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ALTERNARIA LEAF BLIGHT OF MAIZE

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Alternaria leaf blight of maize

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INTRODUCTION

The genus Alternaria, which has a wide distribution and host range, includes species which are pathogens of numerous cultivated plants and are saprophytes living on various substrates (44). Species of Alternaria are common inhabitants of the soil, plant debris, and the phylloplane of plants (18,43). A major component of the air spora is the spores of Alternaria spp., which are major allergens of man.

Alternaria alternata (Fr.) Keissl., the type species for the genus, is variously reported as a polyphagous facultative parasite of "low aggressivity" (64), a weak pathogen capable of attacking only injured or otherwise predisposed tissue (29), or a saprophyte on senescent tissue (43). Isolates of A. alternata are known to produce toxins which participate in pathogenesis and symptom development in certain diseases of crop plants (106).

Recently, A. alternata was reported as a leaf pathogen of maize (Zea mays L.) (56). Young (116), in 1926, reported a leaf spot of maize caused by A. alternata and Joly (43) listed maize as an "occasional host" of this fungus. Martinson (56) found that this fungus caused disease only when inoculated into tissue already damaged physically or physiologically. A. alternata enlarged these necrotic areas producing chlorotic streaking and further necrosis.

The purpose of this research was: 1) to determine the effects of dew period, inoculum density, leaf age, and temperature on disease development; 2) to elaborate the physiology of pathogenesis; 3) to determine the host specificity of the maize isolate of A. alternata;

4) to test the disease reaction variability within maize inbreds to A. alternata; and 5) to determine whether or not a toxin is involved in disease development.

LITERATURE REVIEW

Taxonomy of *Alternaria alternata*

The genus *Alternaria* was established and described by Nees in 1816 on the basis of a single species, *A. tenuis*. Fries, in *Systema Mycologicum*, in 1832, described Nees' fungus under the name *Torula alternata*, citing *A. tenuis* as a synonym. This established the first valid name published for Nees' species (35,50). *Torula* and *Alternaria* were later separated. Simmons (93) redescribed *A. tenuis* and indicated that, under the International Rules of Nomenclature, *A. alternata* (Fr.) Keissl. was the valid name. Between the time Nees described the genus and Simmons determined the valid specific name, incomplete descriptions, variability in spore size and appearance, and large host ranges resulting from facultative parasitism have caused confusion in the classification of *Alternaria* species.

The conidia of *A. alternata* are variable in size as well as form. Conidia are dark olive-buff to olive brown, obclavate, ovate or elongate, muriform with one to nine transverse septa and zero to six longitudinal septa, and somewhat constricted at the septa. Spores are usually smooth with a short, broad beak but are often echinulate and beakless. The spores may appear to a layperson as small "hand grenades" when viewed through a microscope. They range in size from 7 to 70.5 μm in length and 6 to 22.5 μm in width. A succession of spores are formed in acropetal order through the apex of the terminal spore, forming branched chains of up to ten spores (5,24,64).

Aerial mycelium ranges from white to olivaceous black, cottony to felt-like, and may be interspersed with tufts of denser growth. Hyphae can be pigmented and are septate. Conidiophores are septate, branching, pigmented, and erect. Condia arise from pores at the apex of the conidiophore (64). Conidia, conidiophores, and mycelium are predominantly monokaryotic. Germination may occur from any cell of the conidia; more than one germ tube per conidium or per individual cell, can be produced. Anastomoses occur between hyphae in older cultures and nuclear migration has been observed. However, any heterokaryons thus formed would tend to dissociate during spore formation (49).

Condia produced in vitro on agar media are less uniform in size, smaller, and have shorter beaks than those produced on natural substrates. Reduction in relative humidity in petri plate cultures, a reduction in the water potential of the agar media, and lowering temperatures during spore formation and maturation increased the size of condia (68). Saltation is common in culture (64).

The perfect stage of A. alternata is Pleospora infectoria Fckl., which is a polyascal loculoascomycete with three to five septate ascospores (7,35). P. infectoria incites a leaf spot of Marsilea quadrifolia L. (7).

Host Range of Alternaria alternata

Alternaria alternata is widely distributed and, as a weak, non-specialized pathogen, has an extremely wide host range. Neergaard (64) states that A. alternata is "able to live on nearly every plant species if only the parts of the plant infected are dead or debilitated enough".

Joly (43) reported a list of 93 "occasional hosts" for A. alternata. Included in this list were oats (Avena sativa L.), cucumber (Cucumis sativus L.), barley (Hordeum vulgare L.), tomato (Lycopersicum esculentum Mill.), tobacco (Nicotiana tabacum L.), sugar cane (Saccharum officinarum L.), sorghum (Sorghum vulgare Pers.), potato (Solanum tuberosum L.), and maize. Disease usually occurred only on older leaves of these hosts in the form of leaf spots.

The most commonly reported hosts of A. alternata are bean (Phaseolus vulgaris L.) and tobacco. Severe leaf blight and defoliation of beans by A. alternata occurred under favorable conditions (86, 87). Severe flecking of bean pods (1) and a leaf spot of mung bean (P. aureus Roxb.) (36) have also been reported. In several instances, the fungus was found to cause disease in bean plants damaged by blowing sand, insects, or nutrient imbalance (16,17).

Alternaria alternata was reported to cause tobacco brown spot, attacking older weakened leaves early in the season, but, later in the season, causing leaf spots in undamaged, younger tissue (51,75,76). Isolates taken later in the season were shown to be more virulent than those from early season sampling (76).

Alternaria alternata has also been reported as a primary pathogen on sorghum (89). A. alternata f. sp. fragariae has been reported as a pathogen of strawberries (Fragaria chiloensis Duchesne) (20) and A. alternata f. sp. lycopersici causes a stem canker of tomato (34).

Alternaria alternata has functioned as a weak pathogen on most other reported hosts by penetrating through senescent, wounded or

otherwise damaged plant tissue. The fungus incited disease only in already diseased and aging foliage of beets (Beta vulgaris L.) and onion (Allium cepa L.) and attacked pepper (Capsicum frutescens L. var. grossum Sendt.) fruits only through sunscald areas, insect feeding sites, and other disease lesions (10,16). The pathogen was usually found only in old or weakened leaves of carrot (Daucus carota L. var. sativa DC.) (59). Lowered temperatures allowed A. alternata to parasitize the older, lower leaves of geranium (Pelargonium hortorum Bailey) (62). The infection of peppermint (Mentha piperita L.) and sunflower (Helianthus annuus L.) usually occurred at injury sites on the leaves (9,98). A. alternata moved into lesions caused by other pathogens on sugarcane leaves (41,94). Undersized, shaded, or prematurely ripened heads were parasitized by A. alternata, inciting black point of wheat (Triticum aestivum L.) kernels (6,113).

Alternaria alternata has been reported as a weak pathogen of maize, enlarging already necrotic areas and causing chlorotic streaking. Wind blown sand, insect feeding, and other tissue damage preceded the infection and initially incited the necrosis (56). Young (116) described isolates of A. alternata which caused leaf spots on maize. The fungus has also been reported from maize kernels (35).

Pathogenesis by Alternaria alternata

Predisposition of the suscept

As illustrated by its wide host range and "low aggressivity," A. alternata relies heavily on predisposition of the host for establishment in plant tissue. Yarwood (115) describes predisposition as

"environmentally conditioned susceptibility," the "tendency of non-genetic conditions, acting before infection, to affect the susceptibility of plants to disease." Wounding is a type of predisposition that exposes plant tissues to the pathogen and increases the susceptibility of the exposed tissues. This increased susceptibility of the host can permit normally saprophytic organisms to induce pathological processes.

Alternaria alternata is generally considered a saprophyte or weak pathogen. It rarely invades healthy tissue. This may be, in part, due to what Garrett (27) terms the infectivity of spores. The infectivity of spores may be sufficient for infection of senescent or damaged tissue, but inadequate for infection of healthy, green tissue. Garrett (27) states that for "successful invasion and progressive infection of the host, the pathogen requires a certain minimum invasive force, which must be supplied by the inoculum, until a progressive and self-supporting infection is established." Predisposition, through wounding or senescence, enables the fungus to establish a "progressive and self-supporting infection" and assume a virulent, pathogenic role (77). A. alternata is able to gain a foothold in necrotic or senescent tissue and extend into healthy tissue (43,64).

Toxins can play a part in the predisposition of host tissue to infection (115). Alternaria spp. are known to produce toxins and enzymes that weaken, degrade, or kill tissues in advance of fungal growth (43).

Phylloplane activity

Leaves are effective spore traps, the aerial surfaces usually covered with an extensive and varied population of microorganisms (18, 32). Gregory (33) states that a "mass of vegetation can rob an airborne spore cloud several times more effectively than the surface area of the ground on which the vegetation grows." Alternaria spp. produce large numbers of spores which are readily disseminated by the wind (43).

Alternaria alternata is one of the group of fungal pathogens that survive for long periods in the host phylloplane (18). The brown pigmentation and massiveness of the multicellular spores of A. alternata protect it against desiccation and the ill-effects of ultraviolet radiation in sunlight (73). A high rate of sporulation and the multicellular nature of the spores provides for high inoculum potential. Conidia are known to lie on tobacco leaves for several days prior to penetration (103). A. alternata is very frequently isolated from apparently healthy leaf tissue that has been surface sterilized. This suggests that the organism can be present within healthy tissue and make a limited amount of parasitic growth (C. A. Martinson, Dept. of Plant Pathology, Seed and Weed Sciences, I.S.U., personal communication, 1979). Thus, spores of epiphytes can survive in or on leaf tissue until it begins to senesce or is physically or physiologically damaged (18). Many secondary invaders of necrotic tissue are phylloplane residents (48).

Infection courts

There are many examples, with the genus Alternaria and other fungal pathogens, to illustrate the importance of wounding to

pathogenesis. Botrytis cinerea Pers. ex Fr. established a food base in damaged tissue before colonizing healthy areas of strawberry fruits (27). A. alternata was responsible for enlarging necrotic areas of tobacco leaves caused by flea beetle (76) or spider mite feeding (8), by Ascochyta phaseolorum Sacc. (76), and by mechanical wounding or physiological disturbances (40). It also colonized sunscald areas of pepper fruits before moving into undamaged tissue (10). Naturally sand blasted tomato and potato plants were found to be much more susceptible to A. alternata infection than undamaged plants (83,84). Similar findings were also reported with A. alternata and pea (Pisum sativum L.) plants (111). Under high moisture conditions, A. mali Roberts enlarged dead spots on apple (Malus sylvestris Mill.) leaves (80). A. musae Bour. & Bat. invaded banana (Musa sapientum (L.) Kuntze) leaves through small lesions caused by aphid feeding. A drop of plant sap released at the wound site served as a substrate for fungus growth (91).

Lesions incited by other pathogens also served as infection courts for weak pathogens. Besides the previously mentioned case of A. alternata entering Ascochyta phaseolorum lesions on tobacco leaves, A. dianthi Stev. & Hall invaded Xanthomonas esculenti Rangaswami & Easwaran lesions on okra (Hibiscus esculentus L.), increasing the overall damage to the crop (79). It was suggested that Leptosphaerulina australis McAlp. enlarges lesions initiated by Hyalothyridium sp. on maize leaves (47). The stress placed on cotton (Gossypium hirsutum L.) seedlings as well as the wounding caused by root knot nematode feeding predisposed the

seedlings to root infections by A. alternata (15).

Besides entering leaf tissue through wounds, A. alternata penetrated tobacco leaves indirectly through stomata and weakened areas such as leaf margins and basal cells of trichomes (51,75). Direct penetration was less common until later in the season (87). Slightly swollen appressoria were produced but penetration may occur without them (51,87). A. alternata produced pectinolytic and cellulolytic enzymes that aid in penetration (67,76).

Epidemiology

Much of the work on the environmental parameters for A. alternata infection and disease development has been done with tobacco brown spot. The optimum temperature range for growth of A. alternata in culture was 24 to 28 C (96,100). Conidial germination occurred over a broad range of temperatures (16 to 28 C) (103) in the absence of free water, with the lower limit for relative humidity at 85% (63). The optimum temperature for infection was 20 to 21 C (100,103). Relative humidity and dew period were the most important factors for infection; no infection occurred below 90% relative humidity. In moist chamber experiments, 96 to 144 hours of post-inoculation moisture resulted in the most infections. The number of infections was not affected by interruption of the moisture period with dry intervals. Under natural conditions, prolonged and repeated nightly dews provided sufficient moisture for infection (87,103). Waggoner and Horsfall (110) reported similar moisture relationships for A. solani (Ell. & G. Martin) Sor. on potato. Lesions enlarged only when leaves were wet and dew provided

sufficient moisture for infection and lesion expansion. A. alternata isolates from soil were tolerant of water stress with growth extinction occurring at about -150 bars (2).

Development of disease

Lesions on tobacco leaves, incited by A. alternata, were irregularly shaped, averaging 8 mm but ranging from one to 30 mm in diameter (101). The necrotic center was surrounded by a chlorotic halo then a prehalo zone (54). The prehalo zone was visually indistinguishable but differed chemically from healthy tissue (54,102). The lesions expanded rapidly in concentric zones with the prehalo zone becoming chlorotic (54). Fungal hyphae extended no more than 1 mm beyond the necrotic zone (101); hyphae have not been observed entering the intercellular spaces of healthy tissues nor invading living cells (75). It was suggested that toxins or other fungal metabolites diffused into healthy tissue far beyond the necrotic area of the lesion and produced the halo and prehalo zones that are predisposed to fungal invasion (51,54).

