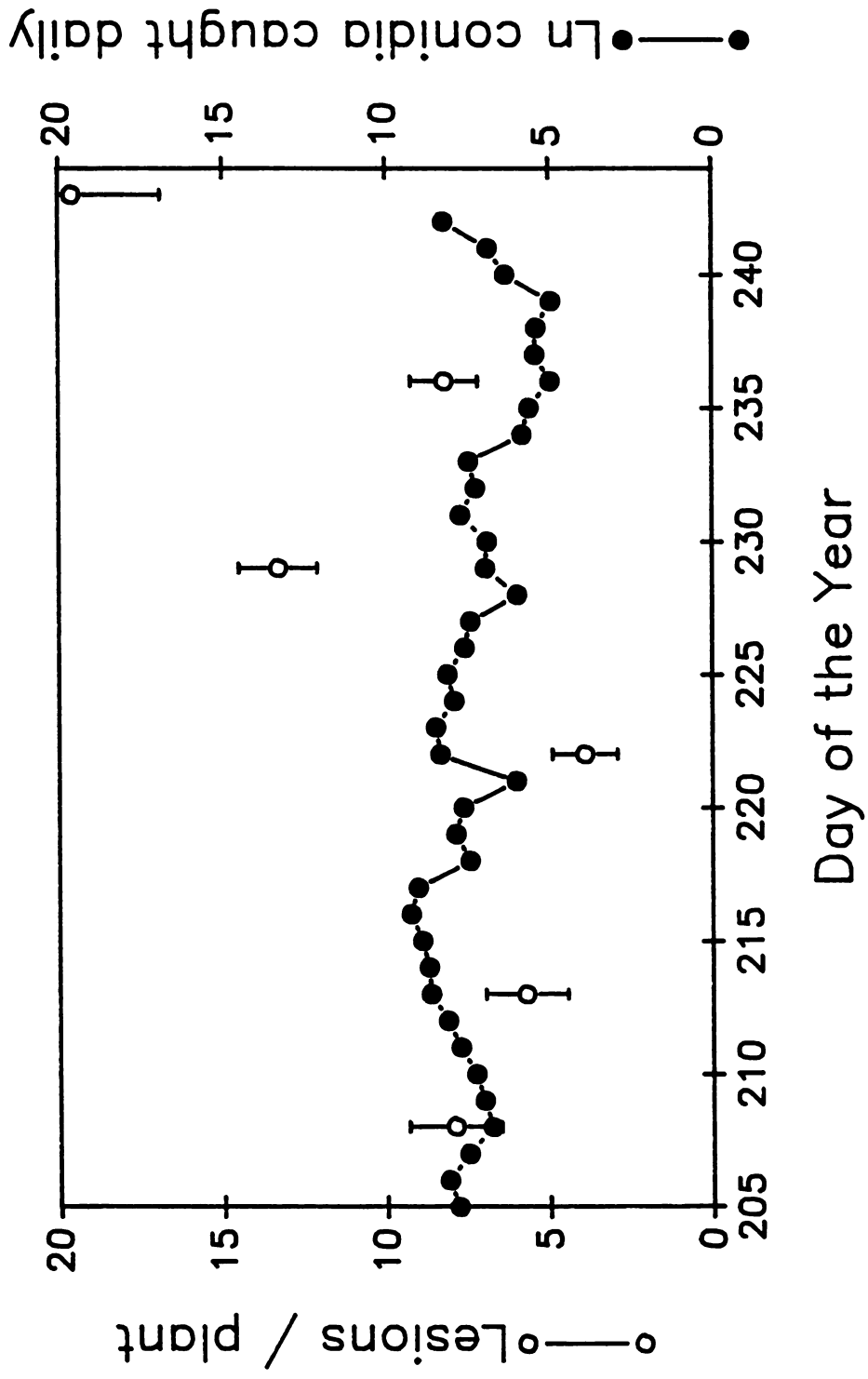
●—●Ln conidia caught daily
o—oLesions / plant
20
15
10
5
0
205
210
215
220
225
230
235
240
Day of the Year

Figure 2.2.

Effects of weather conditions on conidial formation and release

In 1985 conidial release and weather conditions were monitored for 68 consecutive days (June 28 to September 3). In 1987 conidial release and weather conditions were monitored during 48 consecutive days (July 13 to August 31). Conidial release had defined diurnal periodicity (Figure 2.3.). Few conidia were released between 0100 and 0800, the number of conidia trapped began to increase after 0800 and stayed high throughout the afternoon and evening (1200-2200). Because of the diurnal periodicity, the cumulative spore catches were tabulated from 0700 on the current day to 0600 the following morning. Spore catches varied from 0 to 5420 and from 137 to 10433/ 24 cubic meter of air per day in 1985 and 1987 respectively.

Once the weather data were transformed as described previously, hourly data were summed over 24, 12, 6, or 4 hour periods where appropriate, or over other time periods based on observations of weather and conidial release relationships. For example, hourly temperatures for the 12 hour period 0700-1900 were summed and averaged, as well as temperatures during hours of leaf wetness or low (<1.0 mb) VPD. In other cases, a single hourly value was identified as significant (for example, highest hourly VPD on the current day). After weather variables were compiled as described above, they were graphed against conidia released between 0700 on D-1 and 0600 on D-0.

Figure 2.3. Hourly *A. porri* conidial release at the M.S.U. Muck Farm beginning at midnight on August 1 in 1985 and 1987.

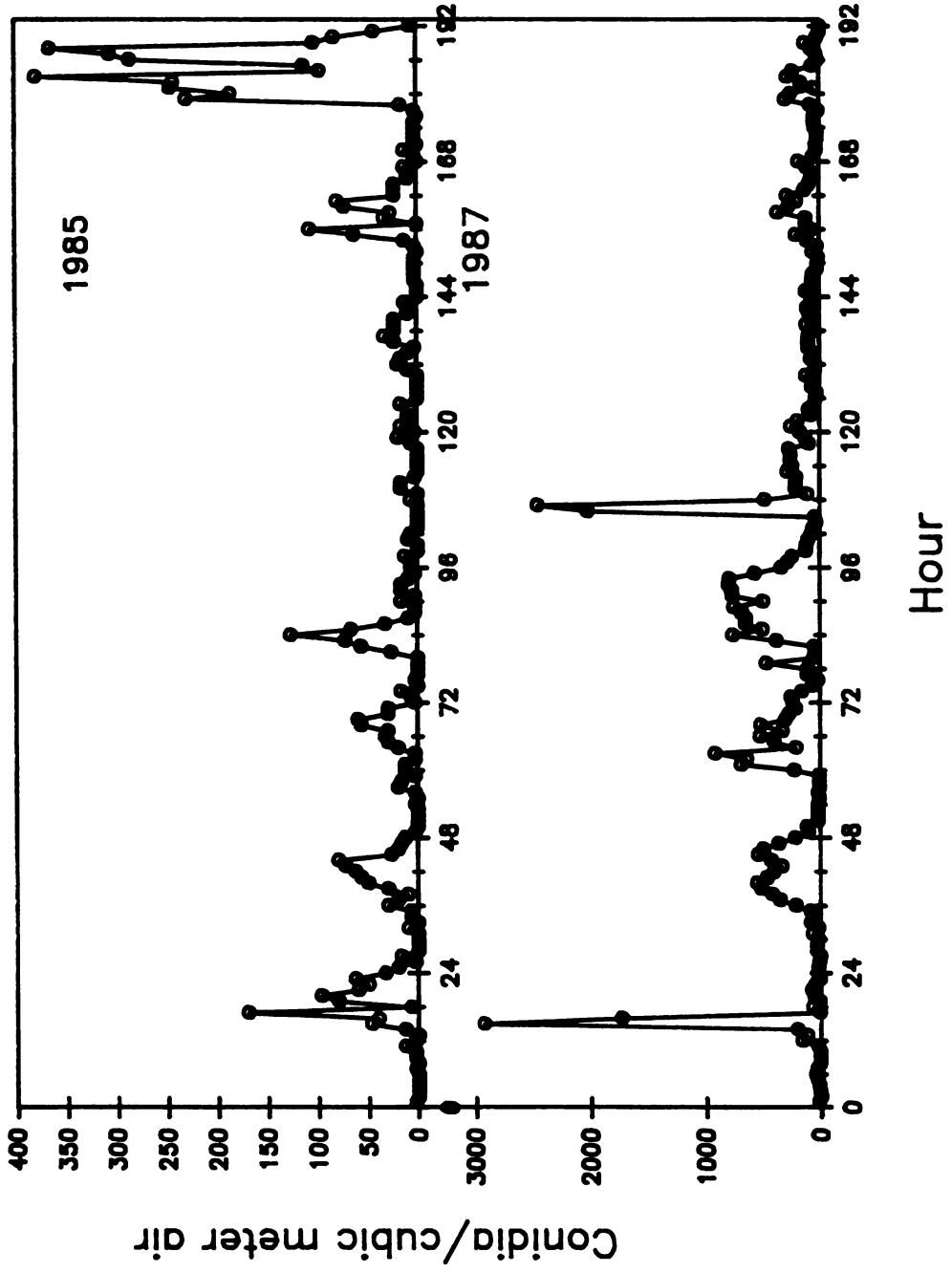
1985
1987
Conidia/cubic meter air
Hour
400
350
300
250
200
150
100
50
0
3000
2000
1000
0
24
48
72
96
120
144
168
192

Figure 2.3.

Graphs were examined for the presence of visual relationships. Examples of typical graphs used to compare these data are shown in Figure 2.4.

The number of hours the vapor pressure deficit (VPD) was < 1.0 mb on D-1, the average temperature during these low VPD hours (D-1), the single highest VPD on D-0, and ln of the number of spores released on D-1 were significantly positively correlated with the ln of the number of spores released on D-0 in 1985 (Table 2.3).

Table 2.3. Environmental factors in 1985 which were strongly positively correlated with the ln of conidia released during the current day (D-0).

Variable	[a]Simple correlation coefficient (r)
Hours of low VPD (<1.0 mb) the previous day (D-1)	.286*
Average temperature during low VPD periods (<1.0 mb) on D-1	.292*
Maximum hourly VPD on D-0	.263*
ln of conidia released during D-1	.535**

[a] Number of days sampled = 67.
Significant at * P = 0.05; ** P = 0.01

In 1987 (unlike 1985) the number of hours the VPD was < 1.0 mb on D-1 was negatively correlated with the ln of the number of spores produced on D-0 (Table 2.4.). The average temperature during periods of low VPD (<1.0mb) on D-1, maximum hourly VPD on D-0, average VPD on D-1, average temperature during hours of leaf wetness on D-1, and the ln of the conidia released on D-1 were also

Figure 2.4. Weather parameters and spore release at the M.S.U. Muck Farm between August 12-27, 1987.

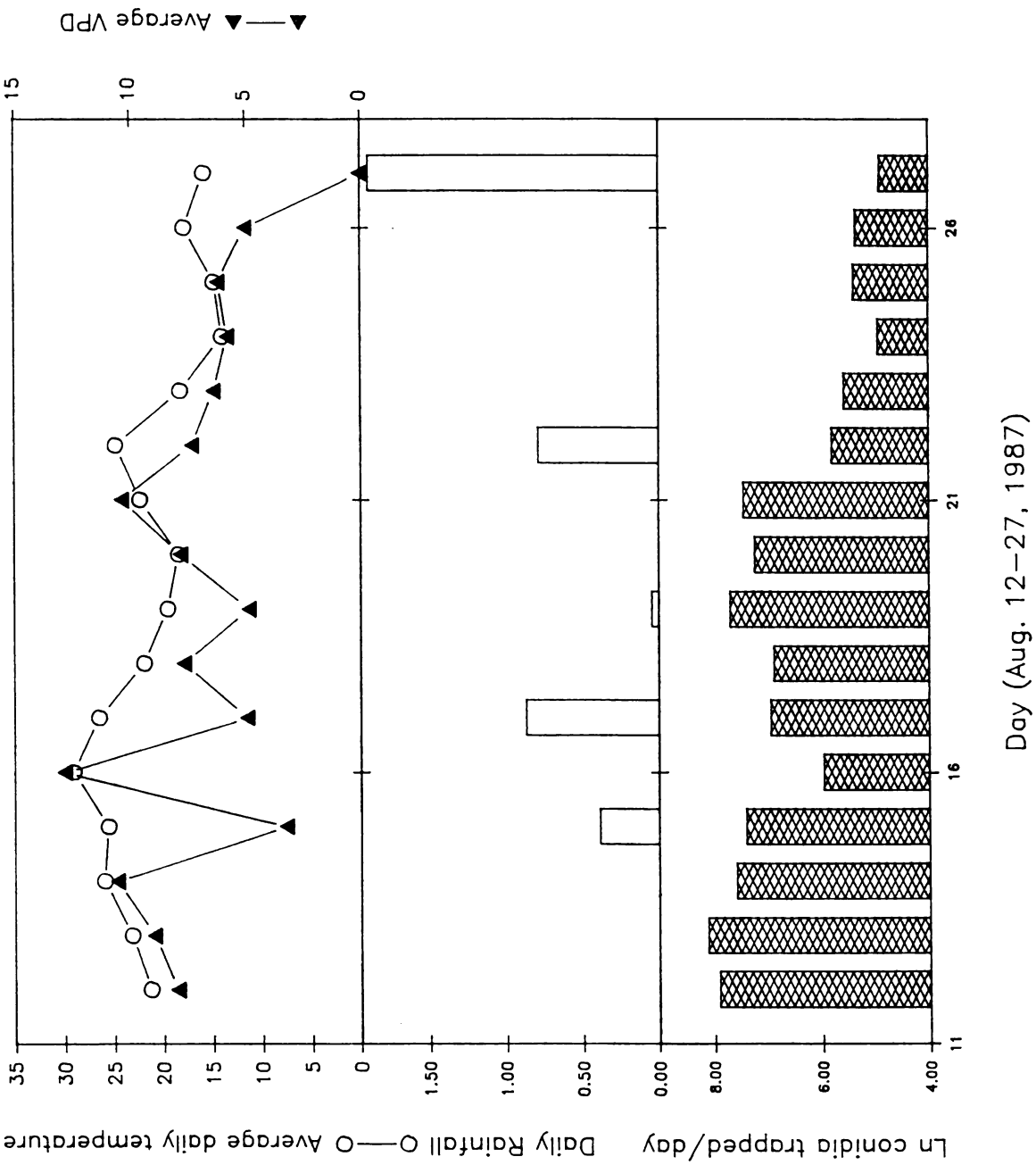
▲—▲ Average VPD
15
10
5
0
35
30
25
20
15
10
5
0
1.50
1.00
0.50
0.00
8.00
6.00
4.00
11
16
21
26
Day (Aug. 12–27, 1987)
Ln conidia trapped/day
Daily Rainfall O—O Average daily temperature (C)

Figure 2.4.

significantly positively correlated with the ln of the conidia released D-0 in 1987.

Table 2.4. Environmental factors in 1987 which were strongly correlated with the ln of the conidia released during the current day (D-0).

