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EPIDEMIOLOGY AND CHEMICAL CONTROL OF POST-HARVEST DECAYS OF BLUEBERRY FRUIT CAUSED BY ALTERNARIA SPP. AND COLLETOTRICHUM GLOEOSPORIOIDES

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EPIDEMIOLOGY AND CHEMICAL CONTROL OF POSTHARVEST DECAYS OF BLUEBERRY FRUIT CAUSED BY *ALTERNARIA* SPP. AND *COLLETOTRICHUM GLOEOSPORIOIDES*

By

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ABSTRACT

EPIDEMIOLOGY AND CHEMICAL CONTROL OF POSTHARVEST DECAYS OF BLUEBERRY FRUIT CAUSED BY *ALTERNARIA* SPP. AND *COLLETOTRICHUM GLOEOSPORIOIDES*

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In a two year study in a commercial blueberry field, conidia of *Alternaria* spp., which could cause blueberry fruit decays, were not trapped from air in large numbers until fruit began to ripen. Inoculation experiments did not provide evidence that latent infections of blueberry fruits by *Alternaria* spp. played a significant role in the disease cycle. Other experiments showed that temperature and moisture conditions such as are found in the Michigan harvest season, as well as damage to fruit such as is caused by mechanical harvesting operations, are favorable to *Alternaria* and other blueberry rotting fungi. Captofol fungicide treatments applied during the growing season controlled *Alternaria* decays after harvest.

Conidia of *Colletotrichum gloeosporioides*, causal agent of blueberry anthacnose disease, were trapped from rainwater washings of infested commercial blueberry bushes from the Bud Swell through Ripe Fruit growth stages of the blueberry bush. Inoculation studies showed that *C. gloeosporioides* incited a blossom blight and that latent infections of fruit established during the bloom period could play a major role in the disease cycle.

ACKNOWLEDGMENTS

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SECTION I

EPIDEMIOLOGY AND CHEMICAL CONTROL OF POSTHARVEST DECAYS OF BLUEBERRY FRUIT CAUSED BY *ALTERNARIA* spp.

INTRODUCTION

The Genus Alternaria (Nees) Fries

The genus Alternaria was established by C. G. Nees von Esenbeck and E. M. Fries in the first quarter of the nineteenth century. The genus was subsequently studied by many mycologists along with other phaeodictyosporic genera, including Stemphyllium, Macrosporium, and Ulocladium (23, 28). Taxonomic chaos resulted by the middle of this century owing to poorly preserved type specimens, inadequate descriptions and illustrations, and an over-reliance on variable mycological characters, including spore shape and dimensions, number and kind of septations in the conidia, number of conidia in a chain, and the host from which a specimen originated (28). Particular confusion arose over the genera Alternaria and Macrosporium. Wiltshire stated that "both genera are used almost indiscriminately for manifestly congeneric species" (38). He declared that the two genera were really one, Alternaria, in the concept of Elliot, Bolle, Mason and Nees (38).

Alternaria can be distinguished from Ulocladium and Stemphyllium as follows (23): Alternaria conidia have an ovoid shape and a clear beak at the distal end, while Ulocladium conidia are obovoid and lack a beak. When Alternaria conidia are borne singly or in chains, the proximal end of each conidium is wider than the distal end; the reverse is true for Ulocladium conidia.

Conidia of the genus Stemphyllium are elliptical with a slight constriction of the center of the spore.

The genus Alternaria was subdivided by Wiltshire into two forms (38). One section included those species which formed conidial chains with short beaks on the individual conidia. The other included those species which formed conidial chains only rarely and whose conidia had long, filiform beaks (28). Neergaard refined this concept by forming sections based on the number of conidia in a chain (23). The Longicatenatae, with conidia in chains of ten or more, were one section. This section included A. tenuis. The Brevicatenatae with conidia in chains of three to five were another section. Alternaria tenuissima was included in the Brevicatenatae although Neergaard acknowledged it as intermediate in type between the two sections (23).

Alternaria conidia are porospores borne at the tip of specialized conidiophores which are morphologically and functionally distinct from the vegetative hyphae. The conidia mature as dictyospores, although the production of longitudinal septa may be delayed in some species or even absent in some individual conidia (28). The size of conidia, length of conidial beaks, number of conidia in a chain, conidial color and segmentation can be variable, even among cultures derived from single spore isolates. These characters are known to be affected by lighting conditions, nutrient content of the substrate, and the age of the particular culture (28).

Alternaria alternata was described by Simmons from a neotype specimen (28). Conidia are ovoid, usually with a basal pore. The

beak represents one-quarter to one-third of the total conidial length. The conidial size ranges from 18-47 μm long by 7-18 μm wide, averaging 31-13 μm. There are three to eight transverse and one to two longitudinal septa per conidium. The conidial wall can be smooth or minutely roughened. Alternaria alternata includes A. tenuis, and is very closely related phylogenetically to A. tenuissima.

History and Occurrence of Alternaria Decays of Blueberry Fruit

Although blueberries (Vaccinium corymbosum L.) have been grown commercially and marketed for several decades, only in the past decade have post harvest decays caused by Alternaria sp. become a problem. Decays caused by Botrytis cinerea Pers. ex Fr. always dominated in the past. Alternaria tenuissima (Fries) Witts was identified as the principle cause of storage decay of North Carolina blueberries in 1971 (18). New Jersey blueberries in 1970 were decayed by Alternaria tenuis Auct. in storage tests but decays caused by Botrytis cinerea and Gloeosporium fructigenum were more prevalent (8). In storage areas and retail sites, Alternaria was continuously present in New Jersey blueberries from 1970-1972. This trend led Ceponis et al. to suspect that Alternaria decays were of increasing importance to the New Jersey blueberry industry (12).

In the years 1973-1976 Alternaria became the most common cause of blueberry decays in New Jersey. This coincided with the beginning of the use of mechanical harvesting for fresh market fruit. The explanation given at the time for the phenomenon was that

mechanical harvesters damaged the fruit and enabled Alternaria, normally a weak pathogen and saprophyte, to invade the fruit (10). Other factors cited which may have contributed to the change in pathogen profile were restrictions on field burning of prunings, inoculum buildup on fruit left on the ground by the harvesters, different blueberry varieties, improper fungicide use, and possible changes in virulence of Alternaria alternata (10). The introduction and widespread usage of benomyl fungicide also coincided with the increase in Alternaria decays of blueberries.

As mechanical harvesting of fresh market fruit was expected to expand rapidly, it was feared that Alternaria decays would become increasingly important. This was especially worrisome because a fungicide which was truly effective against Alternaria was not known. For these reasons development of post harvest methods of disease control were urged (10).

This is the first study of the etiology and epidemiology of post harvest decays of Michigan blueberries. Thus, the alteration of the pathogen profile in Michigan decays cannot be documented, although the shift from predominantly Botrytis decays to Alternaria and Gloeosporium decays is thought to have occurred as it did in North Carolina and New Jersey. In a four year study of Michigan bludberry cankers reported in 1974, Weingartner and Klos reported Alternaria spp. to be present in 48% of blighted or cankered blueberry stems (37). Even though the etiology of blueberry decays had not been studied in Michigan, the pathogen was clearly established in Michigan blueberry fields by 1974.

MATERIALS AND METHODS

The Alternaria Isolate Used in This Study

Several single spore isolates of Alternaria spp. were made from rotting blueberry fruits. Two of these isolates were used for further study, and were sent to Dr. Emory Simmons, University of Massachusetts, Amherst, Massachusetts, for species identification. These were identified as Alternaria alternata (29).

The average length and greatest width of 50 conidia of each isolate grown on PDA with continuous fluorescent lighting were measured by me and found to be consistent with A. alternata as described by Simmons (28). The conidial color, number and kind of septations, and size of the conidial beak were also consistent with A. alternata when observed on conidia grown under the same conditions. Figure 1 is a micrograph of A. alternaria conida and conidial chains from the isolates used in this study when grown on V-8 agar (36).

On rotting blueberry fruits A. alternata appears as a dense green to black velvety mat on the surface of the ripe fruit (Figure 2). The appearance of the fungus in culture was greatly affected by the light regimen used. With continuous darkness the mycelium was olive colored and hirsute. With continuous soft fluorescent lighting the culture became black due to greatly increased sporulation. Sporulation was also greatly favored by growth on V-8 agar as compared to potato dextrose agar.

Effect of Temperature on Fungal Growth

The effect of temperature on the mycelial radial growth rate was studied by incubating cultures at controlled temperatures and measuring colony diameter after seven days. A 7-mm cork borer was used to cut mycelial plugs from the advancing margin of a culture of A. alternata growing on potato dextrose agar (PDA - Difco Products Co., Detroit, Michigan 48201). The plugs were placed aseptically in the center of petri plates containing PDA and incubated at 5, 10, 15, 20, 27, or 30C. Five replicates per temperature tested were used. The experiment was conducted to determine the optimum temperature for fungus growth and development in the field and in storage.

The Effects of Free Water, 100% Relative Humidity, and Temperature on Spore Germination

Spore germination studies were carried out in the laboratory to gain information about how environmental parameters influence spore germination and fruit infection. Spore suspensions were prepared by flooding the surface of PDA grown cultures of an A. alternata isolate, scraping the surface with a bent glass rod, and decanting the resulting spore suspension through four layers of cheesecloth.

Spore germination in free water was studied by placing the spore suspensions in the wells of depression slides and incubating the slides over water at various temperatures. Four replicates per temperature tested were used. At intervals, the slides were removed

from the incubation chambers and the mean percent germination and the mean germ tube length was determined. In each replicate the first 100 conidia encountered were scored as germinated or not germinated and the first 25 germ tubes encountered were measured. This was done at the meniscus of the spore suspension since at the bottom of the depression the spore concentration was too great to count and germination was inhibited. A spore was considered as germinated if a germ tube was visible which was at least as long as the spore was wide. The temperatures tested were 5, 10, 15, 20, 25, and 30C, and the lengths of incubation were 8, 16, 24, 32 and 40 hours at each temperature tested.

To determine if spores could germinate in air at 100% relative humidity spore suspensions prepared as described were sprayed onto glass coverslips with a DeVilbiss No. 15 atomizer. The cover slips were air dried at room temperature. After the spore suspension had dried on the coverslips the coverslips were placed on microscope slides which were in turn placed inside petri dishes lines with wet paper towels. Two petri plates each containing one microscope slide with two cover slips on it were prepared for each temperature/time combination to be studied. This was important because a slide was taken out of its container only once for study. Because of this it did not matter if water condensed on the coverslips after they were removed from the petri plates, as happened with the cover slips incubated at low temperatures.

Field Locations for Alternaria Studies

Epidemiological studies of A. alternata were carried out at a commercial blueberry farm at Grand Junction, Michigan which had a history of postharvest decay problems caused by A. alternata and Colletotrichum gloeosporioides. Weather parameters were monitored, airborne Alternaria spores were trapped, and inoculation studies were carried out in the northeast corner of the 16-hectare (40-acre) field. This area was approximately 0.06 hectare (1/7 acre) in size and contained 18 mature Jersey blueberry bushes in each of six rows. No fungicides were applied in this area during the course of the study.