The lesions incited by A. alternata on beans were similar to those on tobacco; spots were irregular, brown, and expanded in concentric zones (87). A. alternata f. sp. lycopersici caused dark brown stem cankers that exhibit concentric zones. Foliar symptoms included epinasty and angular leaf spots (34).

Alternaria Toxins

"Toxin" is a general term used to denote a poisonous, degradative fungal metabolite that is involved in plant disease; enzymes and growth

regulators are excluded by some authors (52). Dimond and Waggoner (19) state, "In some diseases, toxins are thought to cause a more rapid and extensive invasion by the pathogen than would be the case in the absence of a toxin; it has even been suggested that some parasites would be unsuccessful if the toxin did not kill cells in advance of the fungus and permit it to establish itself continually on dead or dying cells and produce more toxin." Gaümann (28) describes what he calls "short-range toxins," those which act directly on tissue surrounding the focus of infection.

Graniti (32) includes enzymes under the general term "toxin" if they are shown to possess "inherent primary toxicity." These "phyto-aggressins" may include cutinases, macerating enzymes, phenoloxidases, lignin degrading enzymes, cellulases, and pectolytic enzymes not directly toxic to cells. These enzymes mainly affect the plant apoplast. Graniti (32) describes the role of phytoaggressins as "promoting the invasion of host by pathogen by overcoming barriers or depressing defense reactions, enhancing virulence without being directly toxic."

Toxins are generally grouped into three subclasses. Pathotoxins are toxins that play an important causal role in disease development. All symptoms are a result of the direct action of a fungal metabolite. Vivotoxins produce at least a portion of the disease symptoms. Pathotoxins and vivotoxins are host specific, exhibiting the same host specificity as the pathogen. If the ability to produce the toxin is lost, pathogenicity is also lost. The toxin also must be shown to be produced in the host (19,112). The third group of toxins is phytotoxins.

As secondary determinants of disease, phytotoxins are nonspecific, i.e., toxic to hosts and nonhosts of the pathogen. They incite few or none of the disease symptoms and influence the virulence of the pathogen but do not control pathogenicity. Phytotoxins are usually involved in predisposing the host to infection and establishing the pathogen in plant tissue (85,106,112).

Alternaria spp. produce a wide range of toxins, both specific and nonspecific. Most are low molecular weight compounds, usually small peptides or phenols. Some Alternaria toxins produce chlorosis at some stage in the ontogeny of a lesion and are involved in membrane disruption, inhibition of protein synthesis, hormonal imbalances and antimetabolic activity (106). Other Alternaria toxins cause necrosis, damaging or killing parenchyma cells in advance of the pathogen. Necrosis producing toxins are usually produced by localized pathogens (32).

A single species of Alternaria can produce several toxins. A. kikuchiana Tanaka, a pathogen of Japanese pear (Pyrus ussuriensis var. aromatica Rehder), produces a series of host specific vivotoxins, phytoalternarins A, B, and C. The specificity of the toxins is identical to that of the pathogen (39,72,106). This pathogen also produces nonspecific toxins, tenuazonic acid (45), alternariol and its monomethyl ether (109), and altenin (61). A. dauci (Kuehn) Groves & Skolko (35) and A. cucumerina (Ell. & Ev.) J. A. Elliott (24) also produce alternariol and its monomethyl ether.

Alternaric acid, produced by A. solani, causes symptoms in tomato and potato leaves similar to those induced by the pathogen (11). It causes epinasty, necrosis and chlorosis, and wilting (71). However, there is no correlation between pathogenicity of A. solani isolates and the ability to produce alternaric acid nor is the toxin host specific (11).

The toxins produced by A. alternata have been the most studied. A. alternata is capable of adapting its nitrogen metabolism as a function of its life style--parasitism or saprophytism (43). In review articles, Templeton (106) and Miller (58) report the various toxins produced by A. alternata. Alternariol ($C_{14}H_{10}O_5$) and alternariol monomethyl ether ($C_{15}H_{12}O_5$), substituted dibenzo-α-pyrones, induce chlorosis when injected into tobacco and exhibit antibacterial activity (55,58, 69,74). Rosett et al. (82) have reported a number of toxins related to alternariol. These include altenuic acid I, II, and III ($C_{15}H_{14}O_8$), altenusin ($C_{15}H_{14}O_6$), dehydroaltenusin ($C_{15}H_{14}O_8$), and altertenuol ($C_{14}H_{10}O_6$). Pero et al. (70) also isolated altenuisol ($C_{14}H_{10}O_6$) from culture filtrates of A. alternata. Neither their host specificity nor their role in disease development have been elucidated.

Tenuazonic acid ($C_{10}H_{15}O_3N$) is a vivotoxin involved in the brown spot disease of tobacco. The toxin was isolated from tissue in the halo zone of lesions and production of the toxin was correlated with the pathogenicity of fungal isolates (57,82,102). Tenuazonic acid also inhibited root growth of lettuce seedlings (102).

Tentoxin, a cyclic tetrapeptide [cyclo (-L-leucyl-N-methyl-(Z)-dehydrophenylalanyl-glycyl-N-methyl-L-alanyl-)], has been the most extensively elaborated of the A. alternata toxins (14,46). Detailed production and purification techniques as well as the chemical characteristics of tentoxin are described in several publications (23,30, 31,87,88,107,108).

Tentoxin is not host specific but does exhibit some specificity (78). Tentoxin induces stunting, wilting, and chlorosis of many dicotyledonous species, particularly members of the Compositae and Umbelliferae (22,38,87). The Solanaceae, Leguminosae, and Gramineae contain both sensitive and insensitive species (22,38,78). Maize is reported to be insensitive to the toxin (22,106). All species of Cruciferae are insensitive (22,78). The bioassay for tentoxin is based on the sensitivity of some Nicotiana species and the insensitivity of others (14,104).

The mechanisms by which tentoxin affects germinating seedlings are disruption of chloroplast development and inhibition of photophosphorylation (3,38,90) and reduction in transpiration rate (21,23). Tentoxin acts as an energy transfer inhibitor, interfering with the phosphorylation coupling mechanism but having no direct effect on electron transport (3). The activities of chloroplast enzymes are reduced in sensitive species; reduced activities are caused by decreased concentrations of the enzymes rather than selective inhibition of the enzymes (37). These inhibitions are due to the presence, in sensitive species, of a receptor site for tentoxin on chromosome coupling factor 1. Tentoxin

binds to this site, resulting in inhibition of photophosphorylation and light-directed protein and RNA synthesis, leading to disruption of chloroplast development (105).

Transpiration rates are reduced by tentoxin due to suppression of stomatal opening and induction of stomatal closure. Tentoxin stopped or severely inhibited potassium accumulation in guard cells (23).

Alternaria alternata toxins do not appear in the growth medium until after the fungus has reached a peak in growth and autolysis begins (26). Tentoxin is not excreted into the medium but seems to be present within the hyphae. It is unknown whether toxins are produced and infiltrate tissue during the saprophytic growth of the fungus or are excreted during the parasitic growth stage. Alternaria isolates commonly lose their ability to produce toxin after continued culture in the laboratory (114).

MATERIALS AND METHODS

General Procedures

Alternaria alternata isolates

The two isolates were AL-4, isolated from diseased maize leaves in Iowa in 1974 by C. A. Martinson, and isolate V, a tobacco brown spot isolate, obtained from R. D. Durbin, University of Wisconsin, Madison.

The maize isolate (AL-4) was used exclusively in all experiments except toxin studies and one in which the pathogenicity of the tobacco isolate was compared to the maize isolate.

Isolates were maintained by mass transfer to Difco potato dextrose agar (PDA). Spores were detached from the conidiophores by flooding the medium with sterile distilled water, gently rubbing the surface with an L-shaped glass rod, then filtering through cheese cloth. Spore concentrations in inoculation experiments were adjusted to 1.2×10^4 spores/ml, unless otherwise noted.

Maize seed and culture

In all experiments, unless otherwise noted, maize inbred Oh43 was used. Seeds were planted in plastic pots measuring 10 cm diam by 8 cm deep. The soil mixture consisted of three parts clay loam soil, two parts sand, and one part peat. During planting, about 300 g of soil mix plus 5.5 g fertilizer were placed in the pot; five seeds were placed on the soil surface and covered with 300 g of the soil mixture. Osmocote 120 day slow release fertilizer (Sierra Chemical Co., Milpitas,

Calif.) supplied the N-P-K at 19-6-12. The plants were maintained either on a greenhouse bench or in a growth chamber, both with daily watering.

Experimental procedure

Extended leaves on 3 to 4 wk old plants were wounded by briefly (1 sec) touching the leaf blade with the hot flat tip of a soldering iron (3 mm cylindrical tip). The killed area ranged from seven to nine mm diam. About 0.1 ml drop of inoculum (1200 spores/drop) was immediately placed on the killed tissue. Unless otherwise stated, five sites were inoculated/plant, three plants/pot, three pots/treatment. Wounded but uninoculated controls (water drops instead of inoculum) were used to establish the average length of the initial lesion for each experiment.

Inoculated plants were placed immediately in either a nonoperating Percival growth chamber (Percival Refrigeration and Mfg. Co., Boone, Iowa) containing one to two Hankscraft Model 240 humidifiers (Hankscraft Co., Reedsburg, Wisc.) or into a cam-programmed dew deposition environment chamber that maintained temperatures within $\pm$ 0.5 C (12). Temperature and dew period duration were varied according to the intent of individual experiments; however, for most experiments, incubation temperature and dew period were 20 C with six days of 16 hr dew/24 hr (a total of 96 hr dew).

Lesion lengths were measured parallel with the midvein and between the chlorotic edges perpendicular to the midvein.

Environmental Effects on *Alternaria alternata in vitro*

Temperature and media

The effect of temperature and culture medium on growth of the fungus *in vitro* was determined using two media, V-8 juice agar (68) and PDA, and incubation temperatures of 10, 15, 20, 23, 25, 27, 30, and 35 C $\pm$ 0.5 C. Plates were seeded with 9 mm disks cut from the edges of week-old colonies with a sterile cork borer. Diameters of ten colonies per treatment were measured after seven days growth in Precision Thelco Model 6 incubators (Precision Scientific Co., Chicago, Ill.).

Water potential

Osmotic potentials of the culture medium were varied by using Sommers' basal medium (97), which is a glucose-yeast extract-malt extract medium. Different molal amounts of either sodium chloride or sucrose were added to the medium according to the water activity tables of Robinson and Stokes (81) and Scott (92) (Table 1). Water activity is equal to the relative humidity divided by 100 of a closed volume of air in equilibrium with the system. It is a measure of the availability of water in the medium and is interconvertible with water potential using the equation

$$-\Psi = 10.7\ T\ \log_{10}\ (\frac{100}{RH}),$$

where $-\Psi$ is the water potential in bars, T is the absolute temperature, and RH is the relative humidity as a percent (92). The water potential

Table 1. Water activities, water potentials, final water potentials, and molalities of sodium chloride and sucrose at 25 C

NaCl				Sucrose			
m[a]	a_w	Ψ	Final Ψ[b]	m	a_w	Ψ	Final Ψ
.1	.9966	- 4.65	- 5.85	.1	.9982	- 2.50	- 3.70
.3	.9901	-13.79	-16.55	.6	.9887	-15.71	-16.91
.4	.9868	-18.37	-19.57	.8	.9847	-21.32	-22.52
.6	.9802	-27.62	-28.82	1.0	.9806	-27.14	-28.34
.7	.9769	-32.34	-33.54	1.2	.9763	-33.16	-34.36
.9	.9702	-41.85	-43.05	1.4	.9719	-39.43	-40.63
1.2	.9601	-56.39	-57.59	1.8	.9628	-52.50	-53.70
0[c]	.9991	- 1.20	- 1.20	0	.9991	- 1.20	- 1.20

[a] m = molality; a_w = water activity; Ψ = water potential in bars.

[b] The final water potential is the sum of the water potential of Sommers' medium (-1.2) and the solute potentials.

[c] Sommers' basal medium without amendments.

of Sommers' basal medium is -1.2 bars (97). The potential of solute amended media is the sum of the water potential of the medium plus the solute potentials (97). Plastic petri dishes containing 25 ml of medium were seeded with 9 mm disks of either the maize (AL-4) or tobacco (V) isolates. The plates were then enclosed in plastic bags and placed in 25 C incubators. Ten colonies divided into two replications per treatment were measured at seven days.

Relative humidity and spore germination

Leaf disks 10 mm in diameter were placed in plastic petri dishes containing 50 ml of glycerol-water mixtures at various specific gravities (42) (Table 2). Disks were inoculated with a 0.1 ml drop containing approximately 150 conidia. The disks, except for those in 100% relative humidity, were air dried before placement in the dishes (65,66). The disks were incubated for 4, 8, and 12 hr at room temperature, then stained with acid fuchsin in lactophenol. The number of germinated conidia and the number of germ tubes per conidium were recorded.

Table 2. Specific gravities and estimated relative humidities of various glycerol-water solutions (42)

Percent glycerol (by weight)	Specific gravity	Percent relative humidity
0	0.998	100.0
15.5	1.036	97.0
25.0	1.058	92.8
35.0	1.085	85.2
50.0	1.126	73.4

Statistical Analysis

Analysis of variance using SAS76 (4) programs for a randomized complete block design was used to detect possible significant differences in lesion lengths affected by isolate, length of dew period/24 hr, delaying or interrupting the moist incubation period, inoculum density, and leaf age. Analyses of variance (SAS76) for factorial and split plot designs were used to analyze data from the temperature by dew period, inbred variability, temperature by medium, and water potential experiments.