Variable	[a]Simple correlation coefficient (r)
Hours of low VPD (<1.0 mb) on the previous day (D-1)	-.326*
Average temperature during periods of low VPD (<1.0 mb) on D-1	.500**
Maximum hourly VPD on D-0	.466**
Average VPD on D-1	.329*
ln of conidia released on D-1	.701**
Average temperature during hours of leaf wetness on D-1	.532**
Hours rain 0800-2400 on D-0	-.286*

[a] Number of days sampled = 48.
Significant at * P = 0.05; ** P = 0.01

Multiple regressions were run to determine the best equation to predict spore release. Conidial release in 1987 was most accurately predicted (R^2=0.66) based on the natural logarithm of the number of conidia released the previous day, high temperatures during those hours when the VPD was < 1.0 on D-1, and the sum of the numbers of hours when rainfall occurred on D-0 (Figure 2.5.B). Since it is impossible to determine the ln of the number of spores produced yesterday (D-0) during a growing season, regressions were run to determine the best predictive

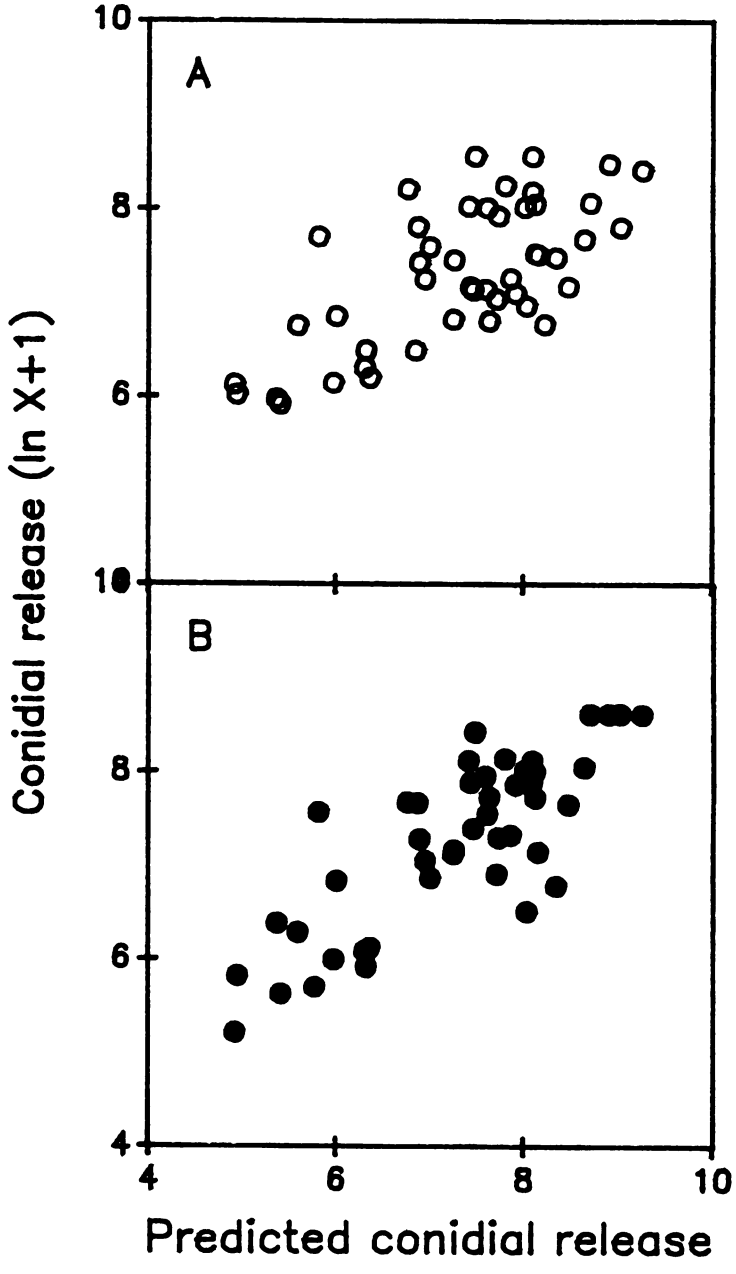

Figure 2.5. Comparison of predicted and observed conidial release (natural logarithm (ln) of the number of conidia trapped + 1) at the M.S.U. Muck Farm in 1987. (A.) Prediction was based on the temperature during hours of VPD less than 1.0 mb on D-1 and the maximum VPD on D-0. (B.) Prediction was based on the ln of the number of conidia released the previous day (D-0), the temperature during hours when VPD was less than 1.0 mb on D-1, and the total number of hours rain occurred on D-0.

equation which could be constructed without this factor. Excluding the ln of conidia produced on D-1, the average temperature during periods of low (< 1.0mb) VPD on the previous day (D-1) was the parameter most highly correlated with the ln of conidia released each day in both years data (Figure 2.6.).

The regression equation which best predicted the number of conidia released on a given day (1987 data) when conidial release the previous day was unknown was based on the average temperature during periods of low (<1.0 mb) VPD on D-1 (X_1), the maximum hourly VPD on D-0 (X_2), and the prediction of ln of the number of conidia released during the previous day (based on temperature during periods of low VPD on D-1, and the maximum hourly VPD on D-0) (X_3) (Equation 2.1.)

Equation 2.1. $\hat{Y} = 2.182 + 0.09296X_1 + 0.03478X_2 + 0.039185X_3$

Where Y = estimated number of conidia released on D-0.

This equation described about 48% (R^2 = 0.478) of the variation in actual conidial release in 1987 (Figure 2.7). There was generally good coincidence between numbers of conidia predicted and those actually trapped; however, the equation generally overpredicted low conidial releases and under predicted high conidial releases (Figure 2.5.a). When the number of conidia released was predicted using the 1985 weather data, there was a positive correlation between predicted and observed conidial release (r=.443).

Figure 2.6. Numbers of *A. porri* conidia released per 24 cubic meters of air in relation to temperatures during periods of low VPD (<1.0 mb) the previous day (D-1).

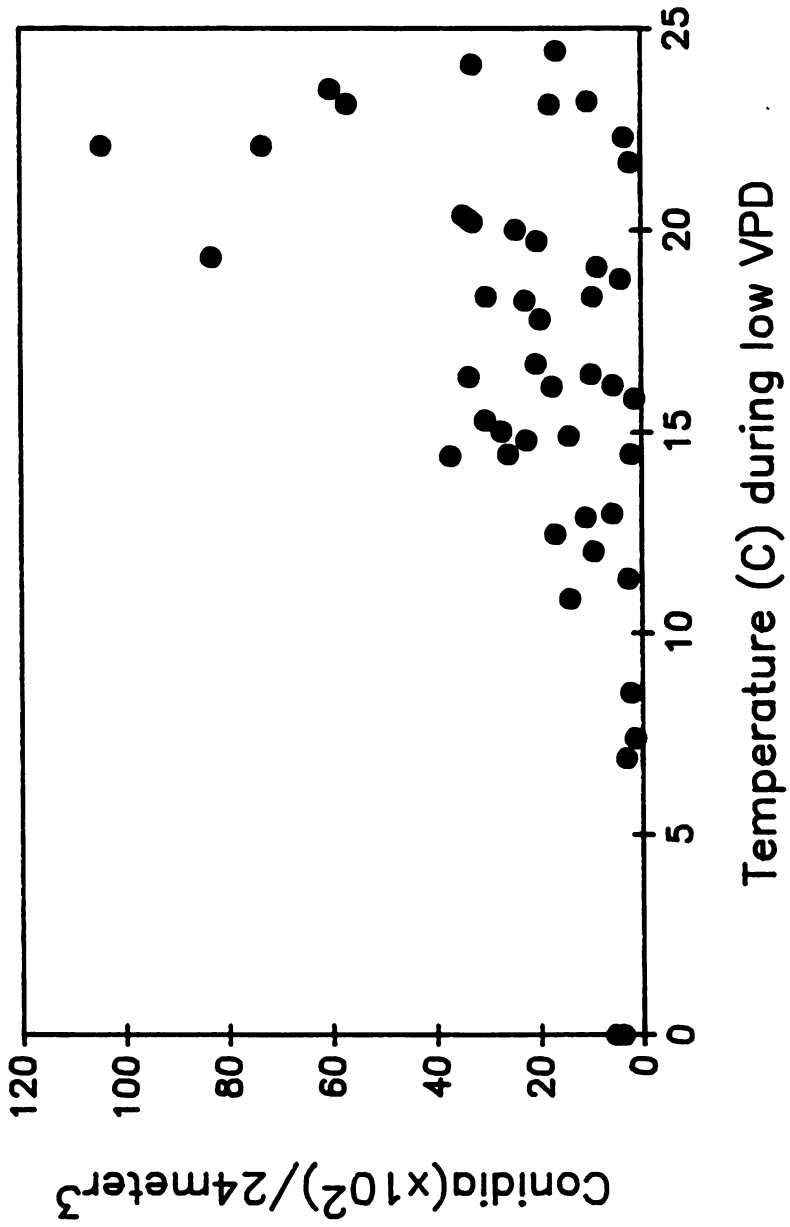
Conidia(x10²)/24meter³
120
100
80
60
40
20
0
0
5
10
15
20
25
Temperature (C) during low VPD

Figure 2.6.

Figure 2.7. The natural logarithm of the number of *A. porri* conidia released and predicted (equation 2.1.) at the M.S.U. Muck Farm during 1987.

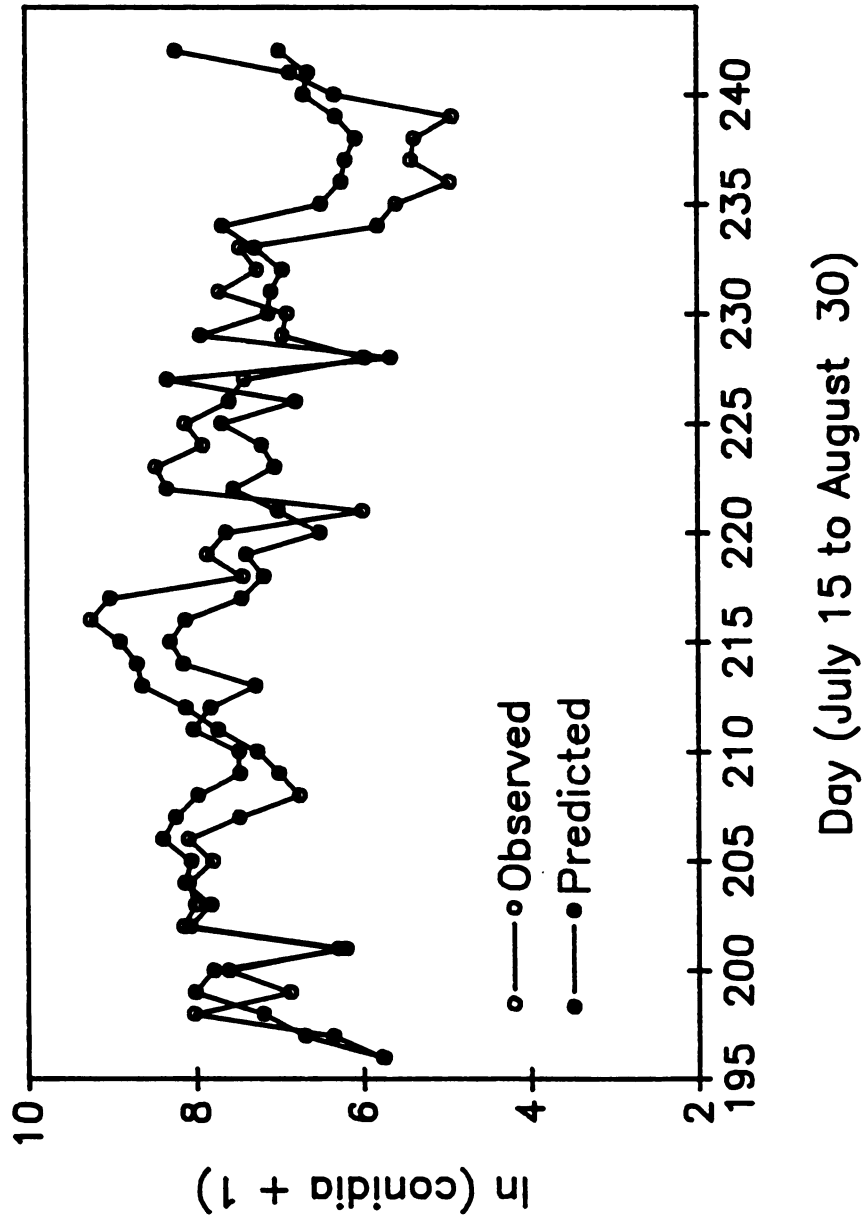
ln (conidia + 1)
10
8
6
4
2
195 200 205 210 215 220 225 230 235 240
Day (July 15 to August 30)
Observed
Predicted

Figure 2.7.

Correlations between temperatures during periods of low VPD on D-1 and conidial release on D-0 were low in 1985, in part because there were many days during that year when the VPD was never <1.0 mb. On those days, X_1 (the temperature during hours of VPD <1.0 mb on D-1) would have a value of 0. (Correlations between temperatures during hours when the VPD was <2.0, 3.0, 4.0 and 5.0 mb on D-1 and conidial release on D-0 were also examined and found not to be significant). Therefore, on days when there were no hours the VPD <1.0 mb, 1/2 the temperature during the hour of lowest VPD was substituted for the temperature during hours the VPD <1.0 mb (X_1). By correcting the temperature in this way, the number of underpredictions of high (>1000) conidial releases were decreased from 15% to 3% in 1985.

DISCUSSION

A. porri lesions appeared late in 1985, coinciding with both the physiological aging of onion plants and a build-up of blighted tissue where sporulation could occur. Examination of lesions showed that numbers of *Stemphylium* spp. conidia also appeared to build up as the season progressed and was frequently (August 2, 7, 21 and 30) associated with the occurrence of *A. porri*. This raised questions about the interaction between the two species of fungi, and the ability of *Stemphylium* to colonize purple blotch lesions and affect lesion area. This question was studied and results are reported in Chapter III. The fact

that an epidemic of *Peronospora destructor* occurred during this growing season resulted in the frequent appearance of sporangia of this fungus. Undoubtedly, *P. destructor* infected first, and the lesion then became colonized by other fungi.

Alternaria, Stemphylium, Peronospora and *A. porri* could sometimes all be found on a single lesion. This probably occurred because catenulate *Alternaria* and *Stemphylium* spp. are good saprophytes and can colonize diseased tissue.

In both 1986 and 1987 older leaves on plants in the field became senescent, lesions coalesced and leaves died from week to week. This led to an apparent decrease in lesion numbers per leaf. One lesion, if located at the base of a leaf, can result in the death of the entire leaf, so each lesion can be very damaging. Miller (1982) found that the amount of leaf area damaged by few (3 or less) or many (4 or more) lesions to be roughly the same. Low lesion numbers, then, do not necessarily reflect that the amount of damage which will result after infection will be low. Lesion location and lesion expansion are more important than lesion number.

There was no strong direct relationship between numbers of conidia released and lesion numbers on onion trap plants in 1987. Undoubtedly lesion formation is influenced by factors other than spore release. The increase in numbers of lesions on trap plants (Figure 2.2)

(which were uniform in age) August 17, 24 and September 1 may indicate that environmental factors were more favorable for infection late in the season.

Because each lesion can cause extensive damage and because of the desire to predict events leading up to infection early enough to issue a spray advisory, it is desirable to determine factors preceding conidial formation and release, and utilize this information to develop a predictive model. This was the approach followed in this study.

In 1985 weather was generally less favorable for purple blotch development and fewer environmental factors were correlated with the natural logarithm (ln) of conidia released than in 1987. This occurred because there were fewer moderate to high conidial releases in 1985 than in 1987, resulting in lower cimulative conidial release in 1985 than in 1987 (Figure 2.8). In 1987 there were several periods of prolonged high conidial releases; however, only two short periods of high conidial release occurred in 1985. As a result, in 1985 there were few days when factors leading to high conidial release could be examined and simple correlation coefficients were lower than in 1987.

Surprisingly, there was a negative correlation between hours of low VPD (<1.0 mb) the previous day and conidial release on the current day in 1987. This does not make biological sense and is probably a direct result

Figure 2.8. Cumulative spore release at the M.S.U. Muck Farm between July 14 and August 19, 1985 and 1987.