Monitoring of Environmental Parameters in the Field

Solar radiation intensity was measured with a solar radiation monitor, rainfall was measured with a tipping bucket rain gauge accurate to 0.25-mm (0.01-inch), and wind speed was monitored using a contact anemometer accurate to 0.1 km/hr (0.16 mile/hr) (Weather Measure Co., Sacramento, California 95841). Duration of leaf wetness was recorded using a leaf wetness meter (M. DeWitt Co., Hengelo, The Netherlands). Relative humidity and air temperature were monitored using a hygrothermograph (Belfort Instrument Co., Belfort, Maryland 21224). All instruments were 7-day continuously recording types and were installed within the rows of the blueberry bushes. All charts were changed at weekly intervals. By comparing the leaf wetness and rainfall charts for the same time periods, the leaf wetness periods were attributed to rainfall or to dew.

Trapping of Airborne Alternaria Conidia

A Burkard volumetric air spore trap (Burkard Scientific Sales Ltd., Rickmansworth, Herts., England) was installed in the field and run continuously from a 12V DC battery power source from April 18, 1978 to September 19, 1978 and from April 18, 1979 to August 28, 1979. The starting date for spore trapping was before bud break and the stopping date was after harvesting was completed in both seasons. The intake orifice was 1 m above the ground and the spore trap tapes upon which the spores were impacted were changed at weekly intervals. The machine was adjusted weekly to draw 8-10 liters (2.1-2.6 gallons) of air per minute.

The spore trap tapes were prepared by mounting the clear Mellinex® tape supplied by the manufacturer on a clock drum with double sided tape and coating it with a Gelvatol® (Monsanto Chemical Co., St. Louis, Missouri 63101) water mixture as per directions supplied by the manufacturer (1). After drying overnight, the tapes were recoated with a viscous mixture of vaseline, parafin, and toluene (1) which was the trapping medium. Spores were trapped for one week in the field and the resulting tape was cut into 48 mm (1.9 inch) sections corresponding to single 24 hr days. Hourly counts of Alternaria spp. conidia were made using a compound light microscope with 10x oculars and a 10x objective lens. No stains were used.

In Vitro Fungicide Tests for Control of Alternaria alternata

A preliminary experiment was conducted to select promising fungicides for field testing by testing their effect on radial growth of the Alternaria alternata isolate obtained from rotted fruit in vitro. The fungicides selected for screening were: Bayleton 50% wettable powder (triadimefon), ferbam 65% wettable powder, Difolatan (captafol) 0.5 kg/l (4 lb/gallon) flowable, captan 50% wettable powder, Benlate (benomyl) 50% wettable powder, Phaltan (folpet) 50% wettable powder, Dithane M-45 (mancozeb) 80% wettable powder, Funginex (triforine) 20% emulsifiable concentrate, and Bravo (chlorothalonil) 75% wettable powder.

The experiment was done by mixing the various fungicides into PDA at concentrations of 0.1, 1, 10, and 100 µg/ml (active ingredient basis). Mycelial plugs were taken from the advancing edge of the PDA-grown fungus culture to be tested with a 7-mm cork borer and placed in the center of each fungicide amended PDA test plate. The cultures were incubated at room temperature under continuous cool white fluorescent light and after four days the mycelial radial growth was measured. Data were recorded as percent inhibition of radial growth by the fungicide as compared to radial growth on unamended PDA plates. Four replicates per treatment were used.

Field Evaluation of Fungicides for Control of *Alternaria* Decays of Blueberry Fruit

Fungicide testing was carried out at the same commercial field described previously. The study was carried out to determine what fungicides, if any, could control *Alternaria* decays post-harvest when applied at various times during the growing season.

The fungicides were applied using a Tecnoma 3-point hitch air blast sprayer (Tecnoma Co., Eppernay, France). One hundred eighty-seven liters/hectare (20 gal/acre) of fungicidal spray was applied at a hydraulic pressure of 10.5 kg/cm^2 (150 lbs/in^2) and a ground speed of 5.8 km/hr (3.6 mph). The experiment was carried out in 1978 using a completely randomized design with four replicates per treatment. Two control treatments consisted of water only from the spray rig. Each replicate consisted of seven bushes in a row.

The fungicides used and the application schedule for 1978 are given in Table 3. The fungicides were applied either throughout the season as eight applications from May 2 (green tip) through July 12 (early blue fruit) or as four late season applications only, from June 2 (5% petal fall) through July 12 (early blue fruit).

The fungicide testing methodology was the same in 1979 except that fewer treatments were applied, and five replicates per treatment were used. The fungicides were applied either throughout the season as seven applications from May 1 (green tip) to July 6 (first blue fruit) or as a single massive application (SAT) on May 1, 1979 (Table 4).

The three center bushes of each replicate of the fungicide trial were harvested as a group with hand-held electrical vibrators (Blueberry Equipment Co., South Haven, Michigan 49090). The weight of the ripe fruit in each replicate was recorded as well as the weight of one level cup of fruit and the number of berries in one level cup. A subsample of 100 berries was taken from each replicate and incubated on moist paper towels in plastic boxes so as not to touch one another and with the stem end upwards. After four days at room temperature the fruit was evaluated based on the presence or absence of sporulating fungus. The fruit was scored as healthy (no sporulation) or rotted by Alternaria. The data were recorded as the percent of fruit in each category on a count basis.

Inoculation Studies on Mature Blueberry Bushes in the Field and at East Lansing

Two single spore isolates of Alternaria alternata isolated from rotting blueberries were used for inoculation studies. The isolates were stored over the winter on half strength PDA (½PDA) at 5C. The cultures for inoculation were grown on V-8 agar (36) in plastic petri dishes under continuous fluorescent light at room temperature for four to eight days. Spore suspensions were made by flooding the cultures with sterile distilled water, scraping with a sterile bent glass rod, and decanting the resulting spore suspension through four layers of cheesecloth. The concentration of A. alternata conidia was adjusted to 7×10^5 conidia/ml sterile distilled water in 1978 and to 3×10^5 conidia/ml sterile distilled water in 1979 using a hemacytometer. The two single spore

isolates contributed equally to each spore suspension used for inoculations.

Inoculation studies were carried out at two locations to determine at which phenotypic stage(s) the blueberry bush was susceptible to infection by A. alternata. At Grand Junction, Michigan, the mature blueberry bushes were of the cv. Jersey. At East Lansing potted 3-4 year old bushes of the cvs. Bluecrop and Berkley were used.

The spore suspension was sprayed onto unwounded plant tissue until runoff occurred. The inoculated branch was then placed in a clear polyethylene bag containing a wet paper towel to maintain free water since preliminary experiments showed that free water stimulated conidial germination. Four replicate branches were inoculated with A. alternata and four with water only, as controls on each inoculation date. The plastic bag covering the inoculated branch was removed after 18-24 hours. Because it was thought that high daytime temperature would interfere with the infection process, the inoculated bushes at East Lansing were kept in a growth chamber (Sherer-Gillette Co., Marshall, Michigan 49068) at 21C while the bag covered the bush.

Inoculation dates and growth stages for the 1978 and 1979 seasons are given in Tables 5, 6, and 7.

The ripe fruit from each inoculated bush or branch was harvested by hand by replicate and placed into separate polyethylene pint bags. In order to prevent cross contamination of the replicates

and treatments separate disposable plastic gloves were used to pick each replicate. Fruit with visible sporulation was discarded in order to prevent mass inoculation of the replicates.

The fruit was placed by replicate on a 1-cm wire mesh screen over water in plastic buckets so as not to touch one another. The buckets were covered to maintain high relative humidity. After four days incubation the fruit was scored as healthy (no sporulation) or decayed by Alternaria (visible sporulation). The data were recorded as the percent fruit in each category on a count basis.

Isolation from Host Tissues to Determine the Overwintering Site of the Pathogen

Experiments were done to determine where Alternaria spp. overwintered in blueberry bushes. In late October of 1977, 35 blighted fruit stems were collected. They were surface disinfested by immersing in 0.25% sodium hypochlorite for five minutes followed by rinsing with sterile distilled water. Tissue transfers were aseptically made to petri plates containing ½PDA. In the same manner 129 apparently healthy flower buds were used for tissue transfers.

In the spring of 1978 more blighted fruiting wood was collected from which to make isolations. Twigs were surface disinfested in 1% sodium hypochlorite with a few drops of Tween-80 added as a wetting agent for 10 minutes with stirring under bench vacuum. Other lots of twigs were isolated from directly without surface disinfestation to determine if the pathogen was located deep within

the twig or at or on its surface. Tissue transfers were made onto PDA and the resulting fungal cultures were identified after four to eight days.

RESULTS

Effect of Temperature on Radial Growth of Alternaria alternata

The optimal temperature tested for mycelial growth of Alternaria alternata in this study was 27C (Figure 3). Alternaria alternata grew more than twice as fast at 20C as compared to 15C, and twice as fast at 15C as compared to 10C. Very little growth occurred after seven days at 5C. The results indicate that Alternaria decays would be favored at high temperatures such as are common during the blueberry harvest season.

The Effects of Free Water, 100% Relative Humidity, and Temperature on Conidial Germination of Alternaria alternata

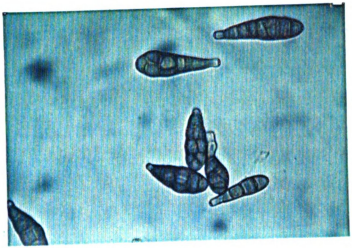
Conidia of the A. alternata isolate used germinated readily at temperatures of 10C or greater at 100% relative humidity (Table 1). Free water was, however, very stimulatory for both conidial germination and germ tube elongation at all temperatures tested, particularly at the lower and higher temperatures (Table 2). Conidial germination was greatest at 20C in free water and at 25C at 100% relative humidity.

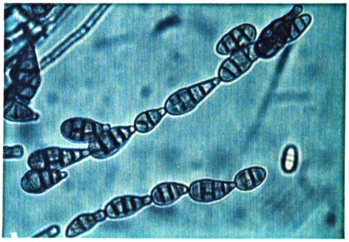
Alternaria alternata conidia produced several germ tubes each in this experiment. This created a wide variation in germ tube lengths, which is reflected by the standard deviations about the mean of germ tube length in Tables 1 and 2.

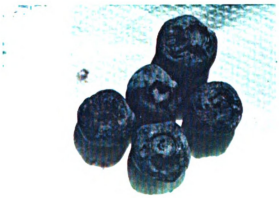
Figure 1-A.--*Alternaria alternata* conidia

Figure 1-B.--*Alternaria alternata* conidia borne in chains.

Figure 2.--*Alternaria alternata* sporulating on blueberry fruit.