When treatments were spaced at equal intervals, linear and multiple regressions, including the subdivision of the sums of squares for the main effects of treatments into polynomial components, were used to obtain predictive regression equations (95).

Duncan's multiple range test (95) for multiple comparisions was used to detect significant differences among the means of lesion lengths for the various treatments.

Toxin Studies

Production, extraction, and purification of suspected toxins

Culture filtrates used for purification and bioassay of suspected toxins were obtained by growing either isolate AL-4 or V (known to produce tentoxin) in 2 liter Erlenmeyer flasks containing 500 ml of medium. The medium was either modified Fries medium (53) or a 4% sucrose-2% yeast extract medium (90). The media were seeded with several 9 mm disks cut from actively growing cultures. The extraction and bioassay methods

were different for each medium.

When modified Fries medium was used, still cultures were grown for four weeks at 25 C under artificial lights with 8 hr of light/24 hr period. The extraction procedure was described by Saad et al. (88). The mycelium was homogenized using a Waring blendor and returned to the culture filtrate. This was centrifuged for 10 min at 10,000 x g and the pellet was discarded. The filtrate was concentrated under reduced pressure at 50 C to one-tenth its original volume then combined with seven volumes of absolute ethanol. The precipitate was removed by filtration. This volume was reduced to one-tenth the original volume under reduced pressure at 50 C and the aqueous solution adjusted to pH 1 with hydrochloric acid. The sample was extracted with an equal volume of diethyl ether in a sepratory funnel and the aqueous phase (AQ2) removed. An equal volume of 5% $NaHCO_3$ was added to the ether phase, shaken, and the aqueous phase (AQ3) removed. The ether phase was evaporated to dryness under reduced pressure at 30 C, then dissolved in 4 ml of water for each liter of original homogenate. No further purification was done; a bioassay was performed using this solution. The aqueous phases were bioassayed also.

Both AL-4 and V isolates were grown in still culture in 4% sucrose-2% yeast extract medium for two weeks at 28 C without light, then extracted following the procedures described by Schadler et al. (90). The culture filtrate was collected by filtration and extracted three times with one-half volumes of chloroform. The combined chloroform extracts were concentrated to a small volume under reduced pressure at 45 C then diluted to 10 ml/500 ml of culture filtrate using chloroform. This was successively extracted with equal volumes of 5% Na_2CO_3,

0.1 N HCl, and twice with distilled water. The volume of the organic extract was reduced under pressure and the AL-4 and V samples applied in 1, 2, 5, and 20 µl aliquots to a 20 cm x 20 cm x 1.0 mm precoated silica gel thin layer chromatography (TLC) plate (Sil G 100, Macherey, Nagel, & Co., Düren, Germany). The plate was developed with ethyl acetate:methanol (95:5) and, after drying, was examined under short wave UV light for toxin. All fluorescence quenching areas were removed separately from the plate and recovered from the gel with 95% ethanol. Part of these ethanol extracts were evaporated to dryness then dissolved in water for use in bioassays. The remaining ethanol extracts were used to obtain absorption spectra (240 nm to 360 nm) using a Beckman DK-1A recording spectrophotometer.

Toxin bioassay

The toxin extracts produced in modified Fries medium and extracted following Saad's method were bioassayed using the cucumber bioassay described also by Saad et al. (88). Fifteen cucumber seeds or ten maize seeds (W64A) were placed in petri dishes containing two discs of Whatman No. 1 filter paper, and 10 ml of either distilled water or toxin solution. These plates were germinated at room temperature at 300 ft-c of continuous illumination for four days. At the end of this time, cotyledons and hypocotyls were weighed and placed in absolute ethanol in a 70 C water bath for three hours. The absorption spectra of these solutions were measured at 663 nm and the chlorophyll content expressed as OD_{663} nm/g fresh wt, with values corrected for absorbance at 720 nm. Germination percentages were recorded also. The three

aqueous fractions (AQ1, AQ2, and AQ3) from the extraction procedures were bioassayed also.

The bioassay of the TLC purified extracts followed the procedures outlined by Durbin and Uchytil (22) and was based on the differential sensitivity of Nicotiana sp. to tentoxin. Nicotiana tabacum L., an insensitive species, and Nicotiana megalosiphon Heurck & Muell.-Arg., a sensitive species, were used. Twenty seeds were placed in petri dishes containing two discs of Whatman No. 1 filter paper moistened with 10 ml of water or an aqueous solution of toxin extract. These were incubated at room temperature under continuous light until the cotyledons had fully expanded. Seedlings were classified as chlorotic or normal. Extracts of both the maize and the tobacco isolates were used. The bioassay was replicated in three separate dishes.

Further bioassays involving extracts produced in both media consisted of attempts to demonstrate toxin activity on mature maize leaves. Detached leaves were placed in moist chambers, spotted with the extracts, wounded with the hot, flat tip of a soldering iron as before, then spotted, injected with the extracts using a 1 cc syringe with a 26 gauge hypodermic needle, or repeatedly punctured with a 26 gauge needle then spotted. Attached leaves were spotted with the extracts or wounded and then spotted before being placed in plastic bags. The treated areas of both attached and detached leaves remained moist through the entire ten day observation period.

RESULTS

Factors Affecting Infection and Disease Development

Wounding and the development of Alternaria blight

Martinson (56) was able to provide sufficient wounded tissue for A. alternata establishment and infection in maize with several cell killing techniques. An experiment was designed to compare the standard hot soldering iron procedure with no wounding prior to inoculation and rubbing the tissue with 600 mesh carborundum prior to or in conjunction with inoculation.

The only procedure that resulted in any yellowing or necrosis was inoculation following injury with the soldering iron where A. alternata infected all the wounded sites. The injury caused by a hot, flat tipped soldering iron provided a discrete amount of injured tissue and an adequate wound for conducting quantitative experiments (Fig. 1D).

In sufficiently injured tissue, after incubation for six days (16 hr moisture daily), lesions expanded far beyond the initial burn wound and exhibited distinct zones. In the center of the lesion, roughly corresponding to the original wound, the fungus sporulated abundantly, producing a black appearance. Some loss of tissue in this area was evident (Fig. 1B). Beyond the lesion center, the tissue was necrotic with some sporulation present (Fig. 1A and B). This necrotic area was surrounded by a chlorotic area, which extended for some distance but was delimited by the leaf veins (Fig. 1A and C). These chlorotic areas soon became necrotic. In some severe instances, nearly the entire

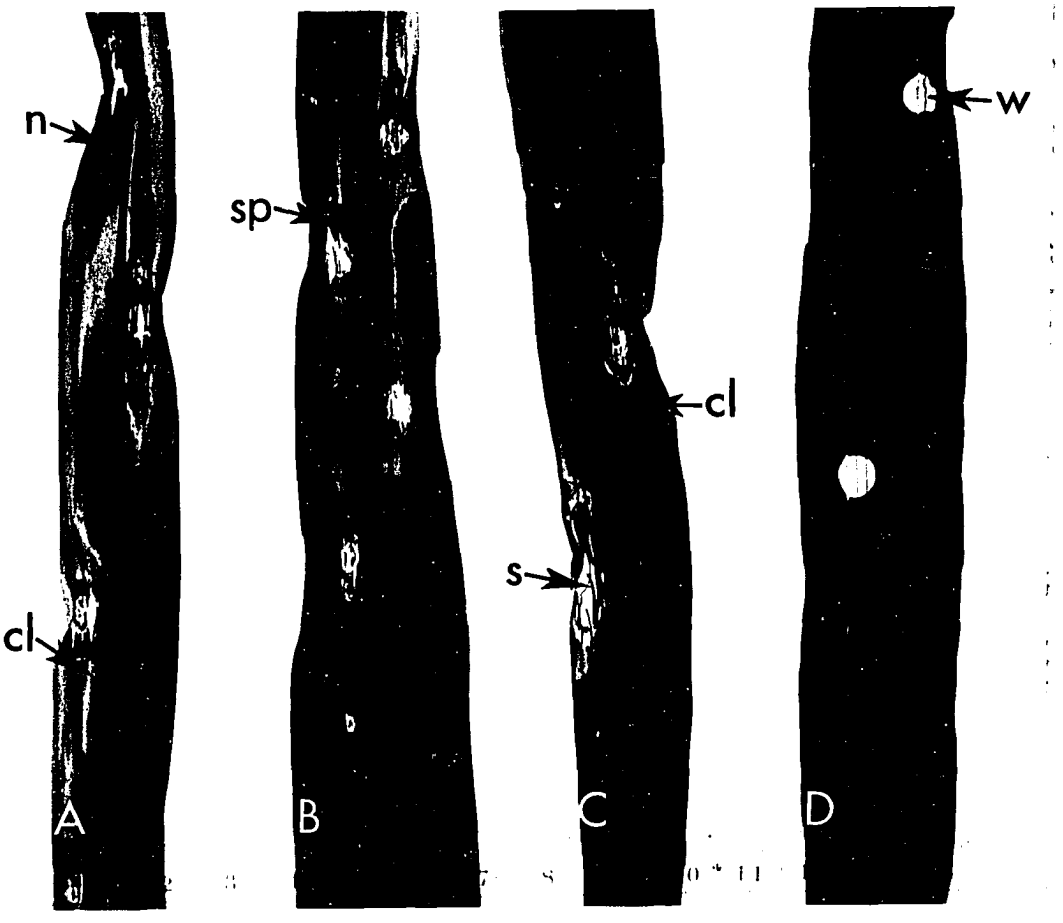


Fig. 1. Lesions on maize leaves expanded by Alternaria alternata showing the initial wound (D) and the necrosis (A), sporulation (B), and chlorosis (C) following inoculation and incubation in a dew chamber for six days (16 hr dew/ 24 hr). Abbreviations: n, necrosis; cl, chlorosis; sp, sporulation; s, shot holing; w, wound

leaf was killed (Fig. 1A).

In an experiment involving the wounding of previously inoculated but healthy tissue, 0.1 ml drops of inoculum containing 1% sucrose were placed on 20 marked but uninjured sites on the third and fourth leaves of three week old maize plants and placed in a growth chamber with humidifier for three successive 16 hr nights. Leaf tissue near the inoculated sites was damaged with the hot soldering iron and the plants returned to the dew chamber for seven days (16 hr dew/24 hr). Disease resulted in 10% of the areas wounded. A. alternata spores survived on moist leaf tissue and infected damaged tissue even when damage and inoculation did not occur simultaneously.

During other experiments in this total study, A. alternata was observed to infect new, accidentally damaged leaf tissue near areas purposely wounded for experiments. Leaf tissue that was accidentally touched by the hot soldering iron, or was killed by contact with the dew chamber lights or the cold walls, was easily colonized.

Alternaria alternata isolates

The maize isolate, AL-4, and the tobacco brown spot isolate, V, of A. alternata were compared for their ability to expand lesions produced by the soldering iron. Both isolates significantly increased lesion size beyond the initial wound. There was no significant difference in expansion by the two isolates (Table 3).

Table 3. Lesion expansion on maize leaves caused by the maize isolate of Alternaria alternata, the tobacco isolate of A. alternata, and an uninoculated water control

Isolate[1]	Lesion length (mm)[2]
Control	7.9a[3]
AL-4	23.8b
V	24.4b

[1]Control = wounded and with no inoculum in water droplet; AL-4 = the maize isolate of A. alternata; V = the tobacco isolate of A. alternata.

[2]Lesion lengths are means of 18 lesions in three replications measured seven days after inoculation (16 hr dew/24 hr period).

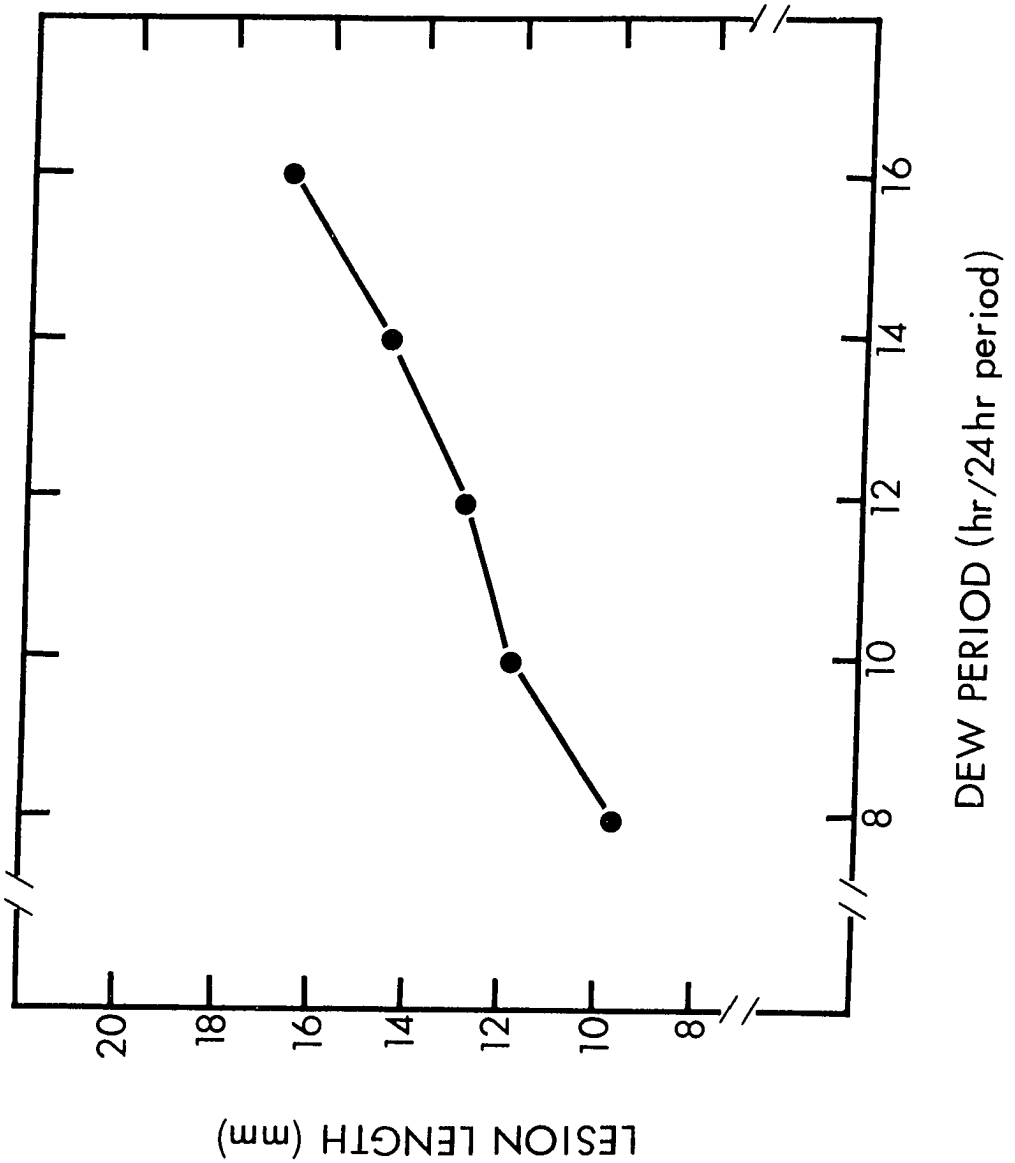
[3]Values followed by the same letter are not significantly different (P = 0.05) as determined by Duncan's multiple range test.