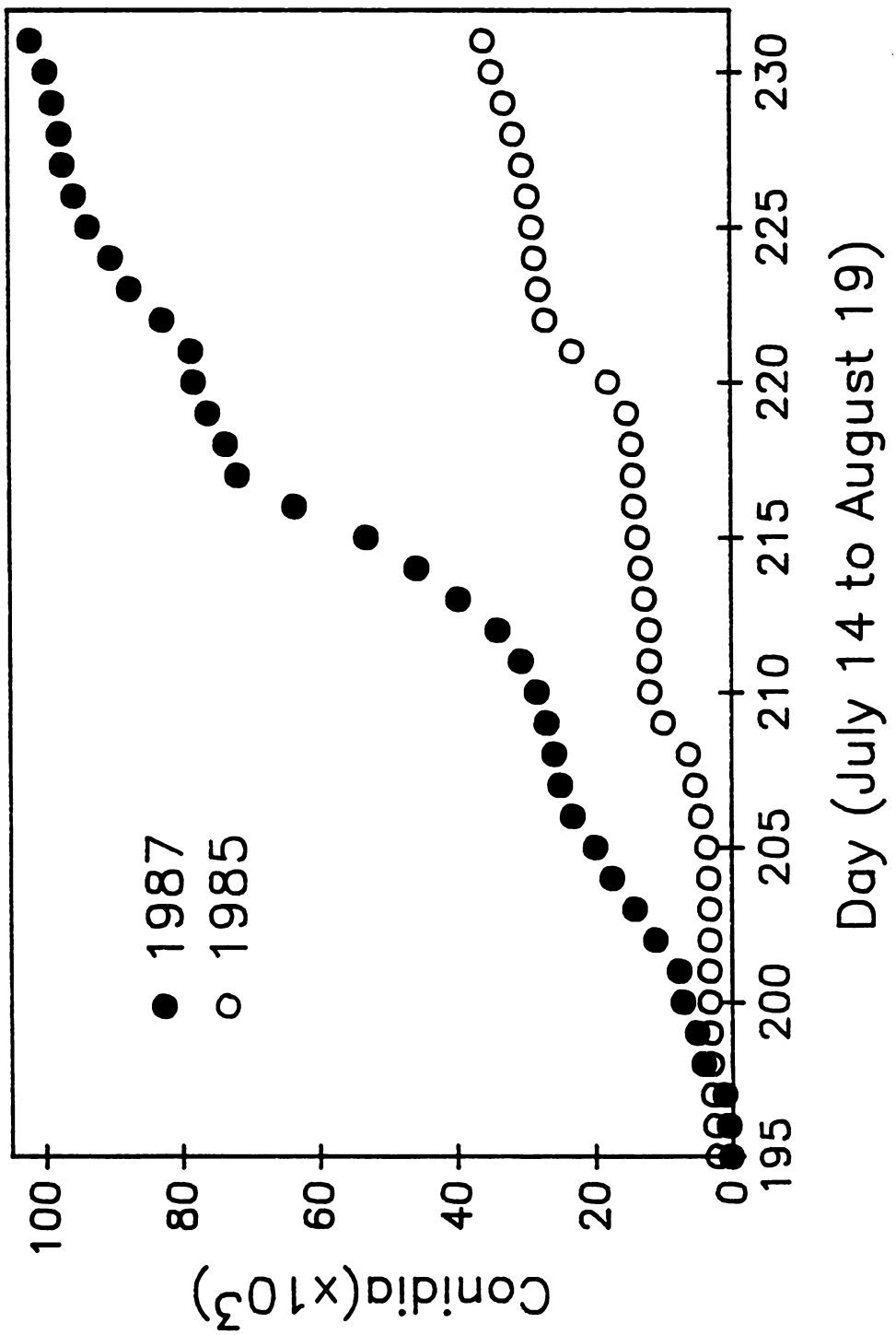
Conidia(x10³)
100
80
60
40
20
0
195
200
205
210
215
220
225
230
Day (July 14 to August 19)
● 1987
o 1985

Figure 2.8.

of the overriding effect of temperature. Warm days tended to favor conidial formation more than cooler days even if the number of hours that VPD was low remained the same.

One of the highest correlations existed between high temperatures during low VPD and spore release the following day (r=.292 and .500 in 1985 and 1987 respectively). However, in Texas a prediction system is based solely on daily hours of leaf wetness (O'Higgins, 1988). Temperatures during the later part of the growing season in Texas are relatively high, and usually favorable for conidial formation and infection of onions by *A. porri* (Miller, personal communication). Moisture however, is limited. In Michigan, especially in humid muck areas, moisture is prevalent but temperature is generally relatively cool (the average daily temperatures at the M.S.U. Muck Farm over the two periods sampled in 1985 and 1987 were 18.8 and 22.8C respectively) to be favorable for conidial formation or infection of onions by *A. porri*. This is not surprising since climate affects plants as well as pathogens (Coakley, 1988) and has long been recognized to influence disease development.

Numbers of *A. porri* conidia predicted by the regression equation (Equation 1) generally increased or decreased as the actual number of conidia observed increased or decreased (Figure 2.7). When the number of conidia released the previous day was not included in the regression, the equation usually underestimated very large

spore releases and overestimated very low spore releases. Future technological advancements (for example the development of a pattern recognition program which could scan a spore trap slide and be included in a computer operated field weather station) may make determination of these values possible. Their inclusion in the model would improve prediction dramatically.

The ln of the number of conidia released on D-1 was significantly positively correlated with the number of conidia released on D-0 (r=.535 and .701 in 1985 and 1987 respectively). Bashi and Rotem (1975) observed that a portion of *Alternaria solani* (Ell. & G. Martin) conidia which formed during a night were not released the following day despite exposure to dry windy conditions (which were conducive to release). It is probable that not all *A. porri* conidia produced each night mature and are liberated the next day, and that the number of unreleased conidia is related to the number originally produced, the time of conidial initiation, and environmental factors such as wind speed or fluctuation of VPD on the following day. This reservoir of unreleased conidia may then be released the following day. A mechanism to retain a reservoir of newly formed conidia would be advantageous to survival of the fungus by increasing the chance that some of the conidia which form on any given night may be released at a later time when conditions may be more favorable for infection.

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CHAPTER III

FACTORS INFLUENCING INFECTION BY *ALTERNARIA PORRI* AND SUBSEQUENT LESION EXPANSION ON ONION LEAVES

INTRODUCTION

Rate of conidial germination, germ tube elongation, lesion expansion, and amount of infection are influenced by environmental factors. Rate of conidial germination and germ tube growth of *Alternaria porri* conidia increased with increasing temperature (Nolla, 1927; Angell, 1929). While the optimum temperature for germination has been reported to be as high as 36C, the optimum temperature for appressorial formation was considerably lower (20-25C) and the optimum for hyphal growth was intermediate (22-30C) (Angell, 1929; Bock, 1964).

Temperature also influenced lesion formation, and a broad optimum for lesion initiation (20-25C) was reported (Bock, 1964). The humidity level and duration of high humidity influenced whether expanding lesions or flecks developed (Bock, 1964). The incidence of sterile white flecks was highest at low relative humidities and the

proportion of purple lesions to flecks increased as relative humidity rose (Bock, 1964).

Conidia can produce more than one germ tube which grow across the leaf surface prior to forming appressoria (Fahim and El-Shehedi, 1966). Appressoria may form over stomata or on the epidermis away from stomata at sites where direct penetration occurs. After direct or indirect penetration, mycelia grow subcuticularly and ramify inter- and intracellularly (Angell, 1929).

As in many diseases caused by *Alternaria* spp., the physiological age of onion tissue influenced the extent of damage caused by a lesion (Miller, 1983). Purple blotch damage increased as leaf age increased and as the plant itself aged.

Frequently lesions on onions in Michigan were colonized with a *Stemphylium* spp. which was identified as *S. botryosum* (Chapter II). Gladders (1981) and Skiles (1953) also observed that *S. botryosum* colonized purple blotch lesions as a saprophyte. *S. botryosum* conidia have a length to width ratio of 1.0-1.5 and a constriction at one transverse septa. They therefore can be distinguished from conidia of a similar species (*Stemphylium vesicarium* (Wallr.) Simm.) which have a length to width ratio 2.0 or more and a constriction at 3 transverse septa (Simmons, 1969). Although *S. botryosum* causes a disease of alfalfa it has not been known to infect onions. *S. vesicarium* has been reported to cause a leaf blight on onions in

Bermuda (Miyabe, 1889) Wisconsin (Teodoro, 1922), India (Rao and Pavgi, 1975), Texas (Miller et. al., 1978) and New York (Shishkoff and Lorbeer, 1987).

The following studies were undertaken to determine how quickly infection and lesion formation occurred on and within onion leaves from *A. porri* and to examine some factors influencing lesion expansion. Because *S. botryosum* was frequently found on purple blotch lesions, it's influence on lesion expansion was also studied.

MATERIALS AND METHODS

Production of *A. porri* conidia

A. porri isolates AAP-1 (which was obtained from Dr. Marvin Miller, Texas A&M) or AAP-3 (isolated from Michigan grown onions in 1986) were used in all experiments because they were highly virulent. [Onion plants inoculated with 2.5 mg of conidia of isolates AAP-1 or AAP-3, subsequently placed in the dew chamber for 24 hours, and moved to the growth chamber for 4 days at 24C, developed 26 and 64 lesions (both expanding lesions and flecks) respectively.] To prepare the fungus for long term storage, it was grown on V-8 agar for six days, transferred to sterilized muck soil in test tubes, incubated for six days at room temperature (shaken daily), and then stored at 5C. For conidial production, infested muck soil from soil tubes was placed on sterile V-8 agar in 9-cm diameter petri plates. The plates were incubated at room temperature in darkness for 6-8 days, exposed to near-UV light (peak

wavelength = 360 nm) for 24 hours to initiate conidiophore formation, and placed in the dark for 1-3 additional days. Conidia were produced abundantly using this system.

Comparison of dry- and wet- harvested conidia

Conidia were collected from V-8 agar plates by dry harvest (with a vacuum aspirator) or wet harvest (by suspending conidia in water) and compared for germinability. Dry harvested 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 but not sucked into the aspirator. To wet harvest conidia, sterile water was poured over *A. porri* colonies on V-8 agar plates and conidia were suspended by agitating with a glass rod. Conidia were collected from the resulting spore suspension on a membrane filter with 0.8 um pore size (Millipore Filter Corp. Bedford, MA, 01730). Water-harvested conidia were then dried for 24 hours over anhydrous CaSO4 before use.

Dry conidia were weighed and dispersed over plants positioned in a settling tower by gently blowing them off a weighing paper with puffs of air from a Pasteur pipette. The settling tower consisted of a galvanized sheet metal cylinder (61 cm diameter by 77 cm deep) mounted on a wooden base. Plants were placed at the base of the settling tower on a revolving platform (5-6 rpm) to allow greater leaf surface area to contact settling spores. A

metal cover was placed over the tower after conidia were dispersed and spores were allowed to settle on the plants for 10 minutes. The base of the settling tower was approximately 2922 cm^2 in area. If 2.5 or 5.0 mg conidia were evenly dispersed over the base of the settling tower, there would have been 3750 and 7500 conidia/cm^2 respectively deposited on the tower base surface. Deposition of conidia on a cylindrical object such as an onion leaf, however, differs from deposition on a flat surface (Ingold, 1971).

Germination, appressoria formation, formation of infection hyphae, and lesion development by *A. porri* on onion leaves

Plants were grown from bulbs in the greenhouse for 5 weeks, then placed in the settling tower for inoculation. The plants were inoculated in the settling tower with 5.0 mg of *A. porri* conidia. Twenty plants were then placed in the dew chamber at 24C, and four plants were removed after 3, 6, 12, 18, or 24 hours of dew. Sections were removed at random from the outermost full-sized leaf of each plant, cut lengthwise, flattened and placed in fixative solution of a 1:1 mixture of absolute ethanol:glacial acetic acid for 24 hours so the tissue was cleared. The tissue pieces were then mounted and stained with 0.1% cotton blue in 85% lactic acid, and at least 150 conidia from each dew period were observed under the light microscope (200 and 1000X) for the presence of germination

tubes, appressoria, infection hyphae or lesion formation. The experiment was repeated twice.

Penetration of onion leaves by *A. porri*

Five-week-old onion plants grown from bulbs in the greenhouse were inoculated with 5.0 mg of *A. porri* conidia in the settling tower as previously described. Plants were then placed in the dew chamber for 48 hours at 24C. The outermost full sized leaf was sampled by fixing the tissue in a solution of 1:1 absolute ethanol:glacial acetic acid for 24 hours, then mounting and staining with 0.1% cotton blue in lactic acid. Leaf tissue was examined microscopically to determine the percentage of conidia which penetrated directly or through stomates. The experiment was repeated twice to insure that the results were repeatable.

Effect of dew period on lesions caused by *A. porri* on onion

Four-week-old plants grown from bulbs in the greenhouse were inoculated with 2.5 mg of *A. porri* conidia in the settling tower. Plants were placed in the dew chamber at 23C. Six plants were removed after each variable dew period of 6, 12, 18, 24, 30 or 42 hours and placed in the growth chamber at 23C with a 12 hour day length. Plants were incubated in the growth chamber for 5 days plus 42 hours, 36 hours, 30 hours, 24 hours, 18 hours, or 6 hours respectively, and were examined 7 days (which included the dew periods plus the periods the

plants were incubated in the growth chamber) after inoculation. Lesion numbers were counted and lesion size measured. The experiment was repeated twice.

Conidial survival on dry onion leaves

To determine the ability of *A. porri* spores to survive on leaf surfaces after dissemination, 20 plants were trimmed so they had 3 or 4 leaves per plant. These plants were then inoculated with 3.0 mg *A. porri* conidia in the settling tower as described above. After inoculation, 4 plants were immediately placed in the dew chamber, and the remaining plants were placed in the growth chamber (24C and 36 to 48% relative humidity). After 24 hours in the dew chamber, the first four plants were moved to the growth chamber and replaced with four different plants from the growth chamber. This process was repeated so that groups of 4 plants had 0, 1, 2, 3, or 4 days exposure to dry conditions not conducive to infection prior to being placed in the dew chamber where infection could occur. Both expanding lesions and flecks were counted 72 hours after plants were placed in the growth chamber following removal from the dew chamber.

Pathogenicity of *Stemphylium botryosum*

Single spores of *Stemphylium botryosum* were isolated from lesions on onion leaves obtained from the M.S.U. Muck Farm in 1987. Cultures of 4 isolates were then grown on potato dextrose agar (PDA) at 28C for 8 days. The resulting mycelia (few spores could be induced to form)

were homogenized at medium speed in an omni-mixer (Ivan Sorvall, Inc., Norwalk, Conn., U.S.A.) in 250 mls of sterile distilled water (dH_2O). This mixture was filtered through cheesecloth to remove large mycelial fragments. Plants were sprayed until runoff with the mycelial suspensions. Plants were placed in the dew chamber (24C) for 24 hours, then moved to the growth chamber (23C) for 9 days. At this time all lesions were counted, excised, and incubated in moist chambers to observe types of conidia formed.

Influence of colonization by *Stemphylium botryosum* on size of purple blotch lesions

Two-week-old onion plants grown from bulbs in the greenhouse were separated in two groups. Half were inoculated with 2.5 mg *A. porri* conidia in the settling tower and half were not inoculated. Plants were then placed in the dew chamber for 21 hours at 24C, moved to the growth chamber for 4 days. *S. botryosum* culture SMB-4 was prepared as previously described except it was grown for 14 days. The fungal mycelia were then suspended in 250 mls sterile distilled water and large pieces of mycelia were removed by filtering the suspension through cheesecloth. The plants were removed from the growth chamber and one-half of the previously inoculated and uninoculated plants were sprayed until runoff with mycelial suspension of *S. botryosum*. All plants were then placed in the growth chamber for 3 days, the leaves were

excised, cut horizontally, flattened and photographed. After the film was developed and photographs were printed, the leaf and lesion areas were determined by tracing leaf and lesion boundaries with a mouse on a digitizer pad and calculating the areas. Once areas were determined, they were recalculated to correct for reductions in sizes from the actual lesions to the reduced sizes in the photograph prints.