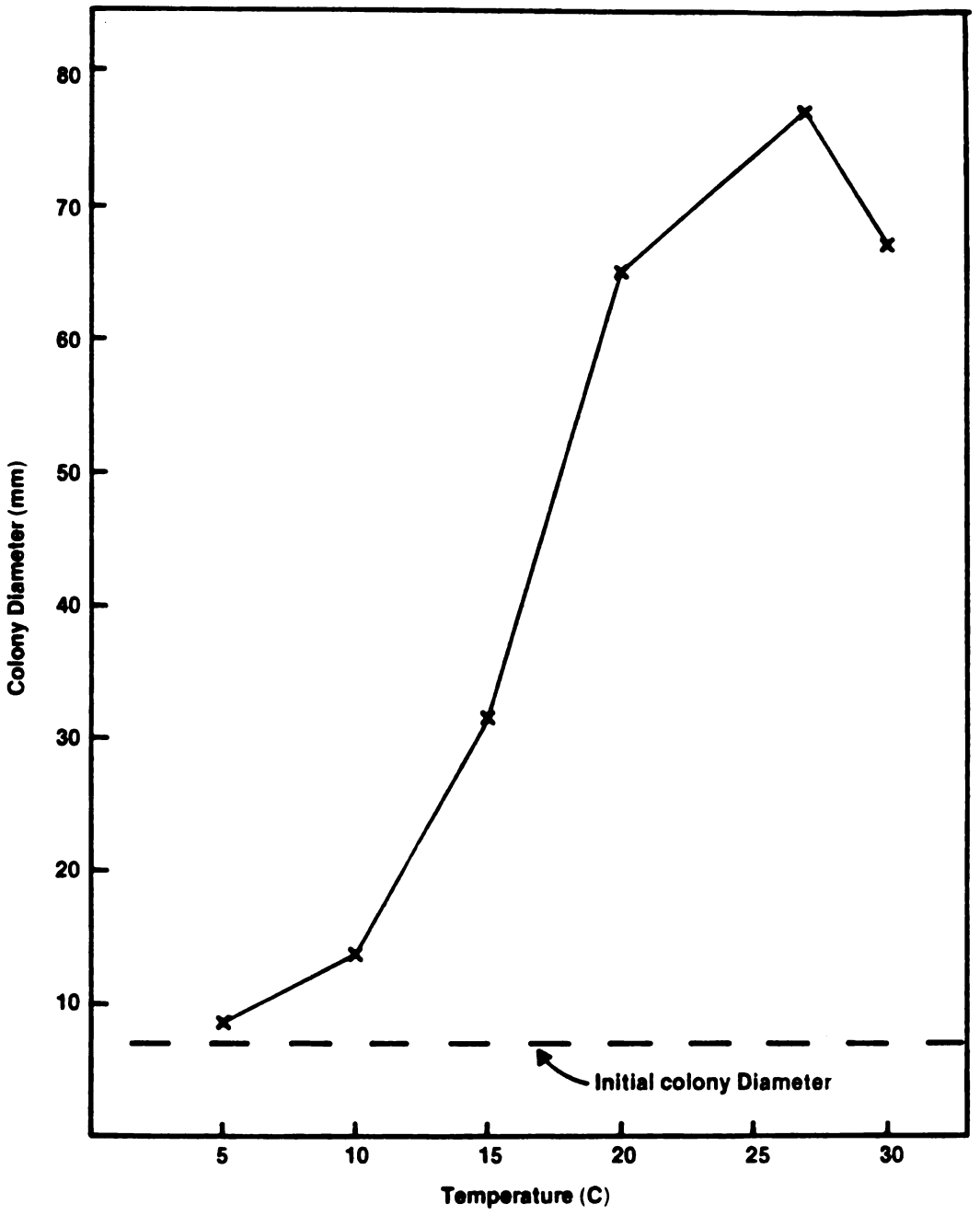


Figure 3.--Mycelial growth of Alternaria alternata on PDA at several temperatures after 7 days.

TABLE 1.--Conidial germination of *Alternaria alternata* on glass slides at 100% relative humidity and various temperatures. Germination percent and germ tube length (μm).[a]

Temperature (°C)	Time Interval							
	8 Hours		16 Hours		24 Hours		32 Hours	
	Percent Germination	Germ Tube Length (μm)	Percent Germination	Germ Tube Length (μm)	Percent Germination	Germ Tube Length (μm)	Percent Germination	Germ Tube Length (μm)
5	0	---[b]	0	---	0	---	11± 6	11± 2
10	1± 2	8± 3	53±11	32±18	75±46	103± 46	83± 5	101± 27
15	37±16	37±25	50±16	85±49	47±24	69± 56	66± 6	132± 72
20	74± 8	78± 50	82± 2	134±93	88± 5	182±125	89± 5	262±171
25	83± 7	49±27	95± 3	110±60	96± 3	126± 80	99± 1	243±100
30	17± 2	74±34	49±13	241±93	51± 4	254±104	40± 5	248±132

[a]Percent germination of first 100 conidia encountered in microscope field. Germ tube length in μm. Mean of four replicates (germination percent) and of 25 germ tubes (germ tube length). Mean ± standard deviation of the mean is recorded.

[b]Missing data.

TABLE 2.--Conidial germination of Alternaria alternata on glass slides in free water at various temperatures. Germination percent and germ tube length (μm).[a]

Temperature (°C)	Time Interval					
	8 Hours		16 Hours		24 Hours	
	Percent Germination	Germ Tube Length (μm)	Percent Germination	Germ Tube Length (μm)	Percent Germination	Germ Tube Length (μm)
5	7± 9	31±20	69± 9	19± 11	81± 6	52± 33
10	10± 3	17± 7	78±19	72± 25	93± 4	118± 52
15	88± 7	---[b]	84±12	---	94± 7	---
20	95± 3	103±38	98± 1	173±102	100	200± 99
25	83± 2	114±45	90± 3	152± 61	94± 3	180±106
30	73± 6	74±30	96± 3	97± 42	100	117± 44

[a]Percent germination of first 100 conidia encountered in microscope field. Mean ± standard deviation of the mean of four replicates. Average germ tube length ± standard deviation of mean of 25 replicates.

[b]Missing data.

Since free water was not required for germination, infection of fruit could occur at 100% relative humidity, although the infection rate would be increased in the presence of free water. Temperatures of 15-25C would be optimal for fruit infection.

Preliminary Screening of Fungicides for Activity Against Alternaria alternata

Radial growth inhibition of A. alternata on fungicide amended PDA as compared to non-amended PDA was determined for nine fungicides and the results for eight of those fungicides are shown in Figure 4. The ninth fungicide tested, Bravo, showed almost no activity against A. alternata.

The fungicides which showed the greatest activity against A. alternata in this study were Funginex (triforine), ferbam, and Difolatan (captafol). Triforine caused more than 90% growth inhibition of A. alternata at a concentration of 100 µg/ml. Captan and Bayleton caused 65% and 55% inhibition, respectively, at the same concentration. Benlate (benomyl) was ineffective against A. alternata in this test.

Difolatan was clearly the most effective fungicide at concentrations lower than 100 µg/ml. The results of this in vitro screening of fungicides were used to select fungicides for field testing for control of postharvest decays of blueberries caused by A. alternata.

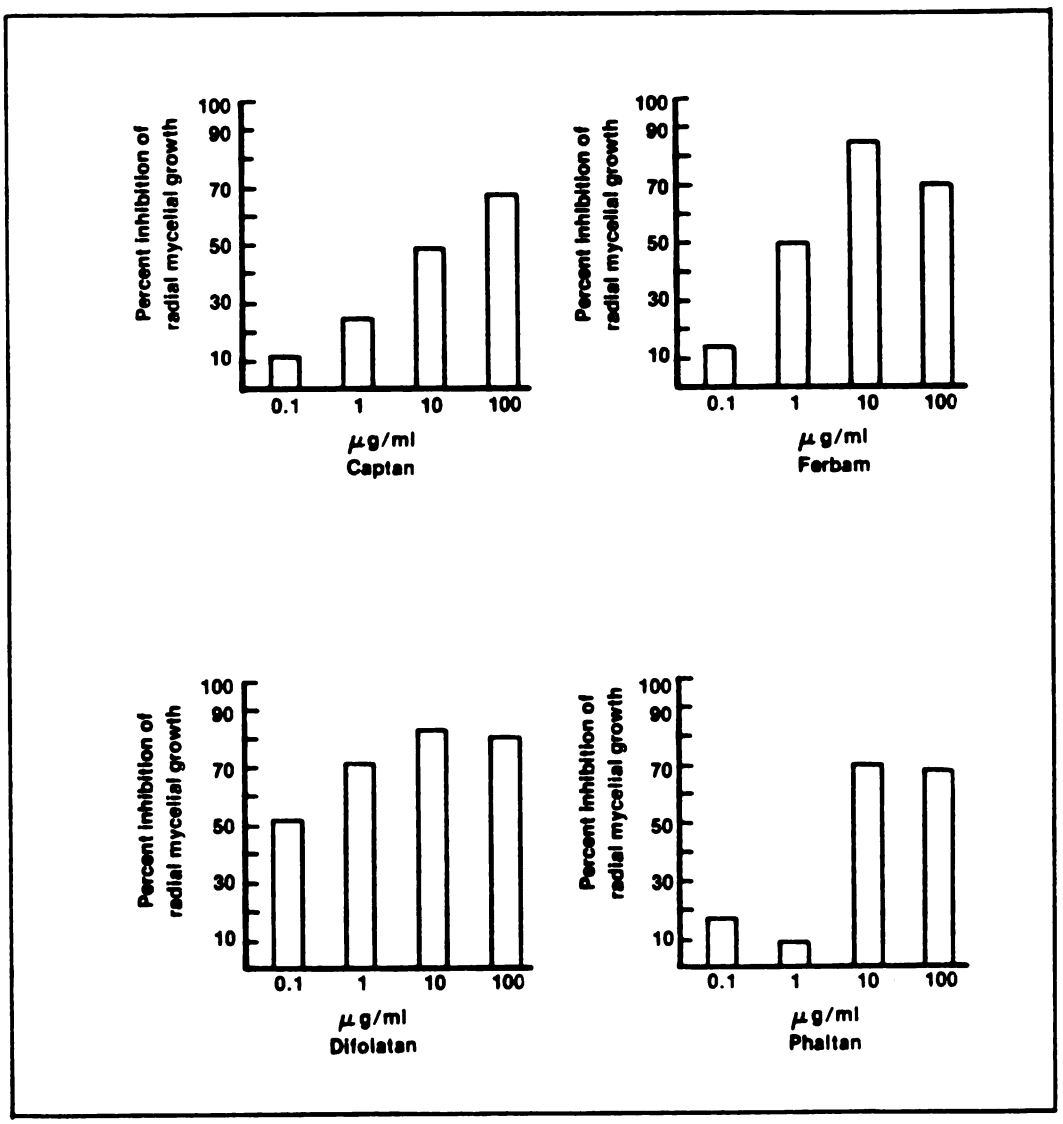


Figure 4.--Growth inhibition of Alternaria alternata mycelium at 96 hr by fungicides incorporated into PDA.