Temperature and dew period

Length of daily dew period The duration of dew period/day necessary for lesion enlargement was investigated by varying the number of hours of dew/24 hr from 8 to 16 hr in two hr increments at 20 C for six days. Plants were removed from the dew chamber at two hour intervals and placed in a dark growth chamber at 20 C. At the end of the dew cycle, all plants were replaced in the dew chamber (without dew) and exposed to normal lighting.

There was a positive correlation (r = 0.993) between the expansion of lesions by A. alternata and the number of hours of dew received/24 hr (Fig. 2). The means of lesion lengths were not significantly different

Fig. 2. Sample regression of the numbers of hours of dew/24 hr (X) and the mean lengths of *Alternaria alternata* lesions (Y). $Y = 2.485 + 0.91X$, $r = 0.993$. Uninoculated control lesions = 8.2 mm


LESION LENGTH (mm)
20
18
16
14
12
10
8
DEW PERIOD (hr/24hr period)
8
10
12
14
16

from 10 through 16 hr (Table 4) but the linear regression of lesion length on dew period was highly significant (Table 5). The sample regression equation was:

$$\hat{Y} = 2.485 + 0.91X.$$

Further regression analysis using the quadratic, cubic, and quartic components was not significant (Table 5).

Table 4. The effect of daily duration of dew on the expansion of lesions by Alternaria alternata on maize leaves

Dew period (hr)[1]	Lesion length (mm)[2]
0	8.2a[3]
8	9.8ab
10	11.9abc
12	12.8abc
14	15.3bc
16	17.2c

[1]Numbers represent the duration of the dew period during a 24 hr period.

[2]Lesion lengths are means of 45 lesions in three replications measured seven days after inoculation.

[3]Values followed by the same letter are not significantly different (P = 0.05) as determined by Duncan's multiple range test.

Table 5. Analysis of variance for lesion lengths on maize leaves incited by Alternaria alternata for five different durations of daily dew periods

Source of variation	df	Mean square	F	Prob > F
Block	2	29.071	0.551	ns
Dew period	4	377.816	7.158	0.0099
Linear DP1,2,3,4,5[a]	1	1490.580	28.240	0.0001
Quadratic	1	2.414	0.046	ns
Cubic	1	1.620	0.031	ns
Quartic	1	16.648	0.315	ns
Dew period x block	8	52.782		
Residual	210	4.520		
Corrected total	224	17.019		

[a]DP1 is 8 hours of dew/24 hr period for seven days. DP2, DP3, DP4, and DP5 are 10, 12, 14, and 16 hr of dew/24 hr period, respectively.

Continuous vs periodical dew Wounded and inoculated plants were exposed to continuous dew for 32, 48, 64, 80, and 96 hr in a 20 C dew chamber. Lesion lengths on these plants were compared to lesion lengths on plants exposed to equal total hr of dew but interrupted by dryness (16 hr dew/24 hr) in a separate experiment. There appeared to be no significant difference between most periods of continuous dew and the corresponding plants exposed to dry intervals (Table 6). The only significant difference occurred at 96 hr dew; six days with 16 hr dew periods resulted in significantly longer lesions than 96 hr of continuous dew. This may, in part, be due to the stress placed on plants in

Table 6. Comparison of the effects of continuous dew and daily 16 hr periods on lesion expansion by Alternaria alternata on maize leaves

Total dew time (hr)[1]	Dew schedule	
	Continuous	Daily 16 hr periods
0	7.9a[2]	7.9a
32	8.4a	8.0a
48	8.9a	8.3a
64	11.3a	11.8a
80	12.3a	12.9a
96	12.0a	22.4b

[1]Numbers represent the time plants were maintained in a dew chamber either continuously or with daily 16 hr periods, i.e., 32 hr of dew or two 16 hr/24 hr applications of dew, etc.

[2]Numbers represent the means of 30 lesion lengths in three replications measured seven days after inoculation. Values followed by the same letter are not significantly different (P = 0.05) as determined by Duncan's multiple range test.

continuous moisture and darkness that resulted in severe root rots and dead or dying plants after several days.

Temperature x dew period interaction The effects of temperature and dew period were evaluated in the dew chamber at 10, 15, 20, 25, and 30 C, and with dew periods of three to six days (16 hr dew/24 hr). Dew periods were investigated in a series of temperature experiments over time.

The linear and quadratic regression of lesion length on total hours of incubation in a dew chamber (16 hr dew/24 hr) were significant

(Table 7) as was the correlation (r = 0.995). The upward slope of the regression line gets steeper with length of dew period; lesion lengths increase more rapidly as the dew period is extended (Table 8, Fig. 3).

Lesion lengths increased with rising temperature to a maximum at 20 C and then decreased above that point (Table 8, Fig. 4). Temperatures below 20 C allowed little expansion of lesions and were more inhibitory than temperatures greater than 20 C. The response of lesion length to temperature followed a bell-shaped curve (Fig. 4) and had significant linear, quadratic, and quartic components (Table 7). The correlation between temperature and lesion length was significant using the linear and quadratic components (r = 0.778); the addition of the quartic component to the predictive equation became too complex to be useful.

The interaction of dew period with temperature to affect lesion length (Table 8) is described by the mathematical prediction equation:

$$\hat{Y} = -73.5 + 6.704T - 0.182T^2 + 1.258D - 0.00394D^2 - 0.069TD + 0.002T^2D,$$

where $\hat{Y}$ is the predicted lesion length, T is temperature, and D is dew period in total hr. The linear regression of dew period increased linearly, quadratically, and cubically with temperature (Table 7).

In summary, the relation of lesion length to dew period is parabolic, the rate of expansion increasing as dew period lengthens. The regression on temperature has linear, quadratic, and quartic components, increasing to a maximum at 20 C and then decreasing. At the higher

Table 7. Analysis of variance for lesion lengths on maize leaves incited by Alternaria alternata at five temperatures and four dew periods

Source of variation	df	Mean square	F	Prob > F
Rep	2	8.3405	0.801	ns
Temperature	4	133.6242	12.825	0.005
L_T: Linear T1,2,3,4,5[a]	1	178.8521	17.166	0.005
Q_T: Quadratic	1	144.3005	13.849	0.025
C_T: Cubic	1	38.5333	3.698	ns
Q'_T: Quartic	1	172.8107	16.586	0.005
Rep x temperature	8	10.4190		
Dew period	3	301.6469	23.137	0.0001
L_D: Linear DP1,2,3,4[b]	1	835.3345	64.071	0.0001
Q_D: Quadratic	1	61.2060	4.695	0.05
C_D: Cubic	1	8.4001	0.644	ns
Temp x dew period	12	40.5611	3.111	0.05
$L_T \times L_D$	1	115.3694	8.849	0.025
$L_T \times Q_D$	1	14.6301	1.122	ns
$L_T \times C_D$	1	3.6348	0.279	ns
$Q_T \times L_D$	1	134.6410	10.327	0.005
$Q_T \times Q_D$	1	9.8601	0.756	ns
$Q_T \times C_D$	1	0.3400	0.026	ns
$C_T \times L_D$	1	86.0331	6.599	0.05
$C_T \times Q_D$	1	28.8120	2.210	ns
$C_T \times C_D$	1	1.5403	0.118	ns
$Q'_T \times L_D$	1	26.0229	1.996	ns
$Q'_T \times Q_D$	1	25.8652	1.984	ns
$Q'_T \times C_T$	1	39.9848	3.067	ns
Residual error	30	13.0376		
Corrected total	59	40.9717		

[a] T1 is 10 C; T2 is 15 C; T3 is 20 C; T4 is 25 C; T5 is 30 C.

[b] DP1 is 3 days with 16 hr dew/24 hr period or 48 hr total dew; DP2, 3, and 4 are 4, 5, and 6 days with 16 hr dew/24 hr period or 64, 80, and 96 total hr, respectively.

Table 8. Length of lesions incited by Alternaria alternata at five temperatures for four durations of dew

Temperature (°C)	Lesion length (mm)[a]					
	Check[b]	Total hr dew[c]				
		48 (3)	64 (4)	80 (5)	96 (6)	Mean[d]
10	7.9	8.8	9.4	10.3	11.5	10.0
15	7.6	8.6	9.9	8.9	12.7	10.0
20	7.8	9.9	13.1	21.7	26.6	17.8
25	7.6	8.4	10.6	12.2	27.6	14.7
30	8.0	9.7	12.4	14.0	18.8	13.7
Mean	7.8	9.1	11.1	13.4	19.4	
$LSD_{.05}$ = 9.4[e]						

[a] Lesion lengths are means of 30 lesions in three replications measured seven days after inoculation.

[b] Check indicates wounded but uninoculated lesions after 96 hr dew at the indicated temperature.

[c] Dew expressed in total hr of dew in increments of 16 hr/24 hr period; i.e., 48 hr of dew in three 16 hr/24 hr applications.

[d] Temperature means exclude the check dew means.

[e] $LSD_{.05}$ = least significant difference at the P = 0.05 level.

Fig. 3. Sample regression of the total number of hours incubation in a dew chamber (16 hr/day) over all temperatures (X) and mean lengths of Alternaria alternata lesions (Y). $\hat{Y} = -20.97 + 0.777X - 0.00394X^2$, $r = 0.995$