RESULTS

Comparison of dry- and wet- harvested conidia

Germination rates of conidia harvested with the vacuum aspirator or in water suspension were similar (Figure 3.1). Because dry harvest yielded far fewer conidia/plate than wet harvest, and dry harvested conidia did not differ obviously from wet harvested conidia, in all subsequent inoculations conidia were wet harvested.

Penetration of onion leaves by *A. porri*

Individual multicellular conidia of *A. porri* formed one to several germination tubes and appressoria and often penetrated at more than one locus. Forty percent of conidia examined penetrated through stomata; sixty-nine percent penetrated directly. (Total penetrations exceeds 100% because some conidia formed more than one germ tube which penetrated.) Germ tubes from individual conidia could penetrate through stomates, directly, or both through stomates and directly. Only one or two germ tubes per conidium were observed to penetrate.

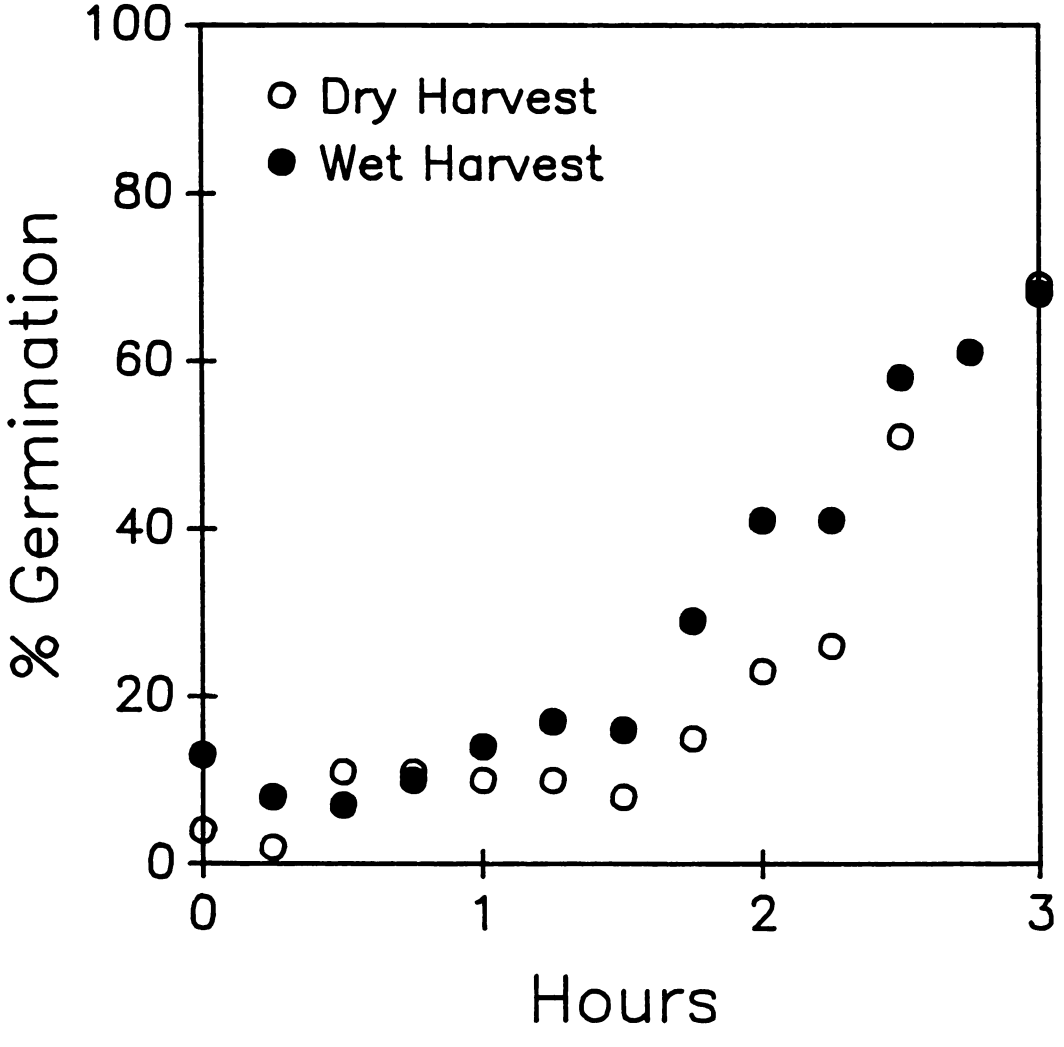

Figure 3.1. Germination of conidia harvested by low air suction (dry) or harvested in water.

Germination, appressoria formation, formation of infection hyphae, and lesion development by *A. porri* on onion leaves

In one of two experiments with similar results, after 3, 6, 12 or 24 hours of dew, 73, 84, 84 and 90% respectively of conidia on onion leaf surfaces had germinated, and 6, 40, 53 and 71% respectively of the germinated spores had formed appressoria (Fig. 3.2.). After 3, 6, 12, or 24 hours of dew, 0, 7, 30 and 36% respectively of the conidia which had formed appressoria had also formed infection hyphae. Twenty, 42 and 68% of the conidia which formed infection hyphae caused visible lesions after 6, 12, and 24 hours of dew respectively (no infection hyphae formed after 3 hours of dew).

Effect of dew period on lesions caused by *A. porri* on onion

Lesion numbers on leaves increased with increasing length of dew period at 23C. There was a significant ($p=<.01$) positive correlation ($r = 0.784$) between length of dew period and lesion numbers (Fig. 3.3.). Length of dew period ($r=-.213$) and lesion numbers per plant ($r=-.154$) were not significantly related to lesion size (data not shown).

Conidial survival on dry onion leaves

Numbers of both lesions and flecks resulting from controlled inoculations were highly variable. However, mean numbers of flecks remained relatively constant whether plants were placed in the dew chamber immediately

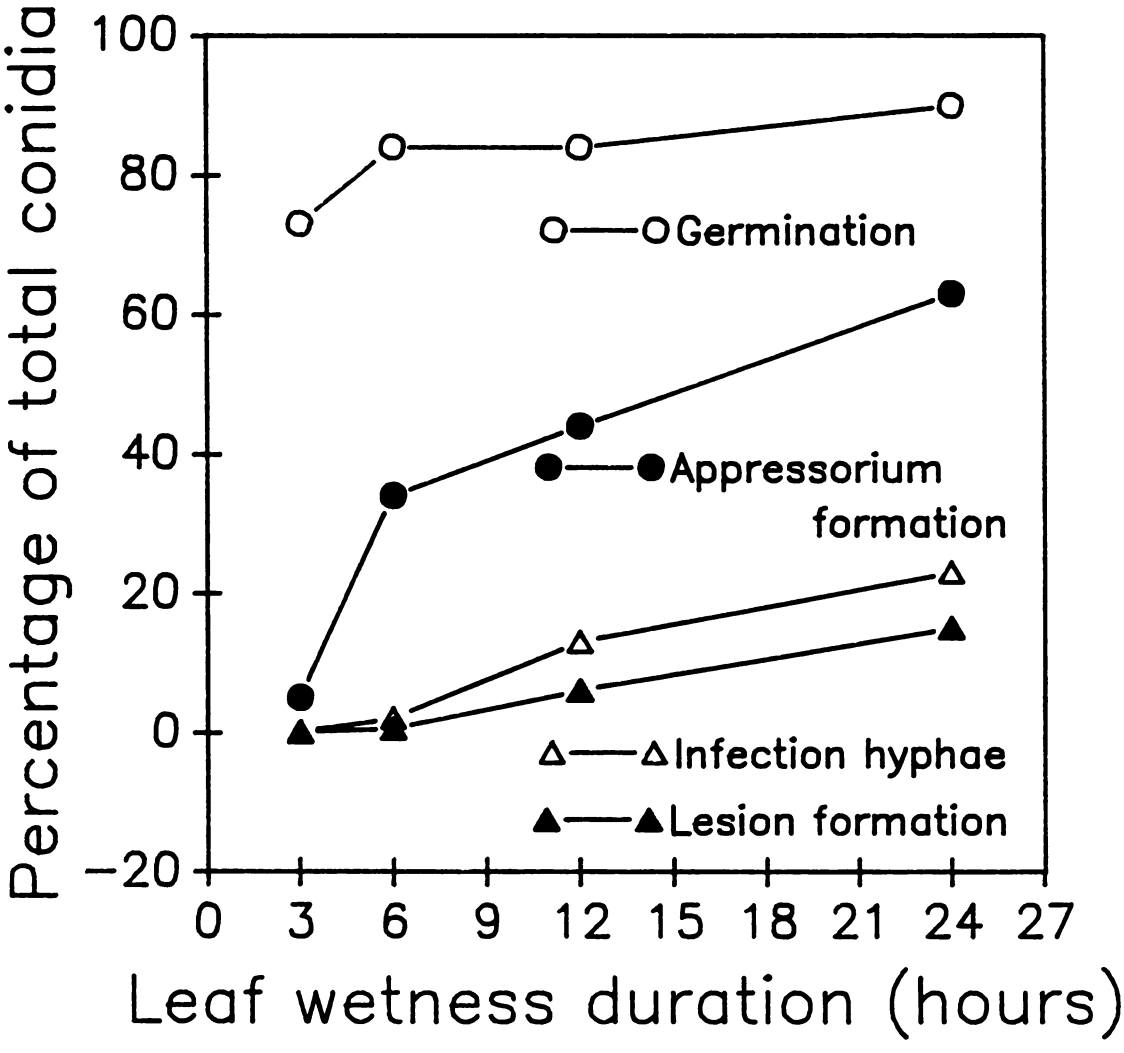

Figure 3.2. Percentage of total conidia to germinate, form appressoria or infection hyphae and visible lesions on onion leaves for variable dew periods.

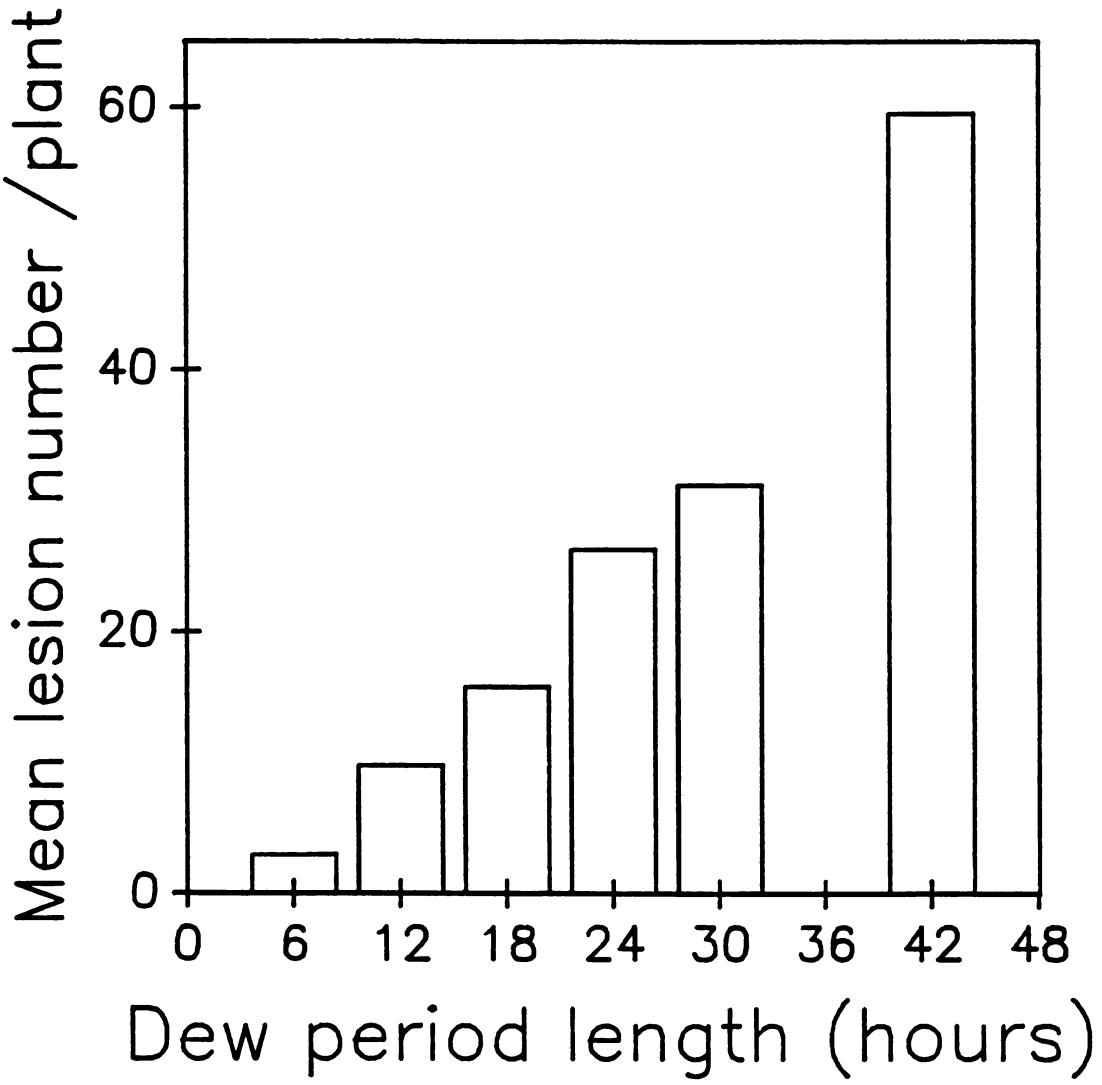

Figure 3.3. Influence of dew period duration on establishment of lesions caused by Alternaria porri.

after inoculation or incubated for four days prior to being placed in the dew chamber (Fig. 3.4.). Flecks were found on inoculated plants but not on noninoculated controls, and one to several spores were found at the center of each fleck. Expanding lesions resulted even when plants were kept in the growth chamber for 1-4 days following inoculation before being placed in the dew chamber. An average of 5.5, 3.5, 2.3, 1.8 and 1.5 lesions per plant formed after plants were incubated in the growth chamber for 0, 1, 2, 3, or 4 days respectively prior to a dew period. However, because of the high variability in lesion numbers per plant, none of these numbers was significantly different from any other.

Pathogenicity of *Stemphylium botryosum*

All four of the isolates of *S. botryosum* tested were weakly pathogenic (Table 3.1) on onion leaves. No lesions developed on control plants. Lesions were small, brown and angular in shape.

Table 3.1. Mean number of lesions on onion plants inoculated with a mycelial suspension of *Stemphylium botryosum*

Isolate	Mean number lesions/plant
SMB1	1.2[a] ± 2.17
SMB2	1.0 ± 1.41
SMB3	0.2 ± 0.45
SMB4	0.4 ± 0.89
Control	0 ± 0

[a] Mean of 5 replicate plants.