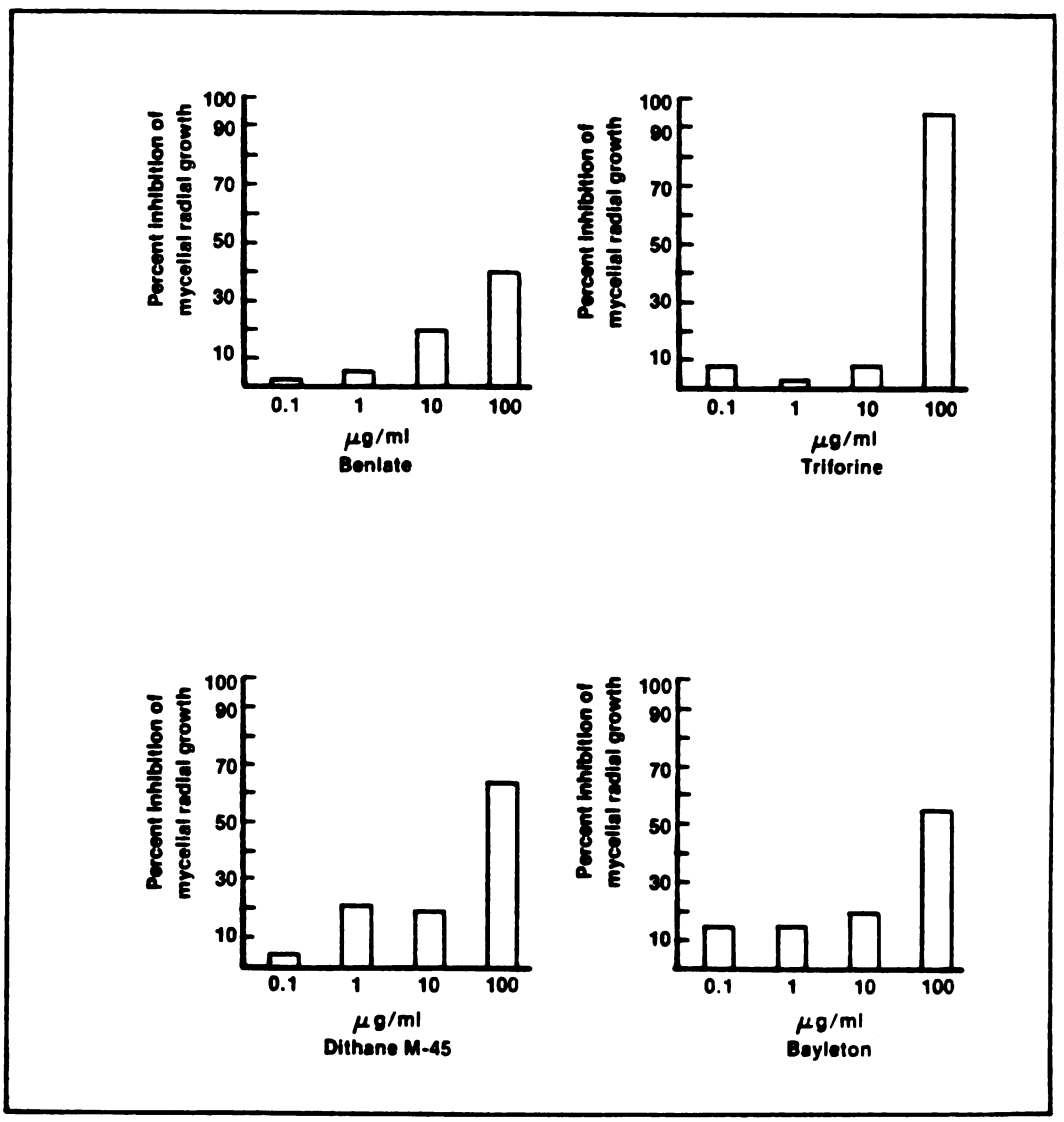


Figure 4.--Continued.

Results of Fungicide Testing in the Field for Control of *Alternaria alternata* Decays of Blueberry Fruits

The results of the 1978 and 1979 fungicide trials for control of *Alternaria* decays of blueberries are shown in Tables 3 and 4, respectively.

In both seasons the four treatments which gave the greatest *Alternaria* decay control all involved Difolatan (captafol). Because of the low decay incidence and variability among the replicates in 1978, the differences in fungicidal control of *Alternaria* were not judged to be significant ($p = .05$) by Duncan's Multiple Range (DMR) test. The differences in fungicidal control of *Alternaria* decays were found to be significant ($p = .05$) by the DMR test in 1979, but not by the Student-Newman-Keul (SNK) procedure. Difolatan 4F at the rates of 9.4 l/ha (4 qt/acre) throughout the season and at 28.2 l/ha (12 qt/acre) on the first application date only (SAT) gave significantly better control of *Alternaria* decays than any of the other fungicides tested in the field in 1979.

Difolatan 4F at the rate of 9.4 l/ha throughout the season did cause a significant reduction ($p = .05$, DMR) in the yield of the bushes harvested for fungicide evaluation in 1979, but not in 1978. Whether this represented a true yield reduction or merely a delay in ripening was not determined.

None of the fungicides tested had any effect ($p = .05$) on the average berry weight or size in either year as determined by DMR test in 1978 and SNK test in 1979.

TABLE 3.--Fungicide trial for control of Alternaria decays of blueberry fruit at Grand Junction, MI in 1978.[T]

Treatment	% Alternaria[U] Decay	Yield[V] Grams
Difolatan 4F[W] 9.4 L/ha (4 qt/acre) E+L[X]	0.12 a	11991 ab
Difolatan 4F 4.7 L/ha (2 qt/acre) L	0.21 ab	11331 ab
Difolatan 4F 9.4 L/ha (4 qt/acre) L	0.23 ab	10182 b
Difolatan 4F 4.7 L/ha (2 qt/acre) E+L	0.38 abc	11205 ab
Funginex (CME 74770) 1.17 L/ha (16 oz/acre) 20% EC[Y] E+L	0.42 abcd	14676 a
Captan 50% WP[Z] 5.6 kg/ha (5 lb/acre) + Benlate 50% WP 1.1 kg/ha (1 lb/acre) E+L	0.43 abcd	10992 ab
Control (untreated)	0.45 abcd	14634 a
That Liquid Sulfur 4.7 L/ha (2 qt/acre) E+L	0.53 abcd	10437 ab
Captan 50% WP 5.6 kg/ha (5 lb/acre) + Benlate 50% WP 1.1 kg/ha (1 lb/acre) L	0.79 bcd	11205 ab

TABLE 3.--Continued.

Treatment	% Alternaria[U] Decay	Yield[V] Grams
Captan 50% WP 5.6 kg/ha (5 lb/acre) + Ferbam 76% WP 6.8 kg/ha (6 lb/acre) E+L	0.82 cd	7347 c
Funginex (CME 74770) 20% EC 1.75 L/ha (24 fl oz/acre) L	0.85 cd	10779 ab
Captan 50% WP 5.6 kg/ha (5 lb/acre) + Ferbam 76% WP 6.8 kg/ha (6 lb/acre) L	0.88 cd	10650 ab
Control (untreated)	0.90 cd	12780 ab
Funginex (CME 74770) 20% EC 1.75 L/ha (24 fl oz/acre) E+L	0.96 d	11121 ab

[T]All fungicides applied on dates and at rates given with a Tecnoma airblast sprayer run at 12.6 kg/cm^2 (180 lb/in^2). All compounds applied in 187 liters of solution per hectare (20 gallon/acre).

[U]Berries were hand harvested and incubated without touching for four days in moist chambers. Mean percent decay of four replicates of 100 randomly selected berries each. Means with letter following in common judged to be not significantly different (p = .05) by Duncan's Multiple Range test.

[V]Fruit was harvested on 8/10, 8/11, 1978. Mean yield of four replicates of three bushes each. Represents total crop yield. Means with letters following in common judged to be not significantly different (p = .05) by Duncan's Multiple Range test.

[W]Flowable.

[X]E+L = Early + Late applications. Dates of early applications were 5/2 (1/16 inch green tip), 5/19 (early pink bud), 5/10 (1/4 inch green tip), and 5/26/78 (1-5% bloom). Dates of late applications were 6/2 (5% petal fall), 6/13-15 (small green fruit), and 6/28 (late green fruit), and 7/12/78 (first blue fruit). L = Late applications only.

[Y]EC = Emulsifiable Concentrate

[Z]WP = Wettable Powder

TABLE 4.--Fungicide trial for control of Alternaria decays of blueberry fruit at Grand Junction, MI in 1979.

Treatment[T,W]	% Alternaria[U] Decay	Yield[V] Grams
Difolatan 4F[X] 9.4 L/ha (4 qt/acre) Through Season	6.2 a	4992 a
Difolatan 4F 28.2 L/ha (12 qt/acre) First Application Only	7.6 a	6628.4 abc
Difolatan 4F 4.7 L/ha (2 qt/acre) Through Season	11.2 ab	6350.2 abc
Benlate 50% WP 1.1 kg/ha (1 lb/acre + Difolatan 4F 4.7 L/ha (2 qt/acre) Through Season	13.6 abc	5862.0 abc
That Sulfur F 4.7 L/ha (2 qt/acre) Through Season	18.4 abc	5600.6 ab
Lime Sulfur 9.4 L/ha (1 gal/acre) First Application Only	18.4 abc	6089.2 abc
Funginex 20% EC[Y] 1.75 L/ha (24 oz/acre) Through Season	20.8 abc	5009.8 a
Control (no sprays)	22.8 bc	6997.7 bc
Procymidone 50% WP[Z] 1.1 kg/ha (1 lb/acre) Through Season	25.4 bc	7423.8 c
Lime Sulfur 9.4 L/ha (4 qt/acre) + Captan 50% WP 5.6 kg/ha (5 lb/acre) Lime Sulfur First Application Only; Captan Only through Season	28.8 c	6384.2 abc

TABLE 4.--Continued.

Treatment	% Alternaria[U] Decay	Yield[V] Grams
Lime Sulfur 9.4 L/ha (4 qt/acre) + That Sulfur F 4.7 L/ha (2 qt/acre) Lime Sulfur First Application Only; That Sulfur Only Through Season	43.4 d	5055.2 a

[T]All fungicides applied on dates and at rates given with a Tecnoma® air blast sprayer run at 12.6 kg/cm^2 (180 lbs/in^2). All compounds applied in 187 liters of solution per hectare (20 gallons/acre).

[U]Berries were hand harvested and incubated without touching for four days in moist chambers. Mean percent decay of five replicates of 100 randomly selected berries each. Means with letter following in common judged to be not significantly different ($p = .05$) by Duncan's Multiple Range test.