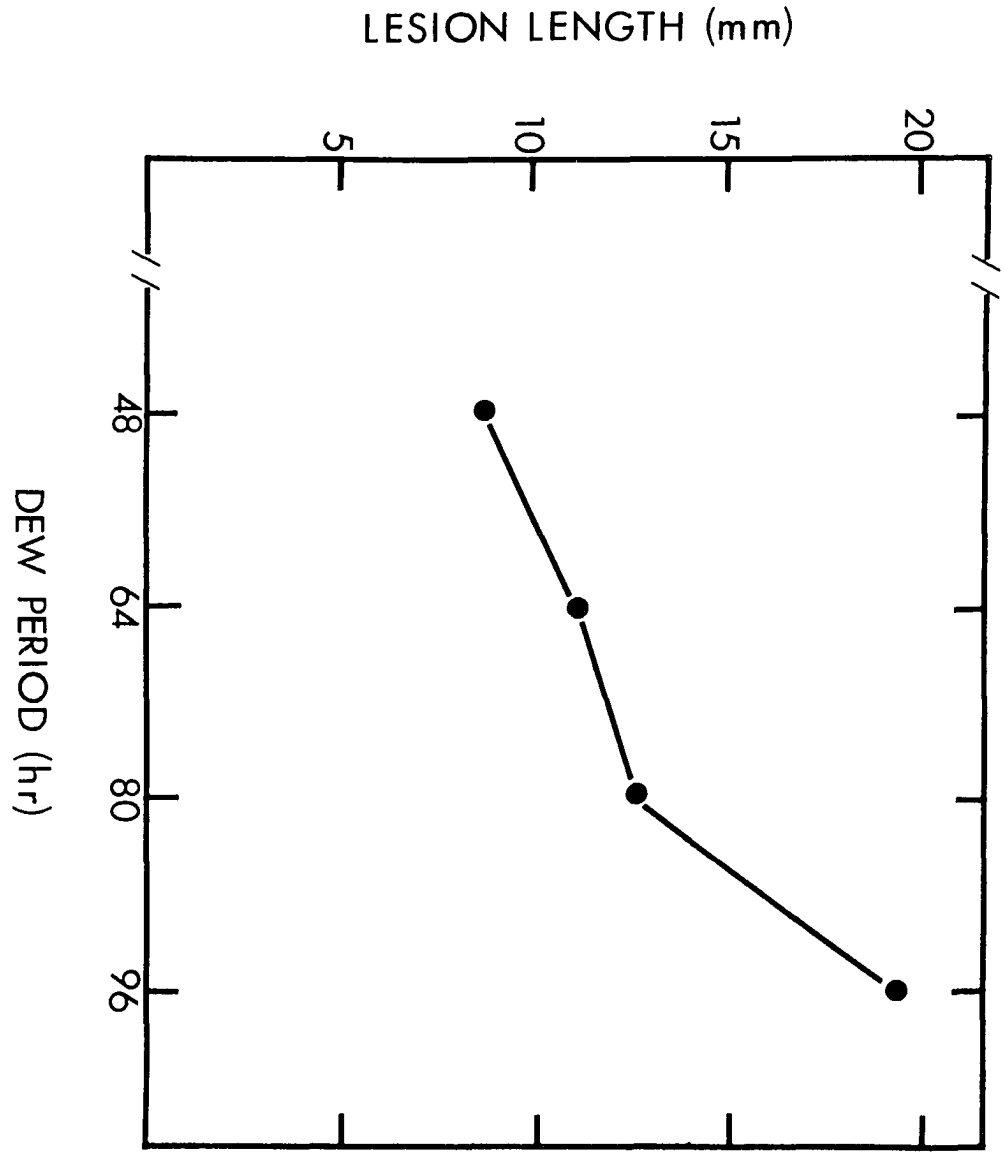
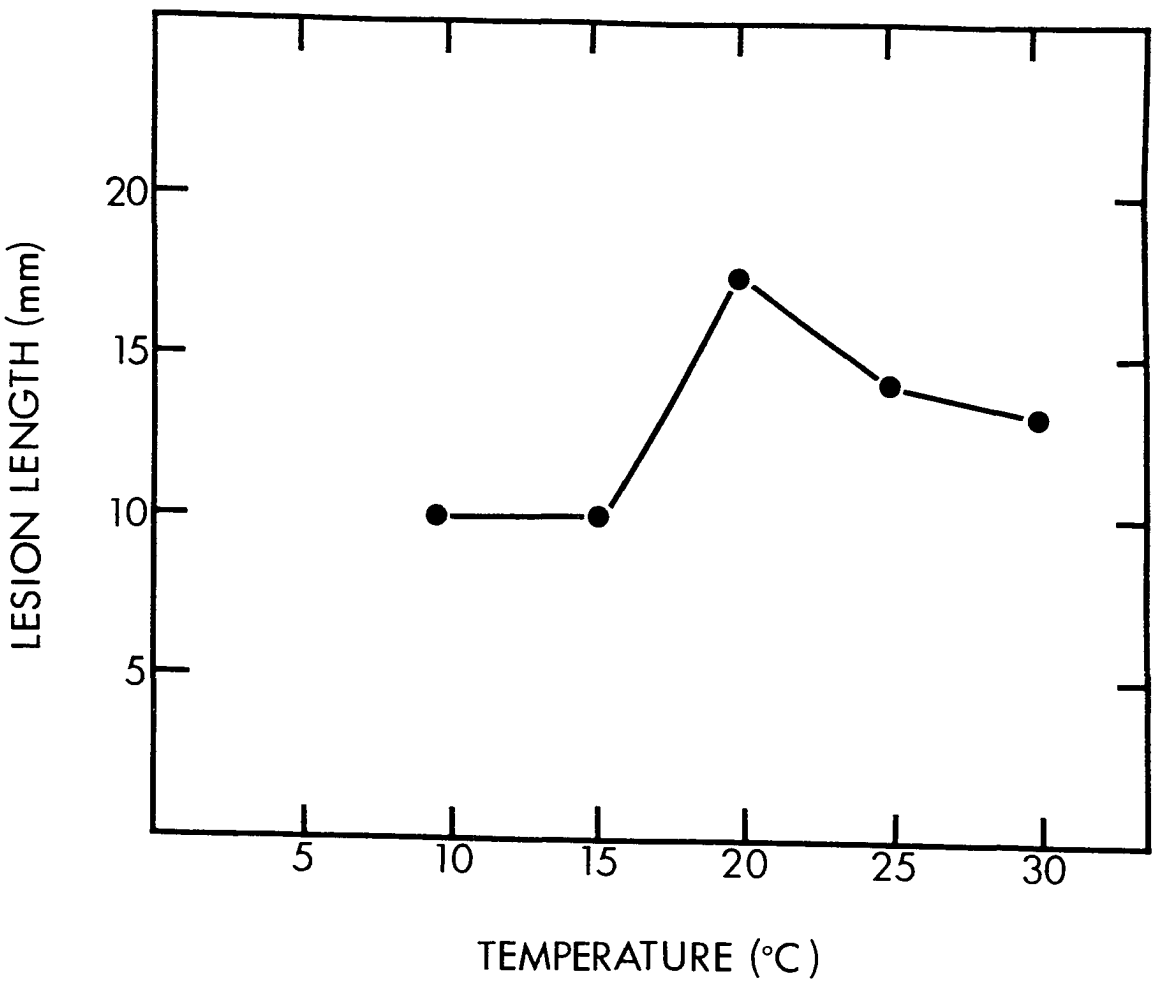

LESION LENGTH (mm)
5
10
15
20
DEW PERIOD (hr)
48
64
80
96

Fig. 4. Sample regression of the incubation temperature over all dew periods (X) and the mean lengths of Alternaria alternata lesions (Y). $\hat{Y} = -4.65 + 1.732X - 0.0372X^2$, $r = 0.778$


LESION LENGTH (mm)
20
15
10
5
5
10
15
20
25
30
TEMPERATURE (°C)

temperatures (especially 20 and 25 C) an increased dew period resulted in much larger increases in lesion length than at the lower and highest temperatures where lesion length increase was linear or slightly cubic (Table 8, Fig. 5).

Interruption of dew period The effect of interrupting the moist incubation period was investigated. Wounded, inoculated plants were placed in a dew chamber for three days (16 hr dew/24 hr) at 20 C then removed from the dew chamber to a 28 to 32 C greenhouse for zero, one, two, four, or six days (plus the normal 8 hr of dryness/24 hr) before returning them to the dew chamber for four additional days (16 hr dew/24 hr).

Interruption of the dew period with dry periods of several days resulted in a significant reduction in lesion lengths (Table 9). Fifty-six hr without dew were adequate to stop further expansion of the initial lesion (Fig. 6).

Delay of dew period The effect of delaying the onset of the moist incubation period after inoculation involved the wounding and inoculation of plants and placement in the dew chamber immediately, or one to seven days after inoculation. The differences between the inoculated lesions and the initial wounds were calculated (Table 10). A delay in the time between wounding and inoculation and the onset of dew had significant effects on the expansion of lesions. Twenty-four hours of dryness after inoculation was sufficient to inhibit expansion of the initial lesion. The decrease in the differences between expanded and initial lesions with increasing number of dry days was parabolic

Fig. 5. The increase in the mean lengths of Alternaria alternata lesions with increase in dew period, comparing five temperatures

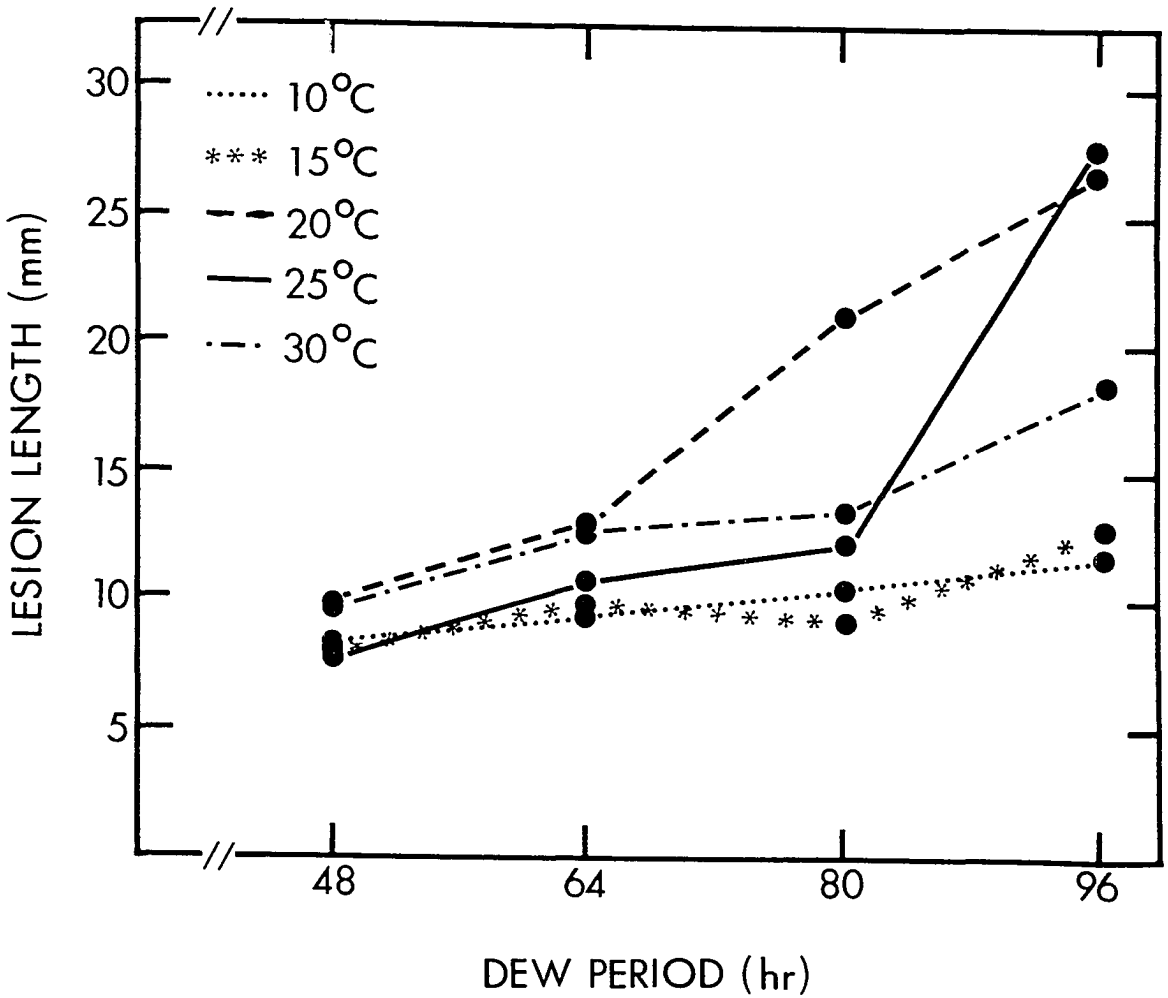

LESION LENGTH (mm)
30
25
20
15
10
5
10°C
15°C
20°C
25°C
30°C
48
64
80
96
DEW PERIOD (hr)

Table 9. The effect of interrupting the daily moist incubation periods with an extended dry period on the expansion of lesions by Alternaria alternata on maize leaves

Extended dry period (hr)[1]	Lesion length (mm)[2]
0(8)[3]	24.4a[4]
32	17.4b
56	12.1bc
104	10.9bc
152	10.2c

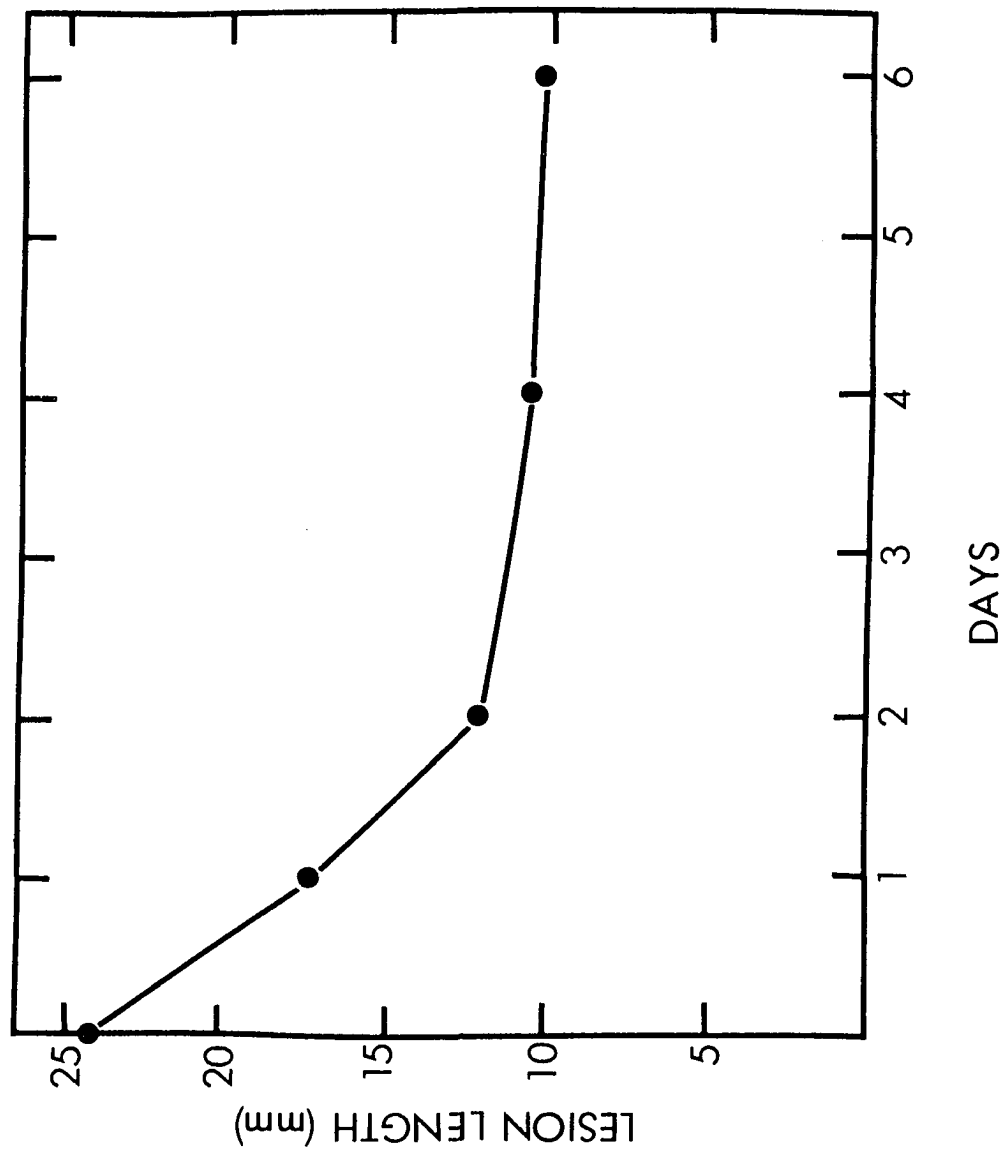
[1]Numbers in this column correspond to the number of hours plants were exposed to dry conditions after 3 days of 16 hr dew/24 hr before replacement in a dew chamber for 4 additional days of 16 hr dew/24 hr.