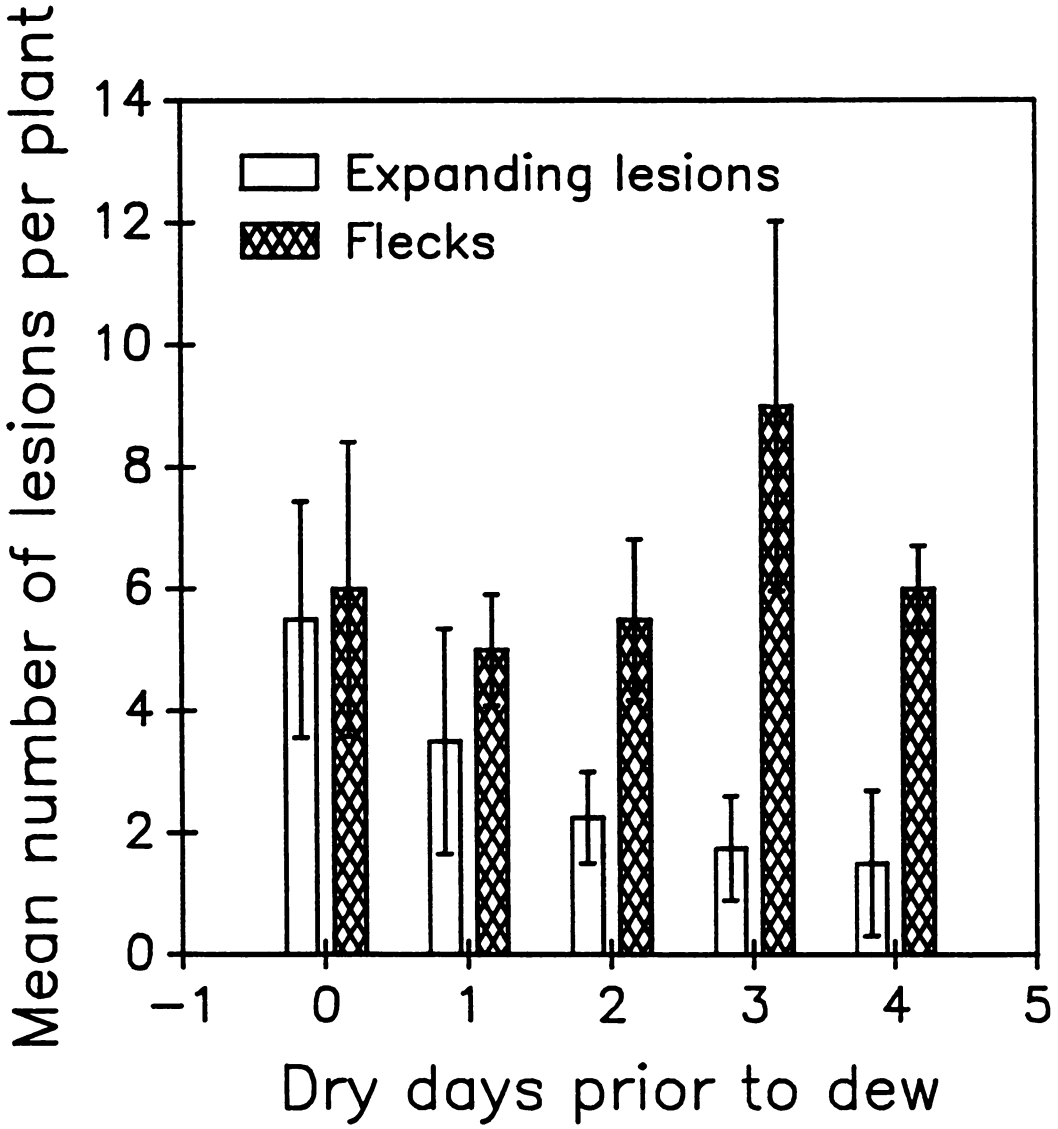

Figure 3.4. Infection of onion leaves by A. porri conidia subject to variable dry periods prior to 24 hours of dew.

Influence of *Stemphylium botryosum* on the number and size of purple blotch lesions caused by *A. porri*

Lesions developed on onion leaf tissue after inoculation with either *S. botryosum*, *A. porri* or with both pathogens. Lesion areas were greater on plants inoculated with *A. porri* alone than those inoculated with *S. botryosum* alone. Lesions were significantly larger when the two fungi were used to inoculate the same plants ($p < .05$) (Table 3.2).

Table 3.2. Mean percentage of leaf area in lesions per plant resulting from inoculation of onions with *A. porri* followed by inoculation with *S. botryosum*.

Treatment	Mean % leaf area in lesions/plant
Control	0[a]
S. botryosum	1.24 ± 0.79
A. porri	8.88 ± 5.93
A. porri + *S. botryosum*	18.13 ± 5.20

[a] Mean of 5 replicate plants.

DISCUSSION

The infection process (development of germ tubes, appressoria, infection hyphae, and resulting lesions) progresses relatively rapidly in purple blotch disease. *A. porri* conidia, which survive well under unfavorable conditions (Angell, 1929), penetrate rapidly and form lesions after as little as 6 hours of dew, enabling the pathogen to penetrate successfully even in areas where moisture is limited and temperatures are high. Bashi and

Rotem (1968, 1974, 1975b, and 1975c) have documented the ability of *A. solani* to survive under semi-arid conditions in Israel. They also found that *A. solani* can withstand interrupted wet periods so that it can utilize wet periods on two different nights to complete penetration. From the data reported in these studies, it seems that *A. porri* may also be well adapted to adverse conditions. It is interesting that the isolate used in these studies was isolated in Michigan where conditions are relatively more favorable for plant disease development than in semi-arid regions such as Israel, yet this isolate had traits which would presumably benefit it in hot dry onion-growing regions. Studies should be undertaken to see if *A. porri* can withstand interrupted wet periods and successfully penetrate.

In chapter II a predictive equation was developed which focuses on weather conditions which influence *A. porri* conidial formation. (This is in contrast to disease and infection prediction models which forecast conditions which result in symptom formation.) The development of the equation resulted from a desire to develop a model on which to base spray advisories. The tacit assumption of this approach is that conditions favorable for spore formation will also favor infection. However, conidial formation of *A. porri* can take place under conditions (humidity less than 100%) which do not favor lesion formation (Chapter VI). Since both conidia production and

subsequent infection are required for disease, and lesion numbers were not well correlated with conidial release (Chapter II), the validity of this approach had to be examined. If spores of *A. porri* were unable to survive and remain viable for several days after dissemination when conditions are unfavorable for penetration, then sprays based only on probable sporulation might be unnecessary. This would occur when a suitable infection period does not follow a sporulation period and disseminated conidia die from exposure to the elements. However, if disseminated spores could survive periods of several days unfavorable for infection, and still be present and ready to infect when conditions became favorable, then sprays based only on conditions favorable for spore formation would be relatively more important than the infection component, since a period favorable for infection would be likely to occur within a few days.

The demonstrated ability of conidia to survive on leaves and cause significant numbers of infections for up to 4 days after inoculation, coupled with the large amount of damage which can result from a single lesion, lends support to the method of basing a spray advisory on sporulation alone. While conditions in the growth chamber were much milder than those in the field, even a few conidia which survived and were were able to infect had the potential to cause a great deal of damage.

Shishkoff and Lorbeer (1987) also inoculated onion leaves with *S. botryosum* and observed "a few lesions which might have been caused by the fungus". The results reported here confirm that *S. botryosum* is weakly pathogenic and was able to cause small lesions on onion. Although *S. botryosum* on onion has not been extensively studied, there are no previous reports that it alone can cause lesions on onion leaves in the field.

In addition to causing lesions on onion, *S. botryosum* increased damage to onion plants infected with *A. porri* in this study. Purple blotch lesions resulting from inoculation with both *A. porri* and *S. botryosum* were larger than those resulting from *A. porri* alone. Presence of *S. botryosum*, commonly considered to be primarily a saprophyte, may be contributing more to onion foliar damage in the field than is presently recognized. In Michigan, most purple blotch lesions observed were colonized by *S. botryosum*, especially late in the growing season (Chapter II).

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CHAPTER IV

THE INFLUENCE OF RELATIVE HUMIDITY, DEW PERIOD, LEAF AGE, AND LEAF SENESCENCE ON SPORULATION OF *ALTERNARIA PORRI*

INTRODUCTION

Environmental factors which affect conidial formation in *A. porri* influence the spread of the pathogen and ultimately disease development. Two of the most important factors appear to be moisture from the atmosphere and free water on the leaf surface. Fahim (1966) found that conidial formation in *A. porri* on agar media was profuse at 90% relative humidity (RH) or above, but at humidities of 81% or lower, conidial formation decreased rapidly and conidia which formed contained fewer cells. Miller (1975) found that eleven or more hours of leaf wetness and 14 or more hours of RH above 90% were positively correlated with an increase in *A. porri* conidia trapped from the air above an onion field the following day. Moisture in the form of rain or irrigation often preceded increases in *A. porri* conidia in the air above an onion field in Nebraska (Meredith, 1965).

Several studies have provided information on the influence of moisture and plant senescence on conidial production and dispersal of *A. solani* (Bashi and Rotem, 1975b; Rotem and Reichert, 1964) and *A. dauci* (Kuhn) Groves and Skolks (Strandberg, 1977; Langenberg, Sutton and Gillespie, 1977), *Alternaria* spp. closely related to *A. porri*. Dew was the principal factor enabling the development of potato and tomato early blight epidemics (Rotem and Reichert, 1964). The number of hours of leaf wetness was correlated with abundance of *A. dauci* conidia in air over a carrot field (Strandberg, 1977). Bashi and Rotem (1976b) found that conidial formation of *A. solani* on tomato leaves increased as senescence increased.

The following studies were undertaken to further elucidate the relationship of humidity, dew period, and leaf senescence on conidial formation in *A. porri* in vivo under controlled and monitored laboratory conditions.

MATERIALS AND METHODS

Effect of dew period length on conidial size

Five-week-old onion plants were grown from bulbs in the greenhouse, inoculated with 2.5 mg conidia (isolate AAP-3) as previously described (Chapter III), and placed in the dew chamber for 24 hours (25C) to allow infection to occur. Plants were then placed in the growth chamber (23C, 12 hour day length) for 1 week, then again incubated in the dew chamber for 9, 12, 15 or 18 hours (25C). Lesions were excised after the variable dew periods, and

then shaken in tubes containing distilled water and a drop of polyoxyethylenesorbitan (Tween 20). Conidial suspensions were plated onto water agar plates and lengths and maximum widths of sixty individual conidia were measured (200X) with a compound microscope.

Effect of dew period duration on conidial germination and infection

To determine the dew period duration necessary for germinable conidia production, four-week-old onion plants were grown from bulbs in the greenhouse and inoculated with conidia of AAP-3 in the settling tower as previously described (Chapter III). Plants were inoculated with 10 mg of conidia so resulting lesion area would be maximized. Plants were then placed in the dew chamber (25C) for 24 hours to allow conidia to infect leaves, moved to the growth chamber for 5 days while lesions developed, then placed in the dew chamber (25C) for 9, 12, 15, 18, 21, or 38 hours. Lesions were excised and developing conidia were suspended as described above, plated onto water agar plates, and incubated in the dark for 4 hours at 24C. Plates were then stained with 0.1% cotton blue in 85% lactic acid and conidia were examined under the microscope for germination. Conidia were considered to have germinated if germ tubes were at least 10 um long.

In addition to examining the ability of conidia exposed to variable dew periods to germinate, the ability of spores to infect after variable dew periods of 12, 16,

or 20 hours was determined. Recently formed conidia were washed from the leaves, and the concentration of conidia in suspension was determined using a hemacytometer. Leaves of 4 onion plants were wiped with a piece of cheesecloth to make the leaves more wettable, then sprayed until runoff with a suspension of 2.0×10^4 conidia per ml. These plants were placed in the dew chamber for 24 hours (24C), then moved to the growth chamber (23-24C). Lesions were counted after 6 days.

Effect of relative humidity on conidial formation by *A. porri*

Five-week-old onion plants were inoculated with 2.5 mg of *A. porri* conidia in the settling tower as described previously (Chapter III). Plants were placed in the dew chamber (25C) for 24 hours and then moved to the growth chamber for 7 days. Plants were placed under near-ultraviolet (UV) light (peak wavelength = 360 nm) for 3 hours, then leaves containing lesions were excised and incubated in sealed glass jars above a reservoir of water or saturated salt solutions of K_2SO_4, KCl, or NaCl (Dhingra and Sinclair, 1985)at 24C. These jars maintained RH at 100, 97.5, 85.5, or 75%. One-half cm^2 tissue was excised from the center of each lesion after 48 hours of incubation and conidia were washed off and suspended in 80% ethanol. Conidia were collected on a millipore filter and counted. The experiment was repeated twice.

Ability of lesions to sporulate repeatedly

One-month-old onion plants were inoculated with 2.5 mg of *A. porri* conidia (AAP-3) as described previously and placed in the dew chamber for 24 hours (25C). Plants were removed to the growth chamber for 6 days, exposed to near-UV light 6 hours, then incubated in the dew chamber for 24 hours. Thirty sporulating lesions were identified on ten plants. These lesions were marked with tags so they could be subsequently identified, and the plants were returned to the growth chamber for three days. The relative humidity in the growth chamber ranged from 36 to 48% during this time, which was unfavorable for spore production. Conidia were removed from the lesions initially by jets of compressed air and thereafter daily by brushing the lesions with a small paint brush to dislodge any remaining spores. After a 3 day dry period, plants were again exposed to near-UV light for 6 hours, and incubated in the dew chamber for 24 hours. The same 30 lesions were examined again for conidial formation.

In another experiment, infected onion plants with well-developed lesions were placed in the dew chamber for 24 hours, then moved to the growth chamber. Ten lesions on ten leaves were examined for the presence or absence of conidia, tagged, and the conidia were removed as described above. After 24 hours in the growth chamber, the plants were placed back in the dew chamber. This process was

repeated 7 times to determine if lesions could continue to sporulate.

Influence of leaf death or senescence and age of leaf tissue on the ability of lesions to sporulate

Many purple blotch lesions, especially on older leaves, expand rapidly, girdle the leaves and cause the leaves to become senescent and die. To determine if *A. porri* had the ability to continue to sporulate on lesions on dead leaves, four-week-old plants were inoculated with 5.0 mg of *A. porri* (AAP-3) conidia, placed in the dew chamber for 24 hours (25C) for infection to occur, then removed and placed in the growth chamber (23C) for 14 days. At this time the outermost infected leaves were completely dead and dry. These leaves were removed from the plants, exposed to UV light for 6 hours, and placed in the dew chamber for four days. Twenty lesions on twenty leaves were examined for sporulation. To determine the influence of leaf age on sporulation of *A. porri*, five-week-old plants grown from bulbs in the greenhouse were inoculated with 2.5 mg of AAP-3 conidia in the settling tower as described previously. Plants were placed in the dew chamber for 24 hours, then placed in the growth chamber at 25C for 6 days while lesions developed. Leaves of different ages with similar sized lesions were excised, labeled and placed in the dew chamber for 3 days. Lesions were sampled by cutting a 1/2 cm^2 from the center of each lesion, and placing them in vials in the refrigerator.

Vials were agitated to suspend as many conidia as possible, then the suspended conidia were collected on filter paper, and counted. The conidia remaining on leaves were also counted. The experiment was repeated twice.

Studies on conidial ontogeny in *A. porri* using the scanning electron microscope

To study in detail the formation and development of *A. porri* conidia, including the timing of spore development, plants infected with purple blotch were maintained in the growth chamber (23C, 12 hr daylength) as purple blotch lesions expanded. Plants were then placed in the dew chamber (24C) in the dark to induce conidial formation. (Although near-ultra-violet (UV) light is required for conidial initiation when *A. porri* is grown on artificial media, conidiophores and conidia developed rapidly on living plant tissue exposed to only fluorescent lights.) Small tissue samples were cut from the lesions at 3 hour intervals beginning 9 hours after placement in the dew chamber, vapor-fixed with OsO_4 for 96 hours in a closed chamber, air dried for two days, and then mounted, coated and examined in a JEOL JSM-35 CF scanning electron microscope.