[V]Fruit was harvested on 8/15/79. Mean yield of five replicates of three bushes each. Represents about 1/3 of total crop yield. Means with letter following in common judged to be not significantly different ($\underline{p} = .05$) by Duncan's Multiple Range test.

[W]First application - 5/ 1/79 - Green Tip
Second application - 5/11/79 - Pre-Bloom
Third application - 5/16/79 - 10% Bloom
Fourth application - 5/23/79 - 40% Bloom
Fifth application - 6/ 5/79 - Petal Fall
Sixth application - 6/21/79 - Green Fruit
Seventh application - 7/ 6/79 - First Blue Fruit

[X]F = Flowable

[Y]EC = Emulsifiable Concentrate

[Z]WP = Wettable Powder

Tables 3 and 4 show that Alternaria decay incidence was ten or more times greater in 1979 than in 1978. This reflects a difference in the criteria used to characterize a blueberry as rotted by Alternaria in each year. In 1978 only berries with abundant green to black velvety mycelium on them were classified as rotted by Alternaria. In 1979 the criteria were expanded to include berries with a small grayish white mycelium at the stem end scar. Isolations showed that these mycelia were also Alternaria, although they were non-sporulating at the time of evaluation.

Inoculation Studies in the Field and at East Lansing Using Alternaria alternata

Inoculations with A. alternata did not consistently increase the incidence of A. alternata decays postharvest above the inoculated control levels in either season in the field or in the potted bush inoculations at East Lansing (Tables 5, 6 and 7). The data from the potted bush inoculation experiment at East Lansing in 1978 were unusable due to problems with insect and avian pests. The results imply that Alternaria decays of fruit are not the result of latent infections established during the growing season.

The isolates of A. alternata used in this study did cause a transient leafspot on succulent blueberry leaves when inoculated on them early in the season. The symptoms consisted of small red spots on the inoculated succulent leaves which faded as the season progressed (Figure 5). The symptom is very similar to one reported by Milholland in North Carolina caused by A. tenuissima (20), and

TABLE 5.--Data from field inoculations of blueberry bushes with *Alternaria alternata* at Grand Junction, MI in 1978.[a,b]

Inoculation Date and Growth Stage	Mean Percent Fruit Rotted by *Alternaria*[c] (Inoculated)	Mean Percent Fruit Rotted by *Alternaria* (Control)
5/2/78 Bud Swell	4.2 ± 1.7	1.5 ± 1.0
5/16/78 Bud Break	8.6 ± 5.5	8.8 ± 2.6
5/23/78 Pink Bud	10.3 ± 1.9	6.5 ± 2.8
5/30/78 Full Bloom	1.5 ± 1.0	0.0
6/6/78 Petal Fall	1.1 ± 0.9	17.7 ± 11.2
6/21/78 Green Fruit	0.0	0.0
7/16/78 Early Blue Fruit	0.0	2.6 ± 1.1

[a]Bushes were inoculated on dates given with a spore suspension of 7×10^5 conidia/ml sterile distilled water or with sterile distilled water only.

[b]Fruit was hand harvested on 8/1/78 and held for four days at room temperature in moist chambers.

[c]Percent visibly sporulating *Alternaria* rots on a count basis. Numbers given are the means of four replicates ± the standard deviations of the means.

TABLE 6.--Data from field inoculations of blueberry bushes with *Alternaria alternata* at Grand Junction, MI in 1979.[a,b]

Inoculation Date and Growth Stage	Mean Percent Fruit Rotted by *Alternaria*[c] (Inoculated)	Mean Percent Fruit Rotted by *Alternaria* (Control)
5/2/79 Bud Swell	10.5 ± 2.5	9.8 ± 2.5
5/9/79 Bud Break	9.8 ± 2.2	---[d]
5/16/79 Pink Bud	20.0 ± 4.2	10.3 ± 1.8
5/23/79 50% Bloom	13.3 ± 2.1	36.3 ± 3.3
6/13/79 Early Green Fruit	13.3 ± 4.2	14.0 ± 4.2
7/5/79 Late Green Fruit	24.5 ± 2.9	18.0 ± 1.1
7/18/79 First Blue Fruit	16.8 ± 2.2	2.8 ± 0.7
8/1/79 First Ripe Fruit	13.5 ± 1.0	9.8 ± 2.1

[a]Bushes were inoculated on dates given with a spore suspension of 3×10^5 conidia/ml sterile distilled water or with sterile distilled water only.

[b]Fruit was hand harvested on 8/15/79 and held at room temperature for four days in moist chambers.

[c]Percent visibly sporulating *Alternaria* rots on a count basis. Numbers given are the means of four replicates ± the standard deviations of the means.

[d]Missing data.

TABLE 7.--Data from inoculations of potted blueberry bushes with *Alternaria alternata* at East Lansing, MI in 1979.[a,b]

Inoculation Date and Growth Stage	Mean Percent Fruit Rotted by *Alternaria*[c] (Inoculated)	Mean Percent Fruit Rotted by *Alternaria* (Control)
4/28/79 Bud Swell	15.4 ± 5.5	13.8 ± 3.7
5/10/79 Bud Break	19.4 ± 6.4	21.2 ± 5.7
5/17/79 Early Pink Bud	22.5 1 5.5	14.4 ± 2.9
5/24/79 Full Bloom	20.6 ± 6.6	19.9 ± 3.6
6/14/79 Green Fruit	19.7 ± 0.7	12.7 ± 3.2
7/19/79 First Blue Fruit	3.3 ± 2.9	2.3 ± 1.5

[a]Potted bushes were inoculated on dates given with a spore suspension of $3x10^5$ conidia/ml sterile distilled water or with sterile distilled water only.

[b]Fruit was hand harvested on 7/31/79 and held 6 days at room temperature in moist chambers.

[c]Percent visibly sporulating *Alternaria* rots on a count basis. Numbers given are the means of four replicates ± the standard deviation of means.

Figure 5-A.--*Alternaria alternata* leafspot 96 hr. after inoculation with 7 x 10^5 conidia/ml sterile distilled water.

Figure 5-B.--*Alternaria alternata* leafspot disappearing after an additional 10 days.

implies that the virulence of the isolates was not attenuated in culture. The fungus reisolated from these spots was indistinguishable in culture from the isolates which were used to inoculate the bushes.

Results of Trapping of Air Dispersed Conidia of *Alternaria alternata* in a Commercial Blueberry Planting in 1978 and 1979

In 1978 the number of *Alternaria* conidia trapped per hour increased rapidly in the last week of June. The daily peak catches of conidia remained relatively high until trapping was discontinued on September 17, 1978. The greatest catch of conidia occurred on September 15, when 909 conidia were recorded in a single hour. These conidia were probably produced on berries which had been left in the field by the mechanical harvester either on the bushes or on the ground, as the field had been mechanically harvested one month earlier. Some may have been produced on weeds or saprophytically on other plant debris.

In 1979 a similar general pattern was observed. The daily peak catches of conidia remained high until trapping was discontinued on August 28 after rising in early July. The greatest catch of conidia occurred on July 18 when 334 were recorded in one hour. In both seasons spore catches never exceeded 50 per hour in May and June and were considered negligible.

The relationships between release of *Alternaria* conidia and environmental parameters monitored simultaneously were investigated by analyzing seven individual episodes of spore release by

statistical methods. Four of these episodes occurred during 1978, and the remainder occurred during 1979. These episodes ranged from three to five days in length and included time periods with both very high and very low numbers of spores trapped per hour. Three of these periods were analyzed graphically and are shown in Figures 6, 7, and 8. The results show that spore release tended to occur at times with higher air temperatures, lower relative humidities, dry leaf surfaces, sunshine, and wind.

Simple correlation coefficients (r) were calculated which related spore release to the various environmental parameters. These data are presented in Table 8. Release of Alternaria conidia was consistently positively correlated with air temperature, solar radiation intensity and wind speed. Release of conidia was negatively correlated with relative humidity.

Chi square analysis of the spore release patterns for the same episodes was also carried out to determine the nature and strength of the associations between low and high rates of conidial production and various environmental parameters. High rates of conidia release were associated consistently with higher air temperatures, low relative humidity, high solar radiation intensity, and higher wind speed, and the absence of water on leaf surfaces. Low rates of conidial release were consistently associated with lower air temperatures, high relative humidity, lower solar radiation intensities and darkness, low wind speeds, and the presence of water on leaf surfaces. These associations were consistently

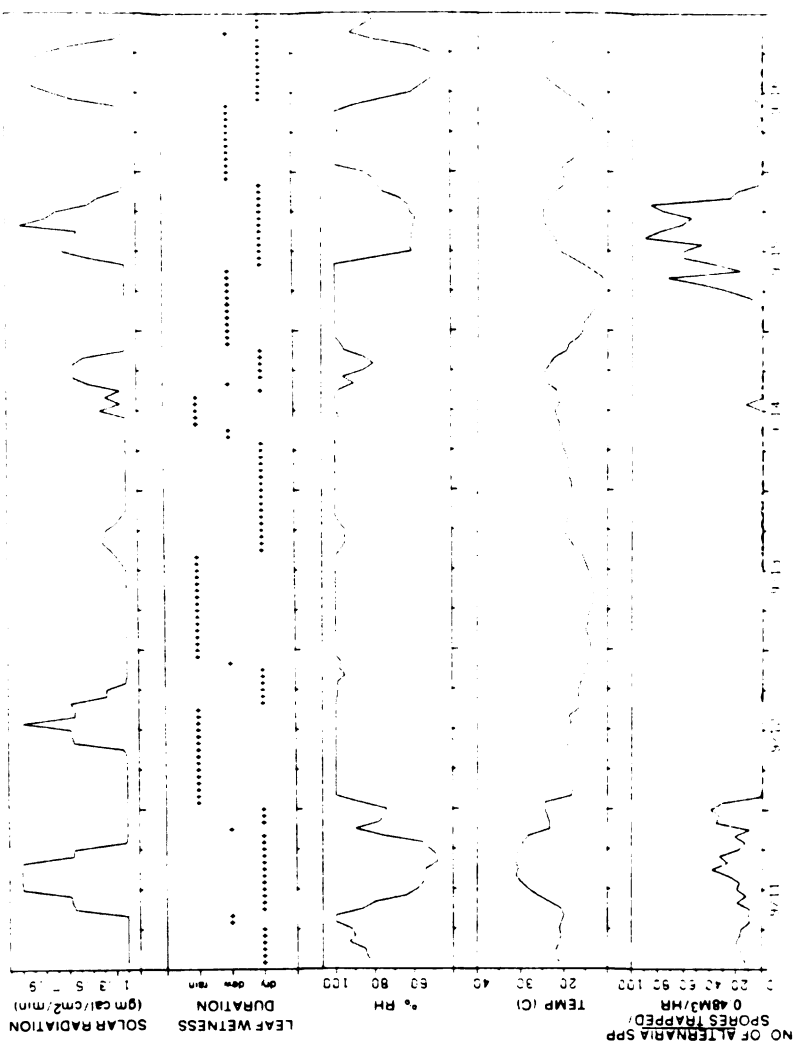


Figure 6.--Relationship between Alternaria spp. conidia trapped per hour and various environmental parameters at Grand Junction, MI. from September 11 to September 16, 1978.