[2]Lesion lengths are means of 45 lesions in three replications measured after a total of seven days in the dew chamber (16 hr dew/24 hr period).

[3]The normal daily dry period (control).

[4]Values followed by the same letter are not significantly different (P = 0.05) as determined by Duncan's multiple range test.

Fig. 6. The decrease in Alternaria alternata lesion lengths with interruption of the moist incubation period for zero, 1, 2, 4, or 6 additional days in addition to the normal 8 hour daily period. Plants had 3 days of 16 hr dew daily before the interruption and 4 similar days before measuring lesion length


LESION LENGTH (mm)
25
20
15
10
5
1
2
3
4
5
6
DAYS

($r = 0.934$) (Fig. 7).

Inoculum density

The effect of spore density in inoculum on lesion expansion was evaluated using 0, 10^0, 10^1, 10^2, and 10^3 spores/0.1 ml drop. The experiment was performed at 20 C with 16 hr dew/24 hr in a growth chamber with humidifiers.

Lesion lengths increased significantly with increasing inoculum density (Table 11). Densities of 10^1, 10^2, and 10^3 spores/0.1 ml were not significantly different but a positive linear trend was evident (Fig. 8).

Leaf age

The effect of leaf age on lesion expansion was determined by inoculating the fourth, fifth, and sixth leaves on maize seedlings where the sixth leaf was the youngest with a fully developed ligule.

Leaf age, as tested, did not show a significant effect on lesion length; however, a trend toward increasing length with increasing age was apparent (Table 12). The sample variances differed significantly from one leaf age to the next age; the sample variance increased with decreasing leaf age. This increased variance explained, in part, the lack of significant differences among the lesion length means.

Host range

A limited host range of the maize isolate (AL-4) was investigated. Leaves on the test plants were wounded with the hot soldering iron and the injured sites were inoculated. The experiment was performed at

Fig. 7. The decrease in the difference (Y) between lesions expanded by Alternaria alternata and control lesions with the number of days (X) between inoculation and onset of moist incubation. $Y = 7.65 + 2.89X + 0.25X^2$, $r = 0.934$

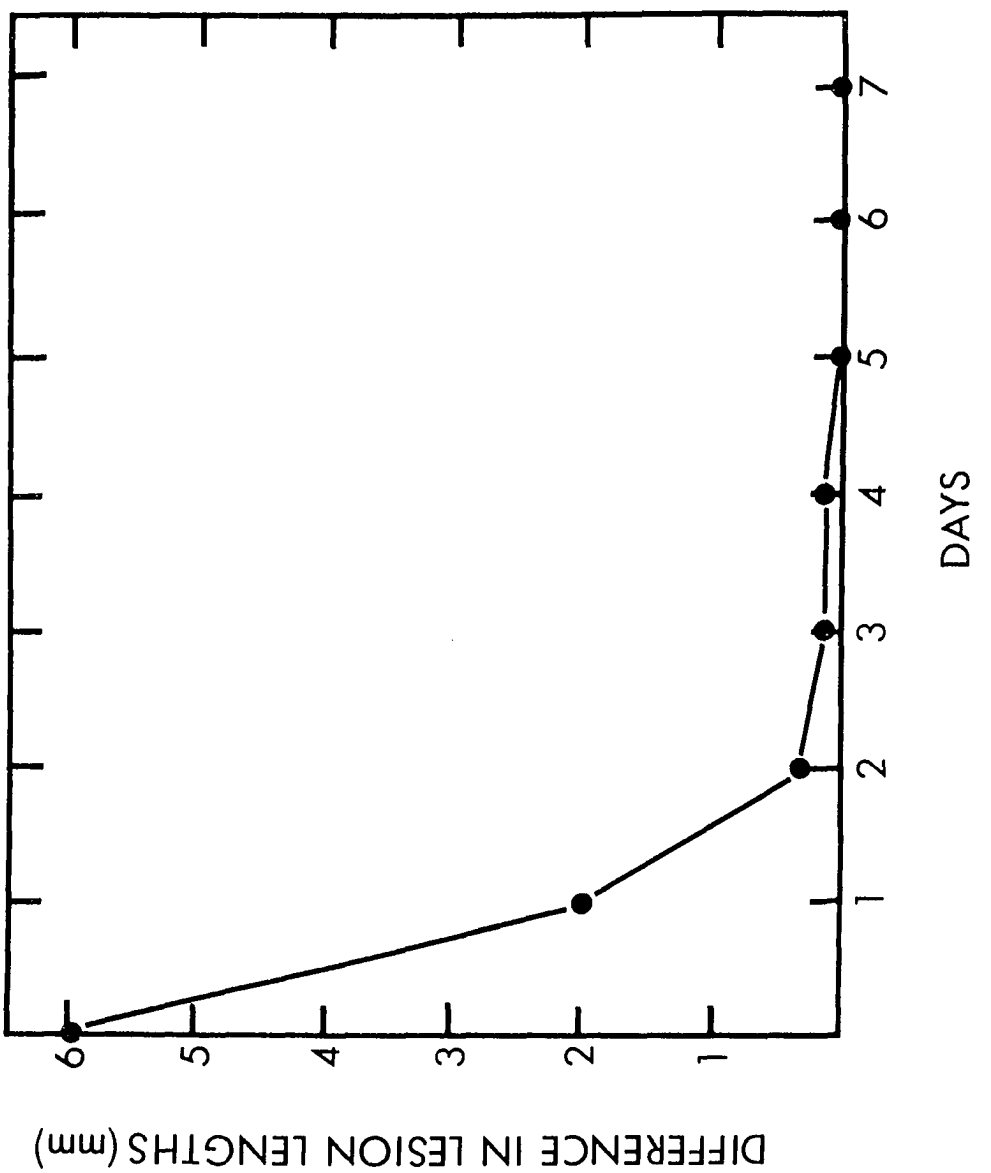

DIFFERENCE IN LESION LENGTHS (mm)
6
5
4
3
2
1
DAYS
1
2
3
4
5
6
7

Table 10. Effect of delaying incubation after inoculation on the difference between Alternaria alternata lesions and the initial lesion on maize leaves

Days[a]	Lesion difference (mm)[b]
0	6.0
1	2.0
2	0.2
3	0.1
4	0.1
5	0.0
6	0.0
7	0.0

[a]Numbers in this column represent the number of days between inoculation of plants and placement in the dew chamber for incubation (16 hr dew/24 hr period).

[b]Lesion differences are means of the differences between 30 initial lesions and their corresponding expanded lesions, in three replications, measured after a total of seven days in the dew chamber.

Table 11. The effect of spore density on expansion of lesions by Alternaria alternata on maize leaves

Spore density[1]	Lesion length (mm)[2]
0	7.6a[3]
1	8.0ab
10	10.4abc
100	10.8bc
1000	12.3c

[1]Spore density was the number of spores/0.1 ml drop of inoculum.

[2]Lesion lengths are means of 60 lesions in four replications measured seven days after inoculation and following seven periods of 16 hr dew/24 hr periods at 20 C.

[3]Values followed by the same letter are not significantly different (P = 0.05) as determined by Duncan's multiple range test.

Fig. 8. The increase in lesion lengths with increasing inoculum density (*Alternaria alternata* conidia/0.1 ml)

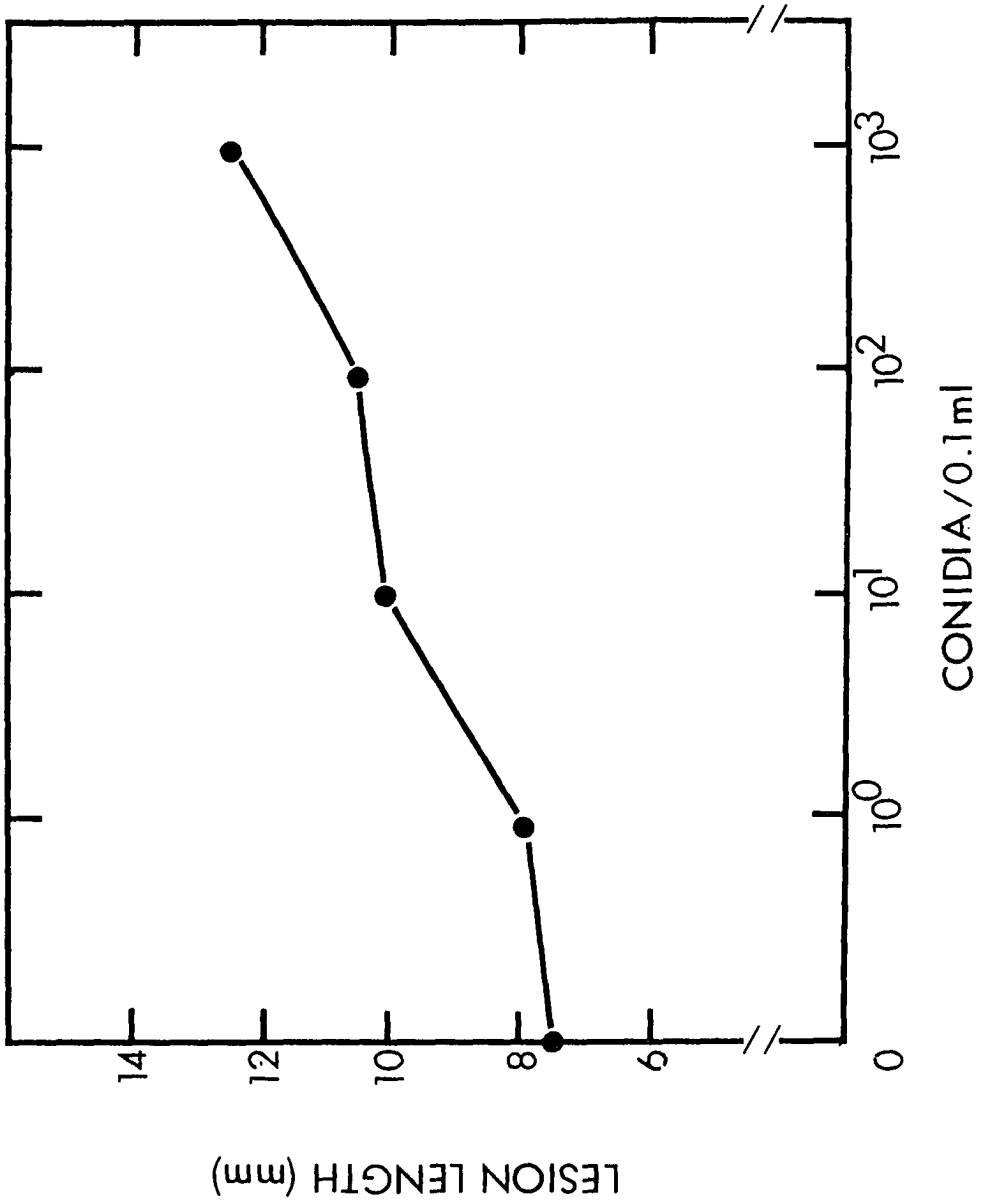

LESION LENGTH (mm)
14
12
10
8
6
CONIDIA/0.1ml
0
10^0
10^1
10^2
10^3

Table 12. The effect of leaf age on the expansion of lesions by Alternaria alternata on maize leaves

Leaf[a]	Lesion length (mm)[b]	S^2[c]
4	22.9	18.97
5	17.1	21.77
6	13.9	37.01

[a]The oldest leaf tested was the 4th leaf; the 6th leaf on the plant was the last expanded leaf.

[b]Lesion lengths are means of 54 lesions in three replications measured after seven days in the dew chamber (16 hr dew/24 hr period). Values were not significantly different ($P = 0.05$) as determined by Duncan's multiple range test.

[c]S^2 = sample variance; the sample variances differ from age to age using Bartlett's test of homogeneity of variance ($P < 0.05$).

20 C with 16 hr dew/24 hr in a dew chamber. The age of the plants and the leaf tissue inoculated varied with the host (Table 13).