RESULTS

Effect of dew period length on conidial size

The average length of conidia examined under the light microscope after 9, 12, 15, or 18 hours of dew were

34.84um, 53.28um, 61.96um, 71.90um respectively (Figure 4.1.). Average width was 4.48um, 5.72um, 6.98um and 7.91um respectively. The maximum total length measured was 125um. Conidial length, width and tail length were highly variable.

Effect of dew period duration on conidial germination and infection

After 9 hours of dew, 26% of conidia formed had germinated (Figure 4.2.). Seventy-two, 91, 93, 96, or 96% of the conidia formed were able to germinate after 12, 15, 18, 21, or 38 hours of dew respectively. Numbers of flecks and lesions on plants infected with conidia exposed to various dew periods increased as the dew period increased. Average numbers of flecks per plant were 60, 687, or 1026 after 12, 16 or 20 hours of dew respectively. Average numbers of lesions formed were 0, 10, and 15.5 after 12, 16 and 20 hours of dew respectively.

Effect of relative humidity on conidial formation by *A. porri*

Conidia formed at all humidities tested (75-100%). However, numbers of conidia which formed increased with increasing humidity (Figure 4.3.). Numbers of spores which formed at each relative humidity tested was highly variable in this experiment (see standard error bars).

Ability of lesions to sporulate repeatedly

All 30 lesions which were examined were able to sporulate more than once. Six of 10 lesion pieces placed

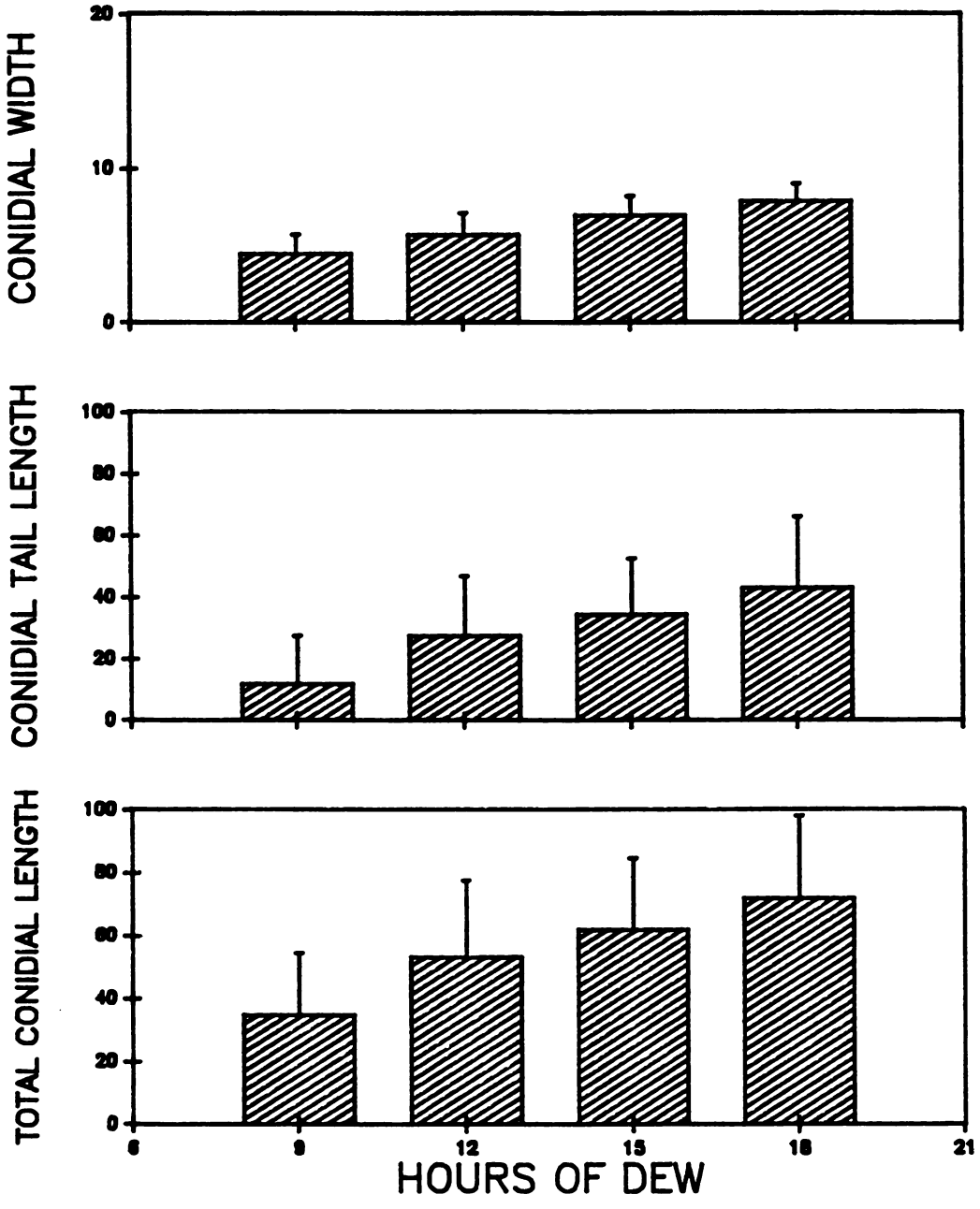

Figure 4.1. Effect of dew period duration on conidial width (A), conidial tail length (B), and total conidial length (C).

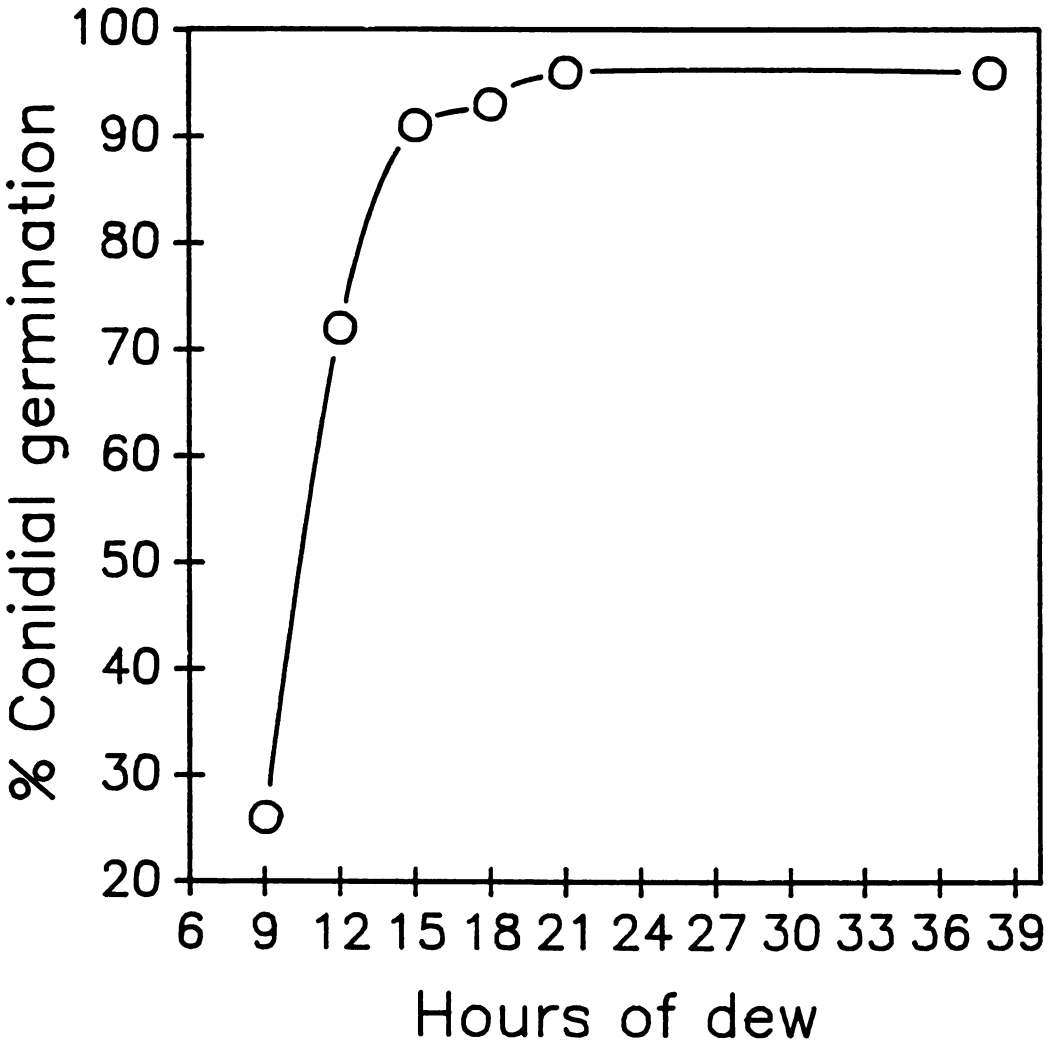

Figure 4.2. Percent germinable conidia which formed on onion leaves after 9, 12, 15, 18, 21, or 38 hours of dew.

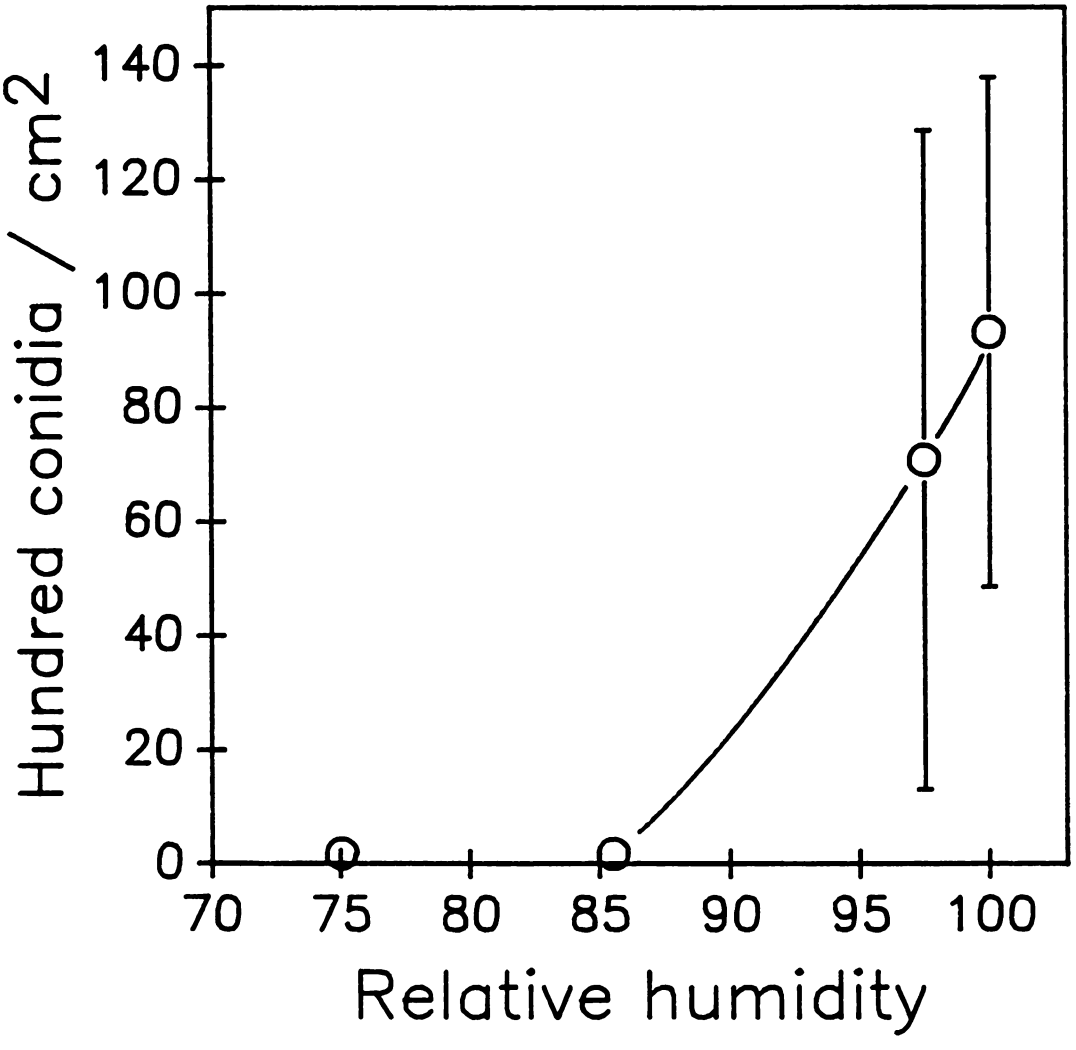

Figure 4.3. The influence of relative humidity on conidial formation on onion leaves.

in the dew chamber continued to sporulate after 8 alternate wet-dry periods. However, after conidial formation occurred on lesions several times, numbers of conidia which formed became increasingly sparse. Fewer than approximately 50 conidia/cm^2 of lesion tissue formed on lesions which had sporulated 7 times previously, whereas lesions formed approximately 1.0 x 10^4 - 1.0 x 10^5 conidia/cm^2 during the first sporulation period.

Influence of leaf death or senescence and age of leaf tissue on the ability of lesions to sporulate

All lesions on dead and senescent leaves formed conidia profusely under the conditions of these experiments.

Leaf tissue age did not influence the conidial density during sporulation (Figure 4.4.). Density of conidial formation was highly variable. For example, lesions on the 2nd youngest leaf bore from 1.5 x 10^4 - 3.9 x 10^4 conidia per cm^2 of tissue.