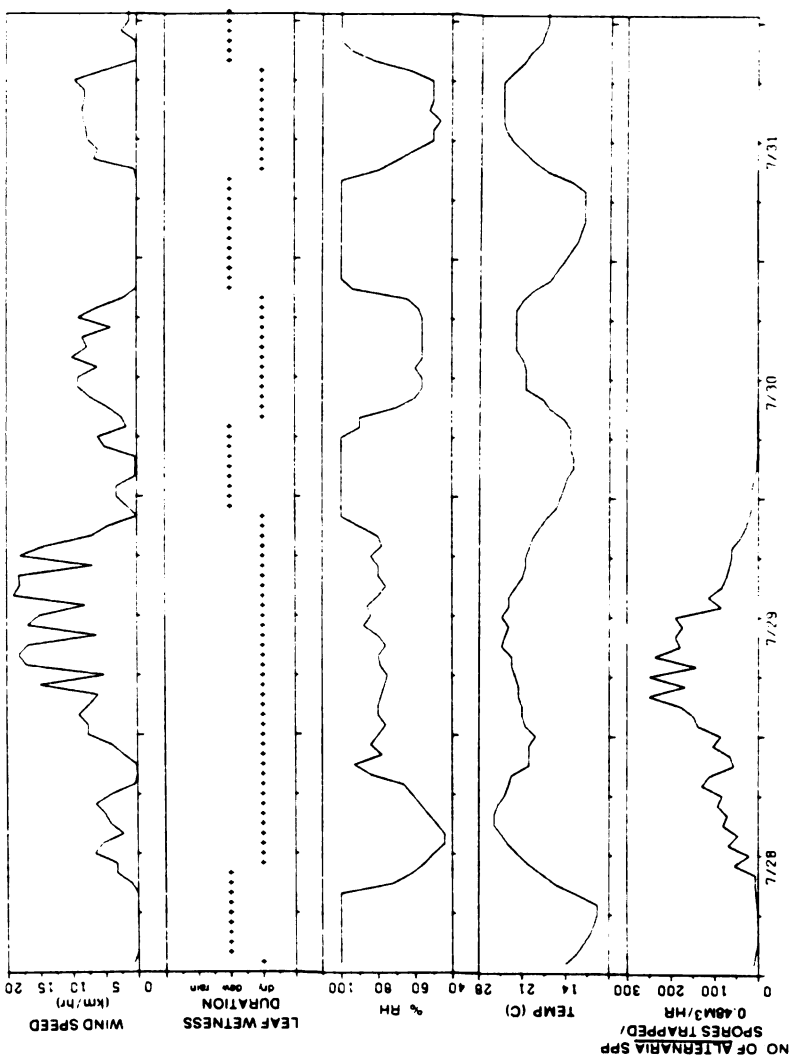


Figure 7.--Relationship between Alternaria spp. conidia trapped per hour and various environmental parameters at Grand Junction, MI from July 28 to July 31, 1978.

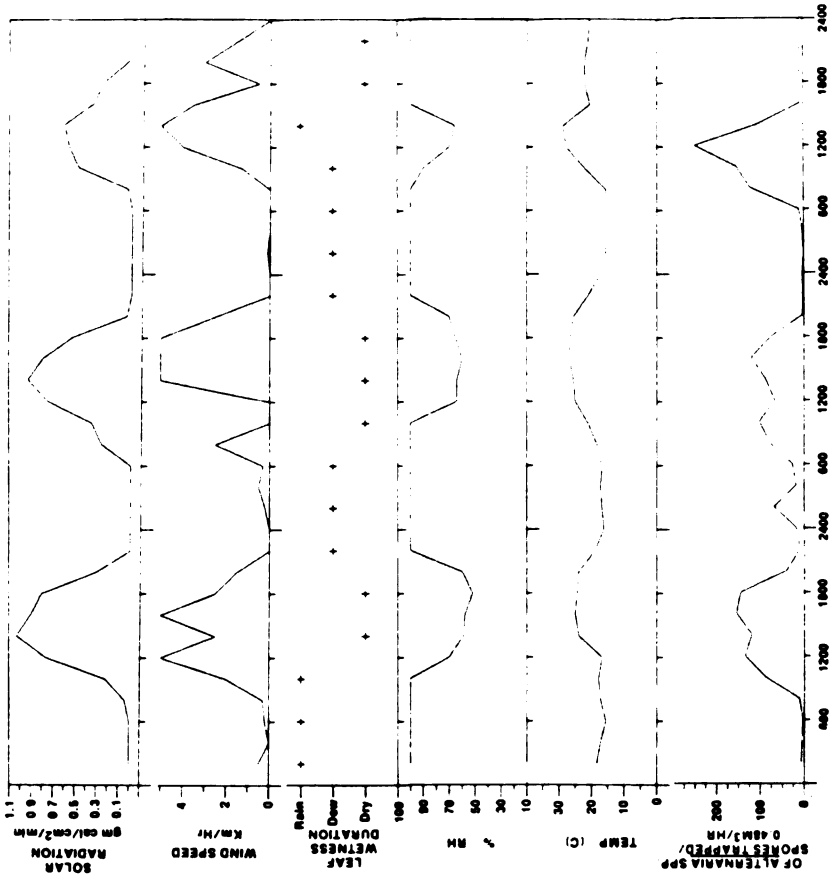


Figure 8.--Relationship between Alternaria spp. conidia trapped per hour and various environmental parameters at Grand Junction, MI from August 2 to August 4, 1979.

TABLE 8.--Correlation of hourly catches of *Alternaria* spp. conidia with environmental parameters for seven episodes of spore release at Grand Junction, MI.[a]

Environmental Factors	Simple Correlation Coefficients[b]						
	1978 Dates				1979 Dates		
	7/24-7/26	7/28-8/01	8/30-9/03	9/14-9/16	7/11-7/14	7/27-7/29	8/02-8/04
Air Temperature (°C)	0.625*	0.502*	0.699*	0.150	-0.094	0.601*	0.501*
Relative Humidity (%)	-0.507*	0.017	-0.658*	-0.461*	-0.548*	-0.554*	-0.613*
Solar Radiation (gm-cal/cm^2/min)	0.279	0.008	0.317**	0.373**	0.610*	0.400**	0.740*
Wind Speed (km/hr)	0.492*	0.524*	---[c]	---[c]	0.393**	0.701*	0.579*

[a]Episodes were selected from spore trapping data from 1978 and 1979 seasons.

[b]Asterisks indicate significant correlation coefficients at P = .001 (*) and P = .01 (**).

[c]Missing data.

significant ($p < .01$) throughout the season, but in two of the seven episodes solar radiation intensity was not associated with spore release and for one episode each there was no association between wind speed and spore release and leaf wetness and spore release. The same associations between the various environmental parameters monitored and spore release were found in chi-square analyses of the data from the entire seasons of 1978 and 1979. These data are presented in Tables 9 and 10, respectively. The chi square analyses of spore release and leaf wetness for 1978 and 1979 are shown in Table 11. The null hypotheses (H_0) tested by the chi square analyses of spore release and environmental parameters shown in Tables 9-11 were that the magnitude of the spore release was not related to (a) air temperature, (b) wind speed, (c) percent relative humidity, (d) solar radiation intensity, and (e) the presence or absence of free water on the leaves. As a result of these analyses, all of the null hypotheses were rejected.

Standard multiple regression analyses were performed using the spore trapping and environmental data which had been simultaneously recorded to determine which, if any of the environmental parameters consistently contributed the greatest amount to the variance explained by the regression equations. The analyses were carried out using the data from the same seven episodes for which simple correlation coefficients had been calculated and chi square analyses performed. All independent variables were entered into the equation simultaneously and the contribution to the explained

TABLE 9.--Chi square analysis of hourly catches of *Alternaria* spp. conidia and environmental parameters in a commercial blueberry planting at Grand Junction, MI in 1978.

Alternaria spp. spores trapped from the air		Air Temperature (C)		Row Total
		Less than 21	22-32	
Less than 50/hr:	Count	710	504	1214
	Row Percent	58.5	41.5	74.6
	Column Percent	92.3	58.6	
	Total Percent	43.6	31.0	
51-909/hr:	Count	59	354	413
	Row Percent	14.3	85.7	25.4
	Column Percent	7.7	41.3	
	Total Percent	3.6	21.8	
	Column Total	769 47.3	858 52.7	1627 100.0

Corrected chi square = 239.8 with 1 degree of freedom
P = 0.0000.

Alternaria spp. spores trapped from the air		Wind Speed km/hr		Row Total
		Less than 4.0	4.1-22.0	
Less than 50/hr:	Count	1095	256	1351
	Row Percent	81.1	18.9	75.0
	Column Percent	77.9	64.6	
	Total Percent	60.8	14.2	
51-909/hr:	Count	310	140	450
	Row Percent	68.9	31.1	25.0
	Column Percent	22.1	35.4	
	Total Percent	17.2	7.8	
	Column Total	1405 78.0	396 22.0	1801 100.0

Corrected chi square = 28.4 with 1 degree of freedom
P = 0.00001

TABLE 9.--Continued.

Alternaria spp. spores trapped from the air		Relative Humidity (%)			
		Less than 73	74-97	98-100	Row Total
Less than 50/hr:	Count	241	247	566	1054
	Row Percent	22.9	23.4	53.7	74.9
	Column Percent	54.8	68.4	93.4	
	Total Percent	17.1	17.6	40.2	
51-909/hr:	Count	199	114	40	353
	Row Percent	56.4	32.3	11.3	25.1
	Column Percent	45.2	31.6	6.6	
	Total Percent	14.1	8.1	2.8	
	Column Total	440	361	606	1407
		31.3	25.7	43.1	100.0

Raw chi square = 213.2 with 2 degrees of freedom
P = 0.00001

Alternaria spp. spores trapped from the air		Solar Radiation (gm-cal/cm^2/min)			
		None	0.01-0.56	0.57-1.12	Row Total
Less than 50/hr:	Count	819	314	218	1351
	Row Percent	60.6	23.2	16.1	75.0
	Column Percent	83.3	68.7	60.4	
	Total Percent	45.5	17.4	12.1	
51-909 hr:	Count	164	143	143	
	Row Percent	36.4	31.8	31.8	25.0
	Column Percent	16.7	31.3	39.6	
	Total Percent	9.1	7.9	7.9	
	Column Total	983	457	361	1801
		54.6	25.4	20.0	100.0

Raw chi square = 87.0 with 2 degrees of freedom.
P = 0.00001

TABLE 10.--Chi square analysis of hourly catches of *Alternaria* spp. conidia and environmental parameters in a commercial blueberry planting at Grand Junction, MI in 1979.