Of the plants tested for susceptibility to A. alternata after wounding, sweet maize and table beets showed expansion of initial lesions by the pathogen with mean lesion lengths of 12.2 and 12.6 mm, respectively (means of 30 lesions) (Table 13). The reaction of sweet maize was similar to that of field maize. Lesions on beet leaves exhibited pronounced purple margins, shot-holing of necrotic tissue, and extensive chlorosis in 25% of the lesions. Expansion of lesions was more extensive on older leaves. Of the remaining hosts tested, alfalfa showed no response to inoculation, soybean showed some slight

Table 13. The plant age, leaves inoculated and cultivars of plants and their reaction to Alternaria alternata inoculation after wounding

Host	Cultivar	Plant age (wks)	Leaves	Reaction
Zea mays L.	Golden Bantam	5	3-4	S[a]
Glycine max (L.) Merr.	Amsoy	6	1st-2nd trifoliates	R
Phaseolus vulgaris L.	Topcrop	5	1st-2nd trifoliates	R/S
Lycopersicum esculentum Mill.	Bonny Best	7	4-5	R
Medicago sativa L.	Ranger	3	1st trifoliates	R
Beta vulgaris L.	Detroit Perfected	3	3-4	S

[a]S indicates a susceptible reaction; R indicates a resistant or no reaction; R/S indicates an intermediate reaction.

expansion and chlorosis of lesions on senescing leaves, tomato showed slight chlorosis on 10% of lesions, and garden bean had some expansion on older leaves, with pronounced purple margins around many of the initial unexpanded lesions.

Inbred variability

Six maize inbred lines (B37, B79, C103, Oh43, W22, and W64A) were tested for reaction to isolate AL-4. Inbred plants were selfed in the field and ears were harvested, shelled, and labeled separately. Ten ears from each inbred line were selected randomly, three pots were

planted with five seeds each and three plants in each pot were inoculated at three sites each. The experiment was repeated four times with four different sets on ten ears/inbred line. In total, forty different ears were tested/inbred line.

There were distinct differences in the reaction of inbred maize varieties to infection with A. alternata (Table 14). Inbreds C103 and Oh43 exhibited the least expansion of lesions by the pathogen while W64A had the largest lesions. The other three inbreds were intermediate in reaction.

While variability was shown between inbreds, there appeared to be little variability for disease reaction within any inbred (Tables 15 and 16). Reactions to the pathogen were homogeneous within each inbred.

Comparisons involving the six inbreds were based on the derivation of the lines. B37, derived from the Iowa Stiff Stalk Synthetic population, and B79, derived from the Iowa 2-Ear Synthetic population, were compared (Table 16). Lesion expansion on B79 was significantly greater than on B37. Lesion expansion on two Wisconsin lines, W22 and W64A, was also significantly different; W64A exhibited significantly longer lesions than W22. When the two Iowa derived inbreds were compared to the two Wisconsin inbreds, the Iowa inbreds had significantly smaller lesions than the Wisconsin inbreds. Oh43 and C103 are both derivatives from Lancaster Surecrop, an open-pollinated variety. Lesion expansion on these inbreds was not significantly different. In the final comparison, B37, B79, and W22 (a derivative from an Iowa inbred) were compared to C103, W64A, and Oh43, three inbreds with less relationship to an Iowa

Table 14. The expansion of lesions by Alternaria alternata on maize leaves of six inbred lines

Inbred	Lesion length (mm)[1]
B79	18.10ab[2]
B37	15.10a
W64A	22.37b
C103	13.28a
W22	17.23ab
Oh43	13.77a

[1]Lesion lengths are means of 120 lesions in three replications in four sets over time. Lesions were measured after seven days in a dew chamber (16 hr dew/24 hr period) at 20 C.

[2]Values followed by the same letter are not significantly different (P = 0.05) as determined by Duncan's multiple range test.

Table 15. The standard errors and variances of the estimates of variability within six inbred lines of maize for lesion expansion by Alternaria alternata

Inbred	Estimate of σ_e^2	Variance	Standard error
B79	1.52	3.67	1.91
B37	-1.23	0.81	0.90
W64A	-0.397	4.42	2.10
C103	-0.81	0.66	0.81
W22	2.66	10.25	3.20
Oh43	-1.16	1.21	1.10

Table 16. Analysis of variance table and comparisons for lesion lengths incited by Alternaria alternata on maize leaves of six inbred lines

Source of variation	df	Mean square	F	Prob > F
Set	3	4418.2660	6.857	0.01
Error a	8	644.3333		
Inbred	5	1307.6059	30.061	0.0001
C1: B79-B37	1	539.3594	12.400	0.0001
C2: (B79+B37) - (W22+W64A)	1	1157.3839	26.608	0.0001
C3: W64A-W22	1	1515.8529	34.848	0.0001
C4: C103-Oh43	1	3.7967	0.087	ns
C5: (B79+B37+W22) - (W64A+C103+Oh43)	1	3321.6364	26.362	0.0001
Inbred x set	15	128.3334	2.950	0.003
Error b	40	43.4984		
Ear (set inbred)	216	17.1893	1.011	0.4587
Error c	407	17.0019		
Corrected total	695	56.4677		

inbred or population. The three inbreds derived from Iowa populations or inbreds had significantly longer lesions than the three remaining inbreds and especially the two Lancaster derivatives (Oh43 and C103).

Environmental Effects on Alternaria alternata in vitro

Temperature and media

The optimum temperature for growth of A. alternata was 27 C on both V-8 juice agar and PDA medium (Table 17). Minimum and maximum

Table 17. The effect of the interaction of temperature and medium on the growth of Alternaria alternata in agar culture

Temperature (°C)	Colony diam (mm)[1]	
	V-8 agar[2]	PDA
10	22.0b[3]	20.2ab
15	34.4e	30.0d
20	40.0f	33.2e
23	50.4i	43.9g
25	53.7j	45.3gh
27	59.9k	53.1j
30	51.8ij	46.4h
35	19.5a	25.8c

[1] Colony diameter was measured after seven days growth and includes the diameter of the initial disc (9 mm). Values represent the means of 10 measurements of colony diameter.

[2] V-8 = V-8 juice agar (68); PDA = potato dextrose agar.

[3] Values followed by the same letter are not significantly different (P = 0.05) as determined by Duncan's multiple range test.

temperatures for growth were not detected in this study. The temperature curves for colony growth sloped upward gradually to the maximum at 27 C then decreased rapidly beyond the optimal peak (Fig. 9). V-8 juice agar supported significantly better lineal growth of the fungus than did PDA (Table 17).

Sporulation was apparent at 20-30 C and was sparse at 10, 15, and 35 C. Colonies were also characterized by more aerial mycelium, a less dense arrangement of mycelium, and more distinct sectoring at 35 C than at lower temperatures.

Water potential

The water potential of the medium, the solute used to obtain the desired water potential, and the fungal isolate chosen affected the growth of A. alternata in vitro (Table 18). The tobacco isolate of A. alternata consistently exhibited better growth at all water potentials and in both solute systems (Table 19).

Sucrose, as the solute controlling water potential, supported better growth than NaCl at all levels (Table 19). Bruehl and Cunfer (13) found similar results using sucrose and KCl to control water potential in the growth medium for snow molds of wheat. The optimum water potential in the sucrose system (-15 to -20 bars) occurred at a higher Ψ than in the NaCl system (-1 to -5 bars). The growth of the fungus dropped sharply at water potentials below -5 bars using NaCl as the solute. However, when using sucrose as the solute, the decrease in growth was more gradual as water potential decreased. The decrease in growth appeared to level off near -40 bars with NaCl but continued

Fig. 9. The colony diameter of Alternaria alternata grown on V-8 juice agar and potato dextrose agar at different temperatures

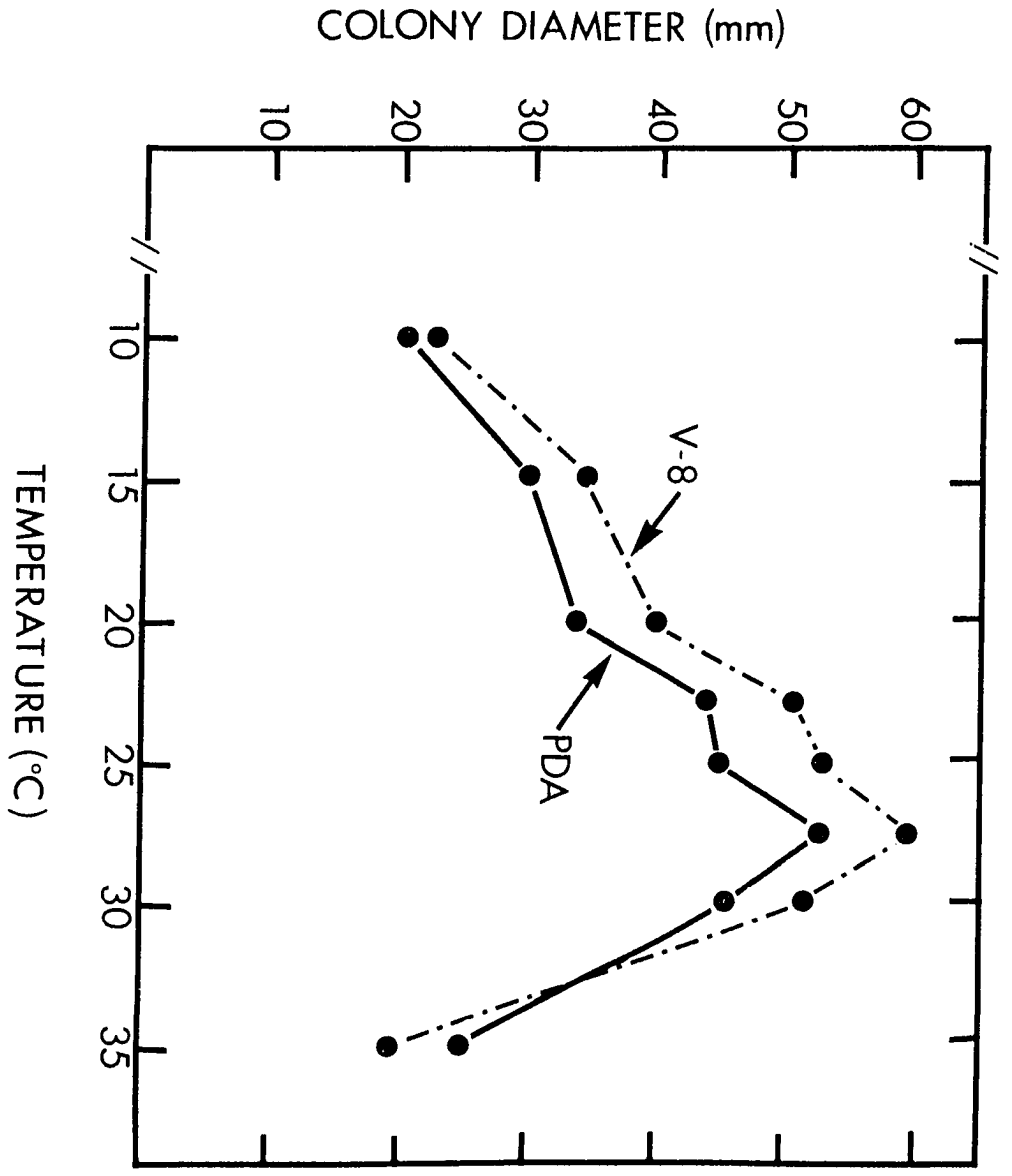

COLONY DIAMETER (mm)
10
20
30
40
50
60
TEMPERATURE (°C)
10
15
20
25
30
35
V-8
PDA

Table 18. Analysis of variance for the growth of Alternaria alternata isolates AL-4 and V grown at eight water potentials (-1.2 bars to -55.6 bars) in agar amended with NaCl or sucrose

Source of variation	df	Mean square	F	Prob > F
Solute[a]	1	7408.90562	46.966	0.0171
Error a	2	157.75063		
Water potential[b]	7	900.52848	138.044	0.0001
Water potential x solute	7	285.05134	43.696	0.0001
Error b	14	6.52348		
Isolate[c]	1	3997.40060	433.940	0.0001
Isolate x solute	1	62.01560	6.732	0.0186
Isolate x water pot.	7	66.59491	7.229	0.0008
Isolate x sol. x water pot.	7	23.78991	2.583	0.0548
Residual	16	9.21187		
Corrected total	63	332.60785		

[a]The solutes were NaCl or sucrose added to Sommer's basal medium (97) to obtain the desired water potentials.

[b]Water potentials were -1.2, -4.8, -16.7, -21.0, -28.6, -34.0, -41.8, and -55.6 bars.

[c]A. alternata isolates AL-4 from maize and V from tobacco.

Table 19. The effect of water potential and solute on the growth of Alternaria alternata in culture

a_w[1]	Water potential[2]	Maize isolate (AL-4)		Tobacco isolate (V)	
		NaCl	Sucrose	NaCl	Sucrose
.9991	1.2	58.2i[3]	57.7i	67.51	75.9m
.9974	4.8	49.2h	59.8i	76.7mn	80.4op
.9894	16.7	37.1de	63.8k	57.8i	83.3q
.9858	21.0	35.5cd	63.3k	46.0g	81.5pq
.9804	28.6	27.8b	62.6jk	41.3f	77.7mn
.9766	34.0	26.8b	48.5gh	41.1f	78.6no
.9711	41.8	25.0a	48.8h	33.0c	60.3ij
.9614	55.6	27.3b	38.8ef	34.2c	47.9gh

[1] a_w = water activity.

[2] Water potential expressed in -bars.

[3] Colony diameter was measured after seven days growth and included the diameter of the initial disc (9 mm). Values represent the means of two replicates of five plates each for isolates from maize (AL-4) and tobacco (V). Values followed by the same letter are not significantly different (P = 0.05) as determined by Duncan's multiple range test.

to drop with sucrose. The water potential of -150 bars where growth extinction reportedly occurs (2) was not included in this study.