Studies on conidial ontogeny in *A. porri* using the scanning electron microscope

Examination of conidia forming on onion leaves exposed to 9, 12, 15, or 18 hours of dew with the scanning electron microscope (SEM) confirmed the rate of conidial development seen in the light microscope. (Direct size comparison cannot be made between conidia examined with light or scanning electron microscopy because samples are

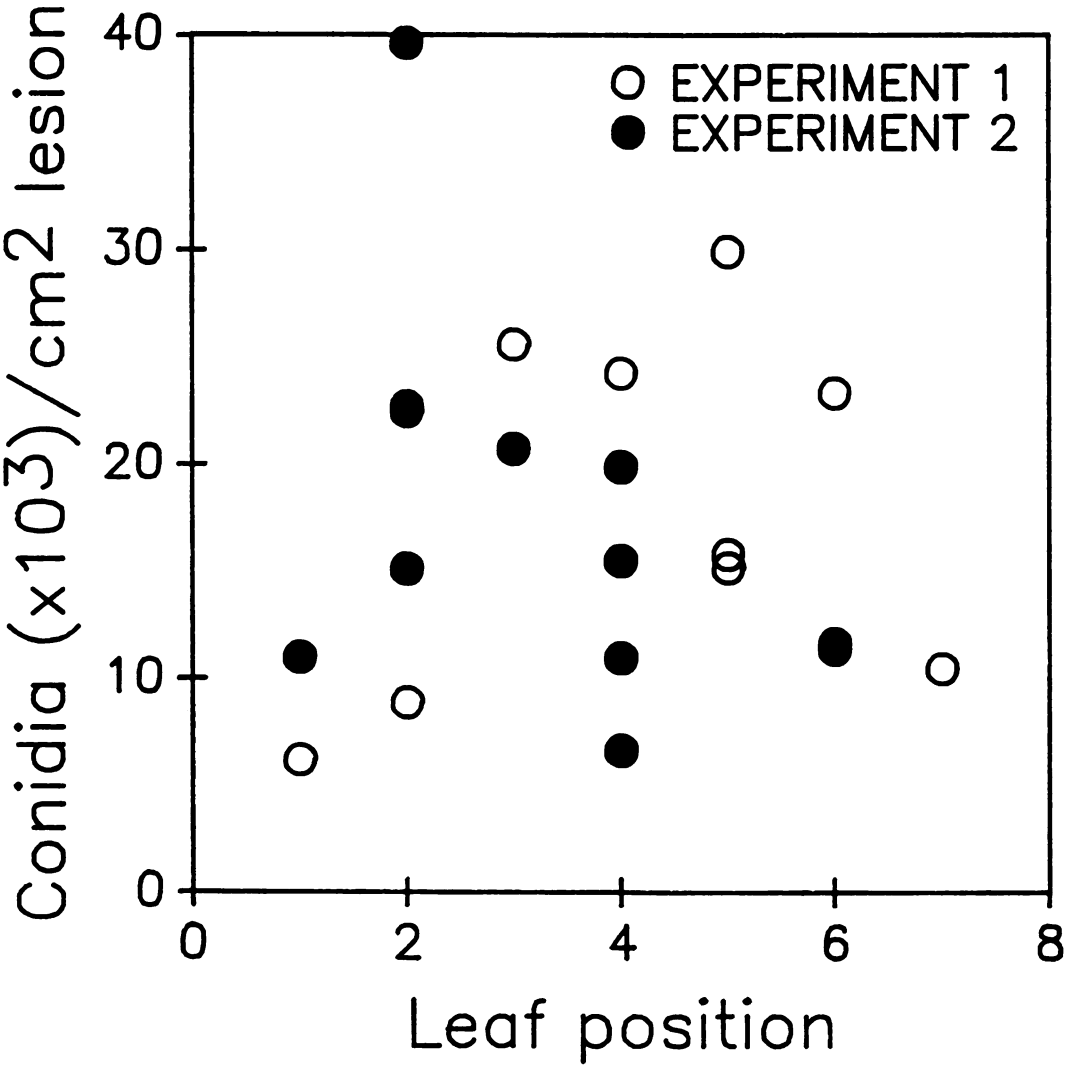

Figure 4.4. Influence of leaf position on density of conidial formation (1 = youngest leaf, 7 = oldest leaf).

shrunk and distorted in fixation for SEM.) After 9 hours in the water-saturated atmosphere of the dew chamber (Figure 4.5a.), conidiophores were well developed and conidial initials could be seen. Even at this early stage, the orientation of conidia could be determined because the bases of the conidia were larger than the tips. After 12 hours of dew the body and tail could be clearly distinguished (Figure 4.5b.); however conidia were observed in different developmental stages at this time. A few transverse septa (crosswalls) could be seen in the bodies of most conidia. After 15 hours of dew the conidia had taken on the characteristic shape of this species (Figure 4.5c). Both transverse and longitudinal septa were present and the tails generally were longer than the bodies of the conidia. Conidia appeared fully mature when exposed to between 15 and 24 hours of dew (Figure 4.5d).

DISCUSSION

Dew period requirements for conidia formation of *A. porri* in this study were in close agreement with requirements of closely related species of *Alternaria* (Bashi and Rotem, 1975; Rotem and Reichert, 1964; Strandberg, 1977; Langenberg, Sutton and Gillespie, 1977). Measurements of *A. porri* conidia of the isolate used (AAP-3) were in the lower range of those for *A. porri* reported in the literature (100-300um long and 15-20um thick) (Ellis). The isolate used in this study (AAP-3) was derived from a single conidium which may have been in the

Figure 4.5. Photomicrographs of formation of *A. porri* conidia on onion leaves. A. Conidiophores with conidial initials after plants were incubated for 9 hours in the dew chamber (X1500). B. Conidia with definite tails after a 12 hour dew period. C. Conidia with both longitudinal and transverse septa (crosswalls) after 15 hours of dew (X440). D. Mature conidia of *A. porri* with characteristic long tails (X550).

4.5

low range of sizes. Conidia appeared well developed and were able to germinate when collected after 9 hours of dew. Conidia formed after 12 hours of dew caused flecks on leaves, and conidia formed after 16 hours of dew could infect and cause lesions. It is possible that conidia may continue to grow in size even after they are fully able to infect.

Lukens and Horsefall (1973) examined formation of *A. solani* conidia after conidiophores had formed. They observed conidial initials at the tips of conidiophores after 3 hours, formation of septa after 6 hours and maturing of conidia after 16 hours (these times do not include conidiophore formation). In our studies, conidiophores and conidial initials formed 9 hours after dew onset and conidia appeared mature after 15 hours of dew. Development of *A. porri* conidia, once conidial initials had formed, progressed faster than development of *A. solani*.

Strandberg (1977) found that *A. dauci* conidia formed on infected living and excised leaves maintained at 96-100% RH. However, *A. porri* conidia formed on agar blocks maintained at lower relative humidities (Fahim, 1966). We also observed conidial formation on onion leaves at relative humidities below 97.5%. Studies on rates of conidial development at different constant relative humidities and rate of development at fluctuating relative humidities are needed to understand the full influence of

humidity on conidial formation especially in a field setting where humidity is usually fluctuating.

Because of problems in accurately measuring relative humidity (or vapor pressure deficit) in the field especially at the leaf surface, dew period may be a more feasible environmental parameter to correlate with conidial formation. Strandberg (1977) felt that though relative humidity could be used to predict time of spore release, dew duration or hours of leaf wetness were better indicators of spore abundance. Conidia formed at relative humidities below 97.5% on onion leaves under the conditions of my experiments, but only in low numbers. Temperature during periods of high relative humidity (low VPD) does influence the numbers of conidia formed (Chapter II).

While 9-12 hours of dew (saturated atmosphere) resulted in fully developed conidia able to infect, shorter periods of high humidities could lead to conidial formation if spores could form following interrupted high humidity periods. This would enable conidia which had begun to form during one night to complete formation during a later dew period. This is known to occur in sporulation of *A. solani* (Bashi and Rotem, 1975c). Sporulation of *A. solani* was enhanced (more spores formed) when dew periods were interrupted rather than continuous. This occurred even if the leaves were wet for the same

total length of time (for example two 6 hour dew periods compared to one 12 hour dew period).

Bashi and Rotem (1975b) found that amount of sporulation of *A. solani* increased as tomato leaves became more senescent and died. Also, sporulation increased each night of the 6 day period they studied, probably due in part to the increasing leaf senescence and lesion expansion. From another study on *A. solani* it was determined that lesion size influenced density of conidial formation. More total spores formed on larger lesions, and conidial formation was more dense on larger lesions than on smaller lesions.

It was determined that *A. porri* formed conidia profusely on dead leaves and could form conidia repeatedly. One consequence of this is that several nights of weather conducive to conidial formation could be followed by releases of high numbers of conidia even if new lesions were not formed. Eventually, of course, the density and total numbers of conidia would decrease if unfavorable conditions persisted.

The fact that leaf age did not seem to influence amount of conidial formation may reflect the fact that all tissue supporting sporulation is functionally dead, and therefore not influenced by tissue age.

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RECOMMENDATIONS

This research project has increased our understanding of many of the biotic and physiological factors which influence lesion formation and subsequent conidial formation and release of *A. porri* onion. In addition to increasing our knowledge of the disease cycle, these studies have made evident several areas where more information is needed.

Field observations, examination of purple blotch lesions and cross inoculations of *A. porri* and *S. botryosum* under laboratory conditions suggested that purple blotch may, in fact, be a disease complex. High levels of damage typically associated with *A. porri*, may be a result of infection by both *A. porri* and *S. botryosum*. Because even low lesion numbers can cause extensive leaf damage by enlarging rapidly and girdling the leaf, the extent to which infection by *S. botryosum* increases lesion size is important. Further research should be conducted to examine the influence of simultaneous inoculations of *A. porri* and *S. botryosum* on lesion size. Whether of not *S. botryosum* can infect onion

plants under field conditions is unknown, and should be investigated.

Once the importance of *S. botryosum* as a contributing factor to purple blotch on onion is known, this component should be included in the model. It is likely that not all conditions conducive to sporulation and release of *S. botryosum* coincide with conditions conducive to *A. porri* sporulation and release.

One object of this research project was to develop a regression equation to predict formation and release of *A. porri* conidia under Michigan onion growing conditions. A predictive equation based on the ln of the number of conidia released on D-1, average temperature during those hours when the VPD was <1.0 mb on D-1, and the sum of the hours during which rainfall occurred on D-0. To increase the applicability of this equation, it should be tested against additional years of data. Specifically, the influence of moisture on conidial formation and release in field situations must be determined so predictions are accurate in less humid years.

Frequent, large underpredictions and overpredictions occurred when a regression equation, which did not include a term for the previous days conidial release, was used to predict conidial release on the current day. This probably results in part from a retention of a portion of mature or partially mature conidia on the purple blotch lesions from one day to the next. Level of conidia

retained from day to day as well as the environmental factors which influence conidial retention should be studied. Inclusion in the regression equation of a factor describing conidial retention may increase accuracy even when the ln of the conidia released the previous day is not known.

Laboratory studies during the course of this research project focused on the influence of constant environmental conditions on conidial formation, survival, infection and lesion formation. While this establishes a baseline of data, environmental conditions in nature are rarely constant. Further studies should examine conidial formation on onion leaves under fluctuating humidity, temperature or interrupted dew periods. Conidial survival and infection of onion leaves should also be studied under fluctuating environmental conditions. Elucidation of the influence of these factors on conidial formation, release, survival, infection and lesion formation under controlled conditions would increase our knowledge of *A. porri* as well as be a valuable aid in interpreting weather data collected in the field.

Increased understanding of a purple blotch disease complex, caused by *A. porri* and *S. botryosum* (if it exists), and the influence of fluctuating humidity and temperature on stages of the disease cycle of *A. porri* should ultimately be applied to a purple blotch predictive model to increase both accuracy and applicability of the model.

APPENDIX

Influence of Interruptions of Dew Period on Numbers of Lesions Produced on Onion by *Botrytis squamosa*

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Michigan Agricultural Experiment Station Journal Article 10793
Accepted for publication 21 February 1985 (submitted for electronic processing).

ABSTRACT

Alderman, S. C., Lacy, M. L., and Everts, K. L. 1985. Influence of interruptions of dew periods on numbers of lesions produced on onion by *Botrytis squamosa*. Phytopathology 75:808-810.

Onion plants inoculated with dry conidia of *Botrytis squamosa* and placed in a dew chamber for 6 hr, followed by a dry period of variable (0.3–24 hr) duration, and returned to the dew chamber for the remainder of the incubation period, had progressively fewer lesions as duration of the dry interruption period increased. Lesion numbers also tended to decline as relative humidity decreased from 90 to 30% during a relatively short 20-min dew interruption period. Plants similarly inoculated showed increasing spore germination, appressorium formation, numbers of lesions, and visible infection hyphae as dew periods were increased from 2 to 24 hr. Plants were inoculated, given an initial dew period of from 2 to 12 hr, a 2-hr dry period, and a subsequent dew period of 12–22 hr for a total incubation period of 26 hr. Since these plants received 24 hr of dew in a 26 hr period, the only variable was the timing of the 2-hr dew interruption. Conidial germination was near 100% after 26 hr, regardless of timing of the dry period. However, appressorium formation and numbers of lesions were both reduced more by dew interruptions occurring after 6 hr of initial dew than by interruptions occurring after 2, 4, 8, 10, or 12 hr of initial dew, presumably because germinating conidia were most vulnerable to drying at this time. Numbers of visible infection hyphae were lowest when dry periods occurred after a 6- or 8-hr initial dew period.

Botrytis leaf blight, incited by *Botrytis squamosa* Walker, is a leaf-spotting and blighting disease which is especially severe under prolonged moist conditions at temperatures of 15–24 C (5,7,9,10). Studies relating dew period and temperature to leaf blight development indicated that greatest numbers of lesions developed at 18–20 C and that lesion numbers increased with increasing leaf wetness durations of up to 48 hr (1,6,8,10,11). Leaf lesions developed within 24 hr after inoculation and incubation under constant leaf wetness at 15–20 C (1). Most lesions remain the same size (1–3 × 2–4 mm), although a small proportion of them may continue to increase in size and lead to blighting if leaf wetness is prolonged (1,3).

Experiments relating interruptions of postinoculation leaf wetness to subsequent lesion numbers produced by *B. squamosa* have not clearly established the relationships of timing or duration of leaf wetness interruptions to lesion development, and no studies have related these factors to pathogen development either upon or within leaves. McDonald (6) examined the influence of wet-dry-wet periods of 4-4-20, 4-8-20, 8-4-16, 0-8-24, and 0-0-24 hr respectively, on lesion development, and found fewer lesions on plants given a postinoculation dry period compared with those provided continuous leaf wetness. Swanton (10) placed inoculated plants under 2- or 8-hr initial wetness periods, 2-, 6-, 9-, or 12-hr dry periods, and a 16-hr resumed wetness period. Increasing lengths of dry periods resulted in decreasing numbers of lesions. However, he failed to include inoculated and uninoculated control plants receiving continuous leaf wetness. Dzikowski (4) examined the influence of 1- or 4-hr dry periods following various initial wetness periods on disease incited by *B. squamosa*. He observed a lower percent diseased leaf area when dew was interrupted (1 or 4 hr) following 5 hr of initial leaf wetness than following 2 hr of initial leaf wetness. However, treatment responses were not quantified in terms of lesion numbers and it is not clear how treatment differences, expressed in terms of percent leaf area diseased, were obtained. All of these investigators used aqueous spore suspensions for inoculations, which we found to give less consistent results than the dry spore inoculation technique (1).

Under field conditions, conidia are subjected to cyclic wetting and drying, often more than once in a 24-hr period, at least in Michigan (*unpublished*). Understanding the influence of these factors on infection of onion by *B. squamosa* could be epidemiologically important.

The objectives of this study were to determine the influence of duration and timing of dry periods interrupting dew periods and of relative humidity (RH) during dry interruptions, on numbers of lesions produced on onion leaves by *B. squamosa*, and to determine the effect of leaf wetness duration and timing of dew period interruptions on conidial germination, appressorium formation, lesion formation, and numbers of visible infection hyphae.

MATERIALS AND METHODS

Botrytis squamosa was grown and spores were collected as previously described (1). One-month-old onion plants (*Allium cepa* L. 'Spartan Banner', 'Granada', or 'Yellow Sweet Spanish') sprouted from bulbs were used. For inoculation, plants were positioned within a cylindrical settling tower 61 cm in diam and 77 cm deep. Dry conidia (2.5 mg, or about 1.25×10^6 conidia) were dispersed near the top of the tower by gently blowing air from a pipet tip over the conidia on a piece of weighing paper. During inoculation, the plants were rotated on a turntable in the chamber at 5–6 rpm (1). After the conidia were dispersed, a cover was placed over the top of the tower for about 5 min to reduce external air currents and allow the spores to settle on leaf surfaces. Because of the limited size of the settling tower, plants within an experiment were randomly assigned to two groups, and each group was inoculated separately. The chamber was vacuumed between inoculations. After inoculation, both groups of plants were placed in a commercial dew chamber (model 1-35 DL; Percival Mfg. Co., Boone, IA), in which visible dew was produced on leaves in less than 1 hr. Lesion numbers on the two groups of plants within an experiment were not significantly different.

Influence of leaf wetness duration prior to dry periods. Forty-two onion plants were inoculated as described above and placed in the dew chamber at 20 C for 2, 4, 6, 8, 10, or 12 hr were moved to a growth chamber (20 C, 65 ± 10% RH) for a 2-hr dry period, and were finally returned to the dew chamber for the remainder of the 24-hr incubation period. Control plants remained in the dew chamber for 24 hr. Lesions visible to the unaided eye on each plant were counted after an additional 6 hr in the growth chamber.