Alternaria spp. spores trapped from the air		Air Temperature (°C)		Row Total
		Less than 21	22-32	
Less than 50/hr:	Count	820	559	1379
	Row Percent	59.5	40.5	94.4
	Column Percent	97.7	89.9	
	Total Percent	56.1	38.3	
51-334/hr:	Count	19	63	82
	Row Percent	23.2	76.8	5.6
	Column Percent	2.3	10.1	
	Total Percent	1.3	4.3	
	Column Total	839	622	1461
		57.4	42.6	100.0
	Corrected chi square = 40.2 with 1 degree of freedom			
	P = 0.00001			

Alternaria spp. spores trapped from the air		Wind Speed km/hr		Row Total
		Less than 4.0	4.1-22.0	
Less than 50/hr:	Count	1232	185	1417
	Row Percent	86.9	13.1	94.3
	Column Percent	95.1	89.8	
	Total Percent	82.0	12.3	
51-334/hr:	Count	64	21	85
	Row Percent	75.3	24.7	5.7
	Column Percent	4.9	10.2	
	Total Percent	4.3	1.4	
	Column Total	1296	206	1502
		86.3	13.7	100.0
	Corrected chi square = 8.2 with 1 degree of freedom			
	P = 0.0041			

TABLE 10.--Continued.

Alternaria spp. spores trapped from the air		Relative Humidity (%)			
		Less than 73	74-97	98-100	Row Total
Less than 50/hr:	Count	330	317	716	1363
	Row Percent	24.2	23.3	52.5	94.3
	Column Percent	86.2	95.2	98.2	
	Total Percent	22.8	21.9	49.6	
51-334/hr:	Count	53	16	13	82
	Row Percent	64.6	19.6	15.9	5.2
	Column Percent	13.8	4.8	1.8	
	Total Percent	3.7	1.1	0.9	
	Column Total	383	333	729	1445
		26.5	23.0	50.4	100.0

Raw chi square = 68.8 with 2 degrees of freedom
P = 0.00001

Alternaria spp. spores trapped from the air		Solar Radiation (gm-cal/cm^2/min)			
		None	0.01-0.56	0.57-1.12	Row Total
Less than 50/hr:	Count	658	407	352	1417
	Row Percent	46.4	28.7	24.8	94.3
	Column Percent	99.1	93.1	87.8	
	Total Percent	43.8	27.1	23.4	
51-334/hr:	Count	6	30	49	85
	Row Percent	7.1	35.3	57.6	5.7
	Column Percent	9.9	6.9	12.2	
	Total Percent	0.4	2.0	3.3	
	Column Total	664	437	401	1502
		44.2	29.1	26.7	100.0

Raw chi square = 61.6 with 2 degrees of freedom.
P = 0.00001

TABLE 11.--Chi square analysis of hourly catches of *Alternaria* spp. conidia and leaf wetness in a commercial blueberry planting at Grand Junction, MI in 1978 and 1979.

Alternaria spp. spores trapped from the air		Leaf Wetness - 1978		Row Total
		Dry	Wet	
Less than 50/hr:	Count	764	588	1351
	Row Percent	56.5	43.5	75.0
	Column Percent	65.4	92.6	
	Total Percent	42.4	32.6	
51-909/hr:	Count	403	47	450
	Row Percent	89.6	10.4	25.0
	Column Percent	34.6	7.4	
	Total Percent	22.4	2.6	
	Column Total	1166	635	1801
		64.7	35.3	100.0
	Corrected chi square = 160.4 with 1 degree of freedom			
	P = 0.00001			

Alternaria spp. spores trapped from the air		Leaf Wetness - 1979		Row Total
		Dry	Wet	
Less than 50/hr:	Count	711	706	1417
	Row Percent	50.2	49.8	94.3
	Column Percent	90.7	98.3	
	Total Percent	47.3	47.0	
51-334/hr:	Count	73	12	85
	Row Percent	85.9	14.1	5.7
	Column Percent	9.3	1.7	
	Total Percent	4.9	0.8	
	Column Total	784	718	1502
		52.2	47.8	100.0
	Corrected chi square = 39.6 with 1 degree of freedom			
	P = 0.00001			

variance (r^2) of each variable was calculated with all the other variables entered into the equation. The dependent variable used was the number of Alternaria spp. conidia caught per hour by the Burkard spore trap. The independent variables used were air temperature (C), wind speed (km/hr), solar radiation intensity (gm cal/cm^2/min), and percent relative humidity. The results of the regression analyses for the seven episodes of spore release are shown in Table 12. The same analyses were performed on data from the entire 1978 and 1979 seasons, starting from the date when more than 50 conidia were trapped in one hour and continuing until spore trapping was discontinued. The results of the multiple regression analyses of yearly data are shown in Table 13.

In six of the seven episodes analyzed, the percent relative humidity contributed the greatest amount toward the variance explained by the regression. For the duration of the episode of 7/29-8/01 1978 the wind speed was very high, nearly twice the seasonal average shown in Table 13 and in this case it contributed the greatest amount to the variance explained by the four environmental parameters.

In the analyses of the seven episodes summarized in Table 12, each of the parameters contributed substantially toward the explained variability on several occasions. A ranking of the variables in order of decreasing contributions to the explained variance was impossible, however, since each variable at times contributed substantially, but at other times only made negligible contributions.

TABLE 12.--Results of regression of hourly spore catches on four environmental parameters for seven episodes of spore release of Alternaria spp. at Grand Junction, MI in 1978 and 1979.

Date of Episode		Air Temperature (°C)	Relative Humidity (%)	Wind Speed (Km/hr)	Solar Radiation (gm-cal/cm^2/min)	Spore Catches (conidia/hr)
7/24 - 7/26 1978	Change in r^2[a]	.10773	.24000	.08250	.02163	
	r^2[b]	.45186				
	Mean[c]	22.3	83.4	4.8	0.368	180
7/29 - 8/1 1978	Change in r^2	.19373	.00002	.41259	.03391	
	r^2	.64024				
	Mean	19.7	80.2	7.2	0.389	56
8/30 - 9/3 1978	Change in r^2	.09767	.42567	---[d]	.04294	
	r^2	.56628				
	Mean	19.3	80.3		0.1186	83
9/14 - 9/16 1978	Change in r^2	.05920	.21229	---	.00096	
	r^2	.27245				
	Mean	19.4	84.5	---	0.1998	201
7/11 - 7/14 1979	Change in r^2	.00002	.30075	.09571	.02772	
	r^2	.42369				
	Mean	23.7	87.5	1.29	0.2849	56
7/27 - 7/29 1979	Change in r^2	.02704	.30709	.19552	.13141	
	r^2	.66106				
	Mean	21.4	74.8	1.41	0.3742	51
8/2 - 8/4 1979	Change in r^2	.02067	.43626	.11067	.22389	
	r^2	.80148				
	Mean	20.5	84.3	1.68	0.3267	58

[a]Portion of total observed variation attributable to parameter
[b]Total portion of variation explained by parameters in the regression equation.
[c]Mean values of parameters for duration of episode.
[d]Missing data.

TABLE 13.-- Results of regression of hourly spore catches of Alternaria spp. on four environmental parameters for two years data from a commercial blueberry planting at Grand Junction, MI.

Dates of Data Collection		Air Temperature (°C)	Relative Humidity (%)	Wind Speed (km/h4)	Solar Radiation (gm-cal/cm^2/min)	Spore Catches (conidia/hr)
7/5 - 9/17 1978	Change in r^2[a]	.11621	.07516	.01669	.01048	
	r^2[b]	.21854				
	Mean[c]	20.9	80.1	4.0	0.3445	53
6/27 - 8/28 1979	Change in r^2	.07668	.07968	.00314	.05982	
	r^2	.21932				
	Mean	20.2	82.8	1.95	0.2887	25

[a]Portion of total observed variation attributable to parameter.

[b]Total portion of variation explained by parameters in the regression equation.

[c]Mean values of parameters for duration of data collection.

In the regression analysis of seasonal data summarized in Table 13, air temperature and relative humidity made the greatest contributions toward the explained variance in 1978. In 1979, however, solar radiation made a contribution nearly equal to that of air temperature and relative humidity.

The low values of r^2 obtained in the seasonal regression analysis summarized in Table 13 as compared to the relatively high values of r^2 found in Table 12 show the importance of applying regression analysis to individual episodes of spore release.

Parameters not included in the regression analysis probably contributed to the unexplained variance. For example, wet leaves were shown by the chi square analysis to be associated with low levels of spore catches, and dry leaves with high levels of spore catches. This parameter was not included in the regression analysis, however, since it was not measured as a continuous variable. Rainfall only rarely coincided with spore release, and so was not included in the analysis. No attempt was made to measure biological parameters associated with spore release such as the amount of Alternaria-bearing substrate in the field. Knowledge of this parameter might greatly increase the value of r^2.

The statistical tests made confirmed observations made by plotting spore catches and environmental parameters against time for three of the episodes. The results are shown in Figures 6, 7, and 8.

Isolation from Host Tissues to Determine the Overwintering Site of the Pathogen

Of the blighted fruiting wood twigs brought to the laboratory in November 1977, 19/35 (54%) yielded *Alternaria* spp. when tissue isolations were made onto PDA. Of the apparently healthy flower buds brought to the laboratory at the same time, only 1/129 (0.7%) yielded *Alternaria* spp. when tissue isolations were made onto PDA. These results suggest that *Alternaria* may overwinter in blighted fruiting wood, but not flower buds.

In the spring of 1978, similar blighted wood was brought to the laboratory, and 5/36 (14%) of the blighted twigs yielded *Alternaria* spp. when tissue isolations were made after surface disinfestation. When tissue isolations were made without surface disinfestation, 19/32 (59%) of the twigs yielded *Alternaria* spp., indicating that *Alternaria* spp. had overwintered and were located predominantly at or near the surface of the blighted wood. The *Alternaria* isolates found in wood at this time of year were not identical to the *A. alternata* isolate from fruit in the same field, but they were members of the "*Alternaria alternata* group" (30) members of which have been reported to cause decays of blueberry fruit (10, 18). The virulence of these wood isolates was not determined. However, it is likely that they could cause a decay of blueberry fruit.

Other fungi isolated from the stems included *Trichoderma* spp., *Penicillium* spp. and *Colletotrichum gloeosporioides*.

DISCUSSION

Postharvest Decays Caused by Alternaria alternata

The detrimental effects of A. alternata on blueberries were shown to be limited to the harvest season. Only relatively insignificant numbers of A. alternata conidia were trapped from air in a commercial blueberry field before the first blue fruit stage of blueberry bush development in both 1978 and 1979. Inoculations of mature blueberry bushes with A. alternata throughout both growing seasons from the bud swell stage through the ripe fruit stage failed to induce Alternaria postharvest decays above natural uninoculated background levels in both seasons. The fact that the inoculations did induce a transient spotting of succulent leaves in both seasons implies that the virulence of the isolates used had not been attenuated in culture. It seems likely that the increase in spore catches reflect sporulating decays of damaged fruit which were infected by Alternaria spp. and immediately decayed. However, it is possible that infection does occur early in the season and that the great increase in spores trapped late in the season is due to sporulation on fruits infected early in the season.