The water potential and solute affected the appearance of the colonies as well as the growth in diameter. As water potential decreased below -5 bars in the NaCl system, sporulation declined, mycelium became less densely packed within the colony, less aerial mycelium was observed,

colony borders became irregular and broken, and colony color changed from greenish brown to orangish tan. In the sucrose system, colony characteristics remained unchanged above -30 bars. At higher osmotic potentials, colony color lightened, becoming yellowish or creamy brown, growth became sparse, few aerial hyphae were observed, and more hyphae appeared submerged in the agar. These effects were less dramatic with the tobacco isolate than with the maize isolate.

Relative humidity and spore germination

Germination of A. alternata spores was poor at all relative humidities studied. A maximum of 80% germination at 100% RH after eight hours was significantly below values expected from reported studies (63) for Alternaria spp. Germination decreased rapidly to 45% at 97% RH, 21% at 92.8% RH, 0.3% at 85.2% RH, and zero at 73.4% RH (means of 600 spores in four replications). A. alternata spores are capable of germinating in the absence of free water. Germ tube elongation and appressorium formation were reduced at relative humidities below 97%.

Toxin Studies

Bioassays--extracts from Fries' medium

Bioassays of the extracts from the culture filtrates of isolates AL-4 and V of A. alternata grown on Fries' medium suggested the presence of a chlorosis inducing toxin(s) in the final extract (Table 20). The isolate V toxin(s), at lower dilutions, induced visual chlorosis of cucumber seedlings but not of maize seedlings; only a slight decrease

Table 20. Germination, chlorosis, and chlorophyll content of four day old maize and cucumber seedlings treated with water or culture filtrates of Alternaria alternata, isolates AL-4 and V

Treatment	Cucumber			Maize		
	Germination	Visible chlorosis	OD663/gm fresh wt	Germination	Visible chlorosis	OD663/gm fresh wt
Control[a]	13/15[b]	no	2.687	10/10	no	0.533
AL4-T 1:100	15/15	yes	1.814	10.10	no	0.796
AL4-T 1:500	14/15	no	3.260	10/10	no	0.670
AL4-A1	14/15	no	3.492	10/10	no	0.982
AL4-A2,A3	0/15	--	--	0/10	--	--
V-T 1:100	14/15	yes	0.649	10/10	no	0.486
V-T 1:500	15/15	yes	2.623	10/10	no	0.782
V-A1	14/15	no	3.116	10/10	no	0.586
V-A2,A3	0/15	--	--	0/10	--	--

[a]Control = distilled water; AL4-T = culture filtrate after extraction, containing toxin produced by isolate AL-4, at two dilutions; AL4-A1,A2,A3 = distilled water fractions used in the extraction of toxin produced by isolate AL-4; V-T = culture filtrate after extraction, containing toxin produced by isolate V, at two dilutions; V-A1,A2,A3 = distilled water fractions used in the extraction of toxin produced by isolate V.

[b]Numbers represent the average number of seeds germinating per seeds treated in three replications.

in chlorophyll content was determined for maize seedlings. The toxin(s) produced by the AL-4 isolate, at higher concentrations, decreased the chlorophyll content in cucumber but not in maize seedlings. Seedling germination was not affected by toxins produced by either isolate. Aqueous extracts did not induce chlorosis of seedlings of either genus but the second and final aqueous extracts completely inhibited germination of seeds of both genera. Low concentrations of the toxin(s) appeared to increase or not affect chlorophyll content in cucumber and maize seedlings.

Bioassays--extracts from sucrose-yeast medium

Thin layer chromatography of the final extracts of both the AL-4 and V isolates grown on the sucrose-yeast medium resulted in two distinct elongated spots per sample that quenched the fluorescence at 254 nm. Recovery of the samples from the silica gel in ethanol and evaporation and resuspension in water preceded the bioassay. The AL-4 preparation from the larger, upper TLC spot (Rf 0.71) produced a yellowish chlorosis of both the tentoxin sensitive species and tentoxin insensitive species of tobacco seedlings. The extract from the small, lower spot (Rf 0.29) caused no chlorosis in either species. The V preparation from the larger, upper TLC spot (Rf 0.67) caused a whitish chlorosis in the tentoxin sensitive species of tobacco seedlings but not in the insensitive species. The extract from the lower spot (Rf 0.21) had no effect on either species.

The ultraviolet spectrum of the upper TLC spot from the maize isolate (AL-4) had an absorption maximum at 275 nm with a slight

adjacent peak at 283 nm. This differed from the UV absorption spectrum for the tobacco isolate (V) that peaked at 280 nm, the same maximum as published for tentoxin (30). The lower spots of both isolates had absorption maxima at 278-280 nm but were not biologically active. The toxin(s) produced by the maize isolate does not appear to be tentoxin in view of the tobacco seedling bioassay and the UV spectrum.

The results of bioassays involving attached and detached mature maize leaves were negative. Extracts from AL-4 and V filtrates had no visible effect on maize leaf tissue. Wounding before application of the extract did not elicit a response to the toxin either.

DISCUSSION

Alternaria alternata has been characterized as a weak, nonspecialized pathogen with "low aggressivity" (64). As a leaf pathogen of maize, A. alternata conforms to many of the generalizations associated with pathogens of low aggressivity: low infectivity of spores, wide host range, residency in the host phylloplane, and infection of damaged or senescent tissue.

Pathogens with low aggressivity rely heavily on predisposition of host tissue for establishment. A. alternata has been reported to enter bean and tobacco tissue damaged by insects and blowing sand (16, 17,76), pepper plants damaged by insects, sunscald, and other pathogens (10,16), tomato and potato plants damaged by blowing sand (83,84) among a number of other examples. In maize, the pathogen invaded only damaged or senescing leaf tissue. The predisposition of the tissue by wounding enabled the pathogen to establish a "progressive and self supporting" infection in moribund tissue (27,77). Without wounding, the pathogen lacked the "minimum invasive force" to invade healthy, green tissue as described by Garrett (27). The foothold in moribund, damaged tissue served as a food base and focus for further ramification of the fungus into adjacent, healthy tissue (43,64).

Alternaria leaf blight became evident in the field in mid-July and widespread from August until harvest. At these times, lower leaves in the crop canopy senesce and become more susceptible to infection. Leaf injury due to insect feeding, blowing sand, or mechanical damage may increase also at these times and predispose leaf tissue to infection

(56). Stress on plants increases also in the form of water stress and the stress associated with grain fill.

The damage caused by the hot soldering iron appeared to be optimum for invasion of maize leaf tissue by A. alternata. The area which was killed remained intact and supplied an adequate food base for extension of the pathogen, and its metabolites, into contiguous healthy tissue. For this study, the killed area was discrete, allowing more precise measurement of pathogen activity.

Inoculum density also had an effect on infectivity. Increasing the number of spores reaching damaged leaf tissue permitted greater expansion of the lesion into healthy areas. Low inoculum density incited little or no lesion expansion. High populations of Alternaria spp. spores are present in the air during the latter part of July and through harvest, providing high inoculum densities for infection of senescing lower leaves in the canopy.

Wounding did not appear to overcome all the defense mechanisms of the host. Of the six maize inbred lines tested, C103 and Oh43, which are two closely related inbreds, exhibited resistance to spread of the pathogen beyond damaged areas. The close relationship between these two inbreds and their similar reaction to A. alternata infection suggest that they possess similar disease resistance mechanisms. On this basis, their reaction to other leaf pathogens might be similar as well.

Variability for disease reaction to A. alternata was shown to be present in maize inbred lines. Iowa derived inbreds showed intermediate susceptibility. Variability for disease reaction within inbred lines

was slight to nonexistent. The maize inbred used as the test plant in all the other experiments in this study was one of the more resistant genotypes. More striking data may have resulted if W64A was the test plant.

The host range of both isolates of A. alternata extended beyond the original host genus. The maize isolate (AL-4) infected dicots as well as monocots after wounding; the tobacco isolate (V) caused lesion expansion in maize as well as tobacco. The ability of the maize isolate to cause disease in diverse host plants supports the identification of A. alternata as a nonspecialized pathogen (64).

The presence of A. alternata spores in the host phylloplane enabled the pathogen to survive on the leaf surface and invade subsequently damaged or senescing tissue (18). The dark coloration and multicellular character of spores contributes to good spore survival on leaf surfaces. The maize isolate of A. alternata (AL-4) infected damaged tissue by means of spores present on maize leaf tissue prior to wounding, and was able to infect damaged tissue from adjacent, established lesions.

The environmental conditions favorable to infection and lesion expansion occur commonly in maize growing areas. The pathogen can survive periods of water stress as shown by Adebayo and Harris (2) and by these studies involving water potential (Table 19). A. alternata spores germinated over a wide range of temperatures and in the absence of free water (63,103). The narrow spacing between maize rows and the denseness of the leaf canopy common to cultural practices in the corn belt result in the possibility of extended periods of relative

humidity above 90%. A. alternata spores germinated at relative humidities as low as 85% which agrees with published data (63). Growth in vitro occurred over a range of temperatures also, with an optimum of 27 C, within the optimum range of 24 to 28 C reported by Sobers and Doupnik (96) and Stavely and Main (100).

Temperature and dew period are important environmental parameters for infection. The optimum temperature for infection and lesion expansion was 20 C, similar to that for tobacco brown spot lesion initiation (100,103), but much lower than the 27 C temperature optimum for growth in vitro. Lesion expansion increased linearly with length of dew period, the rate of increase becoming larger near the temperature optimum. Dew periods less than 10 hr/24 hr and consisting of less than 48 hr total resulted in little lesion enlargement. Daily moisture was necessary for continued lesion enlargement as it is for lesion expansion on potato by A. solani (110). Waggoner and Horsfall (110) found that natural dew provided an adequate duration of moisture for infection and lesion enlargement.

Unlike tobacco brown spot lesions, Alternaria leaf blight lesions on maize were arrested in further development by an extended dry period. An absence of moisture for 24 hr significantly halted lesion expansion, whether the extended dry interval occurred between wounding and the first dew period, or after a progressive infection was established. It appears that growth of the fungus in plant tissue was dependent upon free water or low moisture stress, although growth in vitro was tolerant of osmotic stress. Without moisture to allow fungal growth,

the leaf tissue bordering the lesion evidently developed resistance to invasion or further lesion expansion. An increase in the time interval between wounding and inoculation also decreased the probability of a successful infection. A resistance response triggered by wounding evidently resulted in the accumulation or formation of a fungitoxic barrier and excluded the subsequently inoculated pathogen. The dependence of A. alternata on daily dew (or low moisture stress) would support the classification of this fungus as a saprophytic pathogen of maize. The fungus must be developing primarily or entirely in dead or moribund tissues that cannot tolerate moisture stress.

The similarity between A. alternata lesions on maize leaves and A. alternata lesions on tobacco leaves suggested that a common toxin may be involved in pathogenesis. Alteration of the uninvaded tissue in the prehalo zone beyond the tobacco brown spot lesions was shown to result from a diffusible fungal toxin, tentoxin (54). Maize has been shown to be insensitive to tentoxin in seedling bioassays (106). Filtrates from growth of this isolate in various culture media optimum for tentoxin production elicited no response in maize leaf tissue.

Alternaria spp. have produced a wide range of toxins in a variety of hosts. The toxins produced in culture which caused chlorosis of cucumber and tobacco seedlings could not be shown to take an active part in lesion expansion into healthy tissue. The lack of success in demonstrating a response by maize leaves to culture filtrates could be attributed to a number of causes. The culture media used may not have been optimum for toxin production; certain essential minerals, vitamins,

carbohydrates, or nitrogen forms may not have been present in the media tested. The extraction and purification methods may have destroyed or inactivated the toxin(s) if it was labile. Finally, the bioassay techniques may have been inadequate for detection of the toxin(s). Dimond and Waggoner (19) suggested that fungal metabolites aid in the establishment of the fungus in host tissue by continually supplying the pathogen with dead or dying cells on which it can secure a food base and produce more toxin. The Alternaria leaf blight disease syndrome is suggestive of such metabolites. Even though this study could not confirm it, some fungal metabolite or metabolites from A. alternata, either enzymes or toxins, appeared to precede the pathogen into healthy tissue, killing cells in advance of the fungus.

Weak pathogens are often overlooked as insignificant. From this study with A. alternata on maize, a weak pathogen was found to be destructive of leaf tissue under natural environmental conditions, was found to possess many of the disease inciting mechanisms of more aggressive pathogens, and was seen as a possible tool in understanding host-pathogen interactions which result in either susceptibility or resistance. Determining why a weak pathogen causes disease in weakened tissue and not in healthy may be a step toward understanding resistance mechanisms functioning in other more aggressive interactions. Methods used in this study for wounding could prove useful in studying other weak pathogens such as Fusarium moniliforme.

SUMMARY AND CONCLUSIONS

Alternaria alternata is a weak pathogen of maize, causing leaf blight symptoms under the proper conditions. Wounding is necessary for infection and daily moisture of at least 8 hours is necessary for lesion expansion. Inoculum density was related directly to disease severity and disease was more severe on older leaves. Fungal metabolites appear to play a role in lesion enlargement but none could be related to the disease syndrome or were identified.

The effect of environment on the fungus in vitro did not reflect the effects on disease development. In vitro, A. alternata is tolerant of water stress and has a temperature optimum for growth of 27 C. Lesion expansion, however, is very intolerant of water stress and proceeds most rapidly at 20 C.

Maize inbred lines tested for resistance to A. alternata exhibited a range of reactions from susceptible to somewhat resistant. Variability within any inbred line was very slight.

A. alternata is a pathogen of maize predisposed by wounding or stress. As a weak pathogen, it would serve as an interesting tool for understanding host-pathogen interactions in disease development under stress environments.

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