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Influence of dry period duration on lesion production. Experiments were initiated to examine the influence of brief (0.3–1.7 hr) and extended (2–24 hr) postinoculation dry periods following initial dew periods on numbers of lesions produced by *B. squamosa*. Thirty-six onion plants were inoculated, incubated in the dew chamber at 20 C for 6 hr, then transferred to a growth chamber at 20 C. Dew formed on leaves in the dew chamber as uniform fine droplets in less than 1 hr, and evaporated in less than 5 min after placement in the growth chamber. After a 0.3-, 0.7-, 1.0-, 1.3-, or 1.7-hr dry period in the growth chamber, six replicate plants for each dry period were returned to the dew chamber for the remainder of the 24-hr incubation period. Plants were then placed in a growth chamber for an additional 6 hr, then lesions were counted. Uninoculated controls remained in the dew chamber continuously for 24 hr, then were held in a growth chamber for 6 hr prior to lesion assessment. The experiment was conducted twice.

To examine the effect of longer postinoculation dry periods, 24 onion plants were inoculated, incubated in the dew chamber at 20 C for 6 hr, and then were transferred to a growth chamber at 20 C and 65 ± 10% RH. After 4, 8, or 24 hr in the growth chamber, six plants from each dry period treatment group were returned to the dew chamber for an additional 24 hr. Uninoculated controls remained in the chamber for 54 hr. Experiments were conducted twice.

Influence of relative humidity during dry periods. Following inoculation and 6 hr in the dew chamber at 20 C, six plants were removed and placed in a growth chamber at 30, 60, or 90% ± 10% RH for 20 min and then returned to the dew chamber. Six control plants remained continuously in the dew chamber for 24 hr. Humidity and temperature were monitored with a recording hygrothermograph calibrated and checked before and after the experiment against readings obtained with a sling psychrometer. The experiment was conducted three times.

Influence of dew period duration and timing of dry periods on spore germination and infection. Twenty-eight onion plants were inoculated, then placed in the dew chamber at 20 C for 2, 4, 6, 8, 10, 12, or 24 hr. The third or fourth youngest leaves of four plants were used for sampling because 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 (28 mg of aniline blue, 20 ml of distilled water, 10 ml of glycerol, and 10 ml of 85% lactic acid), mounted on slides, and examined under the light microscope. Germinated and ungerminated conidia, and numbers of appressoria, lesions, and visible infection hyphae were counted and expressed as the percentage of the total number of conidia counted on a leaf sample. Conidia that were washed from the leaves during fixation were collected by passing the fixative through 13-mm-diameter membrane filters (0.33 μm pore diameter; Millipore Filter Corporation, Bedford, MA). These were mounted on slides, stained, and the germinated and ungerminated conidia were counted as above. The conidia were included in the totals for each leaf sample.

A similar experiment was conducted, except that a 2-hr dry interruption of dew was inserted after 2, 4, 6, 8, 10, or 12 hr of initial dew, followed by an additional dew period sufficient to bring the total incubation period to 24 hr. Leaves were sampled as described above. Experiments were conducted twice.

RESULTS

Influence of leaf wetness duration prior to dry periods. Plants given an initial dew period of 2, 4, 6, 8, 10, or 12 hr in the dew chamber, then a 2-hr dry period, followed by a variable dew period to complete the 24-hr incubation period, had an average of 283, 219, 152, 242, 261, or 282 lesions per plant after 2, 4, 6, 8, 10, or 12 hr, respectively, of initial dew period. Control plants held for 24 hr in the dew chamber had 383 lesions per plant. Since a dry period after 6 hr of initial dew had the fewest lesions, a 6-hr initial dew period was used in examining the effect of dry period duration on lesion production.

Influence of dry period duration on lesion production. Inoculated onion plants placed for 6 hr in a dew chamber, transferred to a growth chamber for dry interruption durations of 0.3–1.7 hr, and then returned to the dew chamber for the remainder of the 24-hr incubation period, had significantly fewer lesions than control plants that remained continuously in the dew chamber ($P = 0.05$) (Fig. 1). There was a tendency toward decreasing lesion numbers as the length of the dry period increased. Regression was significant (F test) at $P = 0.10$ but not at $P = 0.05$ ($R^2 = 56\%$).

Plants provided with extended dry periods of 4, 8, or 24 hr had fewer ($P = 0.05$) lesions than those provided with continuous wetness (Fig. 2). Regression was significant (F test) at $P = 0.10$ but not at $P = 0.05$ ($R^2 = 32\%$). There was a tendency toward decreasing lesion numbers as the dry period increased.

Influence of humidity during dry periods. Onion plants given a 6-hr period in the dew chamber, a 20-min dry period at 90, 60, or 30% RH, followed by 18 hr in the dew chamber had fewer lesions as humidities during the dew interruption were decreased (Fig. 3). Regression was significant (F test) at $P = 0.10$ but not at $P = 0.05$ ($R^2 = 25\%$).

Influence of dew period duration and timing of dry period on spore germination and infection. To determine directly the effects

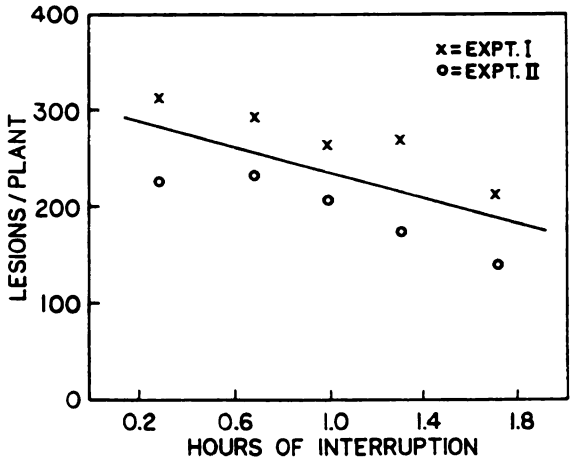

Fig. 1. Effect of dry period duration on number of lesions produced on onion by *Botrytis squamosa*. Plants were given a 6-hr postinoculation wetness period, a variable dry period, and a resumed wetness period to total a 24-hr treatment duration. Regression equation: $Y = 302 - 67.2X$. Control (continuous dew) values were 343 and 450 lesions per plant respectively.

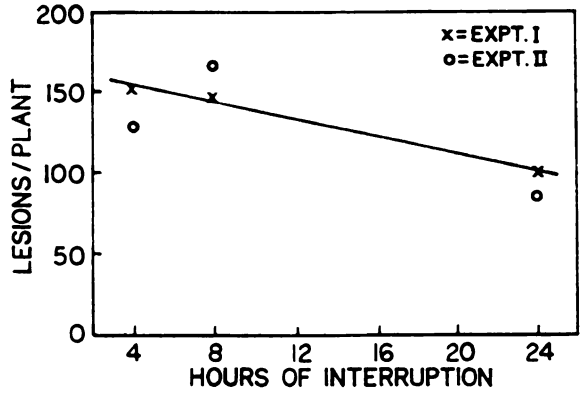

Fig. 2. Influence of 6 hr of postinoculation leaf wetness at 20 C, a variable dry period, and an additional 24-hr dew period on number of lesions produced on onion by *B. squamosa*. Regression equation: $Y = 161 - 2.5X$. Control (continuous dew) values were 413 and 380 lesions per plant respectively.

of dew period and of dew period interruption on the fate of germinating and penetrating conidia, we sampled, fixed, stained, and microscopically examined leaf samples from inoculated onion plants. Increasing duration of continuous dew periods resulted in increasing conidial germination, appressorium formation, and numbers of lesions through 12 hr of continuous dew and in increasing numbers of visible infection hyphae through 24 hr of continuous dew (Fig. 4). Regression of germination on dew period was significant at $P = 0.10$ ($R^2 = 41.3\%$), regression of appressorium formation on dew period was significant at $P = 0.05$ ($R^2 = 67.6\%$), regression of numbers of lesions on dew period was significant at $P = 0.05$ ($R^2 = 69.3\%$), and regression of visible infection hyphae on dew period was significant at $P = 0.01$ ($R = 76.7\%$).

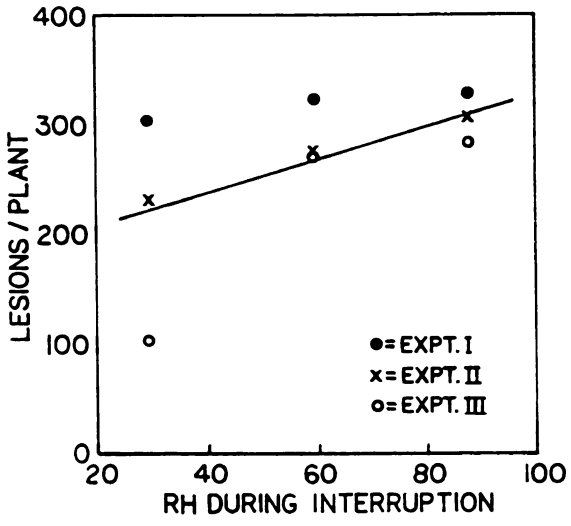

Fig. 3. Influence of a 6-hr postinoculation dew period, a 20 min dry period at 30, 60, or 90% RH, then an 18-hr dew period on numbers of lesions produced on onion by *B. squamosa*. Regression equation: $Y = 176 + 1.5X$. Control values (continuous dew) were 388, 426, and 376 lesions per plant respectively.

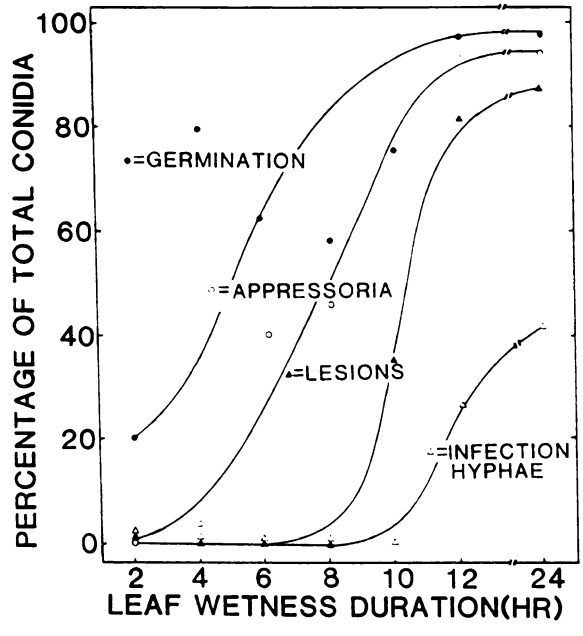

Fig. 4. Effect of variable dew periods on percent conidial germination, appressorium formation, lesions, and visible infection hyphae (all expressed as percentages of total conidia counted on leaf samples). Regression equations were: for germination, $Y = 45.0 + 2.6X$; for appressorium formation, $Y = 6.3 + 4.5X$; for lesions, $Y = 15.4 - 4.7X$; and for visible infection hyphae, $Y = -10.4 + 2.14X$.

Interruption of dew period for 2 hr did not significantly affect percent conidial germination, regardless of the timing of the interruption (Fig. 5). Interruptions after 6-hr initial dew periods resulted in the largest reductions in percent appressoria and lesions formed, and interruptions after 6 or 8 hr resulted in greatest reductions in percent visible infection hyphae (expressed as percent of total conidia counted on the leaf sample).

DISCUSSION

Plants given a 6-hr initial dew period followed by a 2-hr dry period and resumption of dew for the remainder of the 24-hr incubation period had fewer lesions than plants given a 2-, 4-, 8-, 10-, or 12-hr initial dew period. These data suggested that germinating spores were particularly vulnerable to drying after about 6 hr of dew, when germination had been initiated but before most germ tubes were protected by having penetrated the leaf, and we confirmed this hypothesis later with more detailed experiments (Fig. 5). The 6-hr initial dew period was then used to examine the effect of dew interruption duration on lesions produced. Even short dry interruptions following a 6-hr dew period reduced numbers of lesions on onion leaves, and numbers of lesions continued to decline with increasing lengths of dry periods (Figs. 1 and 2) in more or less linear fashion. This indicates that dry periods following the minimal length of dew period necessary for any visible lesions to occur (1,8) reduced infection efficiency at an increasing rate as dry periods lengthened, and underscores the epidemiological importance of uninterrupted leaf wetness periods. Our data generally support those of previous studies (4,6,10) with some quantitative refinements. Rather surprisingly, RH during a short 20-min dew interruption seemed to have some effect on lesion production (Fig. 3).

Our histogical observations on leaf samples revealed some interesting aspects of the effects of dew period and dry interruption

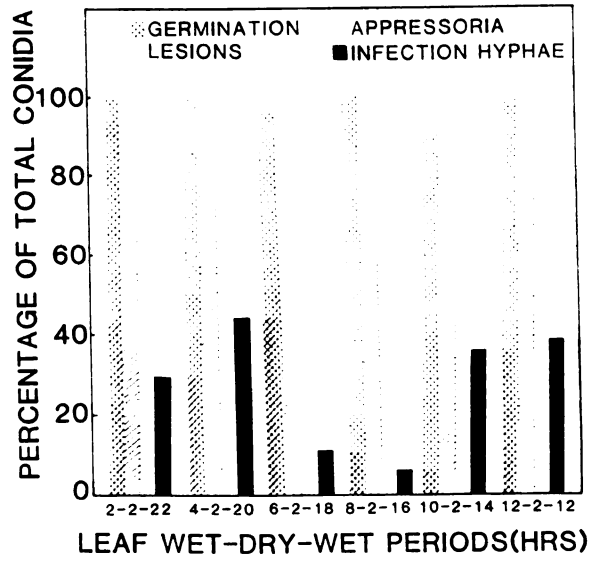

Fig. 5. Effect of timing of a 2-hr interruption of dew on percent conidial germination, appressorium formation, lesions, and visible infection hyphae (all expressed as percentage of total conidia counted on leaf samples).

period timing on the dynamics of spore germination, appressorial formation, lesion numbers, and visible infection hyphae. As expected, increasing dew period duration led to increasing percent conidia germinated, appressoria formed, lesions formed, and visible infection hyphae, and the time sequence of these events was clearly visible (Fig. 4). Conidial germination occurs first, followed by appressorium formation, then visible lesions, and finally visible infection hyphae within the lesions. Numbers of lesions were usually about 10% lower than percent conidia germinating, indicating that most germinating conidia establish lesions. The reasons for the low numbers of infection hyphae within lesions are obscure, but we (1) and others (3,7) have noted that the majority of the lesions of *B. squamosa* do not expand beyond a certain size (1–2 × 3-5 mm), and that the proportion of those in which hyphae continue to develop and lesions expand, leading to leaf blighting, is influenced by the length of continuous dew periods (1,3). The failure of *B. squamosa* to continue to grow in many lesions is suggestive of a hypersensitive-type reaction, and is supported by the generally recognized difficulty of isolating *B. squamosa* from lesions. Infection hyphae of *B. squamosa* grew about 3 times faster in senescent than in healthy onion leaf tissue (2), further suggesting that healthy onion tissue is resistant to ramification of infection hyphae.

The reduction in percent appressoria, lesions, and visible infection hyphae by dry periods occurring about 6–8 hr after onset of the initial dew period confirmed our hypothesis that, at these times, most conidia have germinated but have not yet penetrated and formed lesions (Fig. 4), and that the germ tubes are very likely vulnerable to drying if they have not yet penetrated. In our microscopic observations, we noted that some conidia formed appressoria and penetrated beneath the conidium with little or no germ tube visible, and these conidia may be less vulnerable to drying than those that form longer germ tubes. The timing and lengths of dry periods following onset of dew would thus seem to be important influences on numbers of lesions formed from a given inoculum load.

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