The relationships between A. alternata spore release and meteorlogical parameters monitored in this study are consistent with those observed by others for related species (15, 16, 27, 32) and for A. alternata (26). Spore release was positively correlated

with air temperature, solar radiation intensity, and wind speed and negatively correlated with relative humidity. Large spore catches were very strongly associated with dry leaves, and low spore catches with wet leaves. These patterns are consistent with the hypothesis that A. alternata conidia are violently discharged into air in response to changes in relative dumidity and light intensity as has been demonstrated for A. tenuis (17).

Although the general pattern of Alternaria spore release had been previously established, the strength of the correlations between the meteorological factors and spore release had not been determined. Multiple regression analysis of the spore trapping and environmental data of this study revealed that relative humidity was the factor which had the greatest influence on the magnitude of spore release. Wind speed, solar radiation intensity, and air temperature also contributed substantially toward explaining the observed magnitude of spore release in several of the episodes studied. The variance unexplained by the regression analyses was probably due to the contributions of variables not entered into the regression analyses, such as leaf wetness duration preceding spore liberation and the amount of spore bearing substrate in the field.

Since A. alternata causes only a postharvest decay problem and is not very active in the field prior to the blue fruit stage of fruit maturity, application of fungicides early in the season to control Alternaria decays postharvest would likely be of little value. Application of fungicide only late in the season would have a good chance of controlling Alternaria decays. Difolatan was

clearly the best of the fungicides tested for controlling Alternaria decays in 1979, when a single massive application of Difolatan (28.2 l/ha) at the green tip growth stage of the blueberry bush in 1979 controlled Alternaria decays after harvest. It is likely that a single application of Difolatan (captafol) made 21 days preharvest (the maximum legal interval) would control Alternaria postharvest decays.

The cause of the observed association between mechanical harvesting and increased Alternaria decay incidence (10) is in part due to the greater damage occurring to fruits when harvested by machine as compared to hand. This damage increases Alternaria decays (Appendix, p.). Growers using mechanical harvesting equipment often try to harvest the crop in as few passes over the bushes as possible. Because of this the fruits tend to become soft and over-ripe which can predispose them to decays by Alternaria spp. which are generally considered to be non-aggressive pathogens (10).

SECTION II

EPIDEMIOLOGY AND CHEMICAL CONTROL OF POSTHARVEST DECAYS OF BLUEBERRY FRUIT CAUSED BY *COLLETOTRICHUM GLOEOSPORIOIDES*

INTRODUCTION

The Genera Gloeosporium Desm. and Mont. and Colletotrichum (Penz.) Sacc.

Many diseases of fruits, vegetables, and ornamentals share a similar etiology and symptomatology, and are commonly known as "anthracnoses." Typical symptoms include fruit or vegetable rot, and blossom or foliage blight (22). When anthracnose decays of blueberries were first studied and reported, the workers reported the causal fungus to be Gloeosporium (8, 10, 12, 33, 34), the imperfect form of Glomerella cingulata (Stonem.) Spaulding and Schrenk.

The genus Gloeosporium was erected by Desmaziers and Montagne in 1849 with G. castagnei Desm. and Mont. as the type species. According to von Arx (2), Saccardo used the genus Gloeosporium to include Melanconiaceous fungi growing on leaves with continuous, hyaline conidia. This treatment was inconsistent with the type specimen (2). Gloeosporium was separated from Colletotrichum by Saccardo because Gloeosporium had glabrous acervuli whereas Colletotrichum had setose acervuli (2).

Glomerella cingulata was thoroughly reviewed by von Arx in order to resolve taxonomic confusion arising from a multiplicity of synonyms for both the perfect and imperfect stages of the fungus (2). Von Arx dismembered the genus Gloeosporium entirely because it was too heterogeneous and reassigned the species to several genera, one

of which was Colletotrichum. In the present study the nomenclature of von Arx (2) has been followed, and the fungus causing anthracnose decays of blueberries is called Colletotrichum gloeosporioides (Penz.) Sacc. in place of Gloeosporium fructigenum.

Acervuli of C. gloeosporioides are found on necrotic regions of clearly defined lesions, vary from setose to glabrous, are irregular in shape, and can be as large as 500 micrometers in diameter. The acervular spore mass is salmon colored (22). Conidia are cylindrical with obtuse ends, hyaline, aseptate, uninucleate, and range in size from 9 to 24 micrometers long by 3 to 6 micrometers wide. They are borne on unicellular, hyaline or faintly brown phialidic conidiophores. Conidia are dispersed by splashing water (22).

On PDA, colonies of C. gloeosporioides are grayish-white to dark gray with aerial mycelium varying from sparse to uniform and mat-like. The reverse side of the colony is unevenly white to gray (22).

A closely related species, C. acutatum Simmonds, differs from C. gloeosporioides in colony color which is orange to pink and in its fusiform conidia which are slightly smaller, especially in width, than conidia of C. gloeosporioides. Perithecia have not been observed for this taxon. It has been reported only once in North America (13).

In the review of the literature on post harvest decays of blueberries caused by C. gloeosporioides which follows, C. gloeosporioides will be referred to as Gloeosporium sp. as it was originally reported.

History and Occurrence of Gloeosporium Decays of Blueberry

In spite of the commonly held belief that the most important fungus involved with blueberry (Vaccinium corymbosum L.) decays was Botrytis cinerea Pers. ex Fr., the incidence of decays caused by a Gloeosporium sp. was five times greater than that of B. cinerea in New Jersey studies conducted from 1970 to 1972 (12). Decays caused by this Gloeosporium were greatly favored by extremely wet weather in 1972. As would be expected, decay incidence increased from the field to storage and to retail outlets (12).

In another New Jersey study carried out in 1971 the frequency of Gloeosporium fructigenum rots was greater than the frequencies of either Botrytis cinerea rots or Alternaria tenuis rots for the season as a whole (4.0, 3.0 and 2.4%, respectively). Significantly, the ratio of Gloeosporium decays to Alternaria decays and Gloeosporium decays to Botrytis decays shifted from approximately 2:1 in early season to 6:1 in mid-season to 45:1 in late season. This trend was consistent with field observations that Gloeosporium decays became more common as the season progressed (8).

Glomerella cingulata was shown to cause both a blossom blight and a postharvest decay of cranberry (Vaccinium macrocarpon L.) fruit in New Jersey (35). Blossom blight of blueberry and latent fruit infection were reported from New Jersey but no data were presented (34).

Regular application of fungicides to control Gloeosporium decays had not been practiced in New Jersey because the disease had

been considered to be of minor importance. However, in 1970 Gloeosporium spp. caused the decay of five percent of the blueberries in storage and marketing channels (12). Field fungicidal spray programs were initiated, on the assumption that control of Gloeosporium in the field would diminish its destructive potential in the marketplace (10). A spray program using captan was preferred. A ferbam-captan experimental spray program was ineffective as were post harvest dips with benomyl (10). The ineffectiveness of the benomyl dips is interesting because benomyl is generally effective against Gloeosporium spp. (25). Very promising results were obtained using captafol (Difolatan) in field tests (10).

In one study, very soft, leaky berries accounted for 31.5% of all decayed fruit, but no visible sign of a pathogen was present. Isolation onto PDA revealed Gloeosporium in 20%, Alternaria in 22%, and Botrytis in 35% of these fruits (10).

Milholland has described disorders of blueberry bushes caused by Gloeosporium minus Shear in North Carolina (19, 21). Leaf lesions resulted from inoculation of succulent blueberry leaves (cv. Croatan) with conidial suspensions of 10^6/ml. Spore germination and appressorial production on inoculated leaves was increased 2.5-fold when the inoculum was suspended in 50% glucose as compared to distilled water. This may account for the presence of large G. minus lesions at blueberry hydathodes because of the sugars present in solution there (19).

The leaf lesion isolate was shown to be identical mycologically and equal in pathogenicity to isolates causing severe cankering and dieback on blueberry stems (21). Cankers were observed to begin at the leaf scar or flower buds. Acervuli were produced over the entire canker. It was not clear whether the fungus penetrated the leaf scar directly or via attached infected petioles. Gloeosporium minus hyphae grew intracellularly in the cortex, xylem and phloem of infected stems (21).

MATERIALS AND METHODS

Colletotrichum gloeosporioides Isolate Used in This Study

A single spore isolate of the causal organism of anthracnose decay was made from rotting blueberry fruit and used for all further studies. On rotting fruit the fungus was macroscopically visible only as salmon colored spore masses (Figure 1). Similar salmon colored spore masses were found on blighted blossom clusters (Figures 2 and 3). Hyaline setae were microscopically visible in the acervuli on rotting blueberry fruit (14).

On PDA the colonies produced numerous acervuli with salmon colored spore masses. The mycelium was generally dense. Colony morphology and sporulation was apparently unaffected by constant soft fluorescent lighting or constant darkness. The fungus produced a large amount of a pink-purple pigment in culture on PDA, which was especially visible from the reverse side of the petri plates. No setae were observed in acervuli produced on PDA (14).

Conidia grown on PDA measured 18.0 μm long by 7.8 μm wide (mean of 50 conidia measured). They were cylindrical to slightly elliptical and hyaline with granular appearing inclusions (Figure 4).

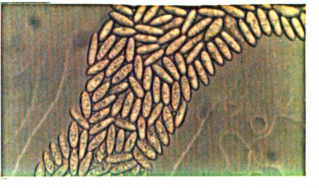
The mycelial characters were consistent with those described for *Colletotrichum gloeosporioides* (Penz.) Sacc. (22). The pink to

Figure 1.--Blighted blossom cluster and rotting fruit caused by inoculation of blueberry bush with 10^6 Colletotrichum gloeosporioides conidia per ml sterile distilled water at the pink bud growth stage.

Figure 2.--Colletotrichum gloeosporioides entering fruiting wood via blighted blossoms.

Figure 3.--Spore masses of Colletotrichum gloeosporioides on a blighted blossom after 48 hr in a moist chamber.

Figure 4.--Colletotrichum gloeosporioides conidia (x 400)



purple pigment produced on PDA has been reported for C. acutatum Simmonds ex Simmonds (13) previously, and for Glomerella cingulata from blueberry (33). Since conidial measurements clearly favored C. gloeosporioides rather than C. acutatum, the fungus was identified as C. gloeosporioides (Penz.) Sacc.

Effect of Temperature on Fungal Growth and Spore Germination in Free Water and in Air at 100% Relative Humidity

These experiments were carried out in exactly the same manner with the C. gloeosporioides isolate used as described in Section I with the Alternaria alternata isolate except that radial growth of the fungus was measured after 10 days instead of after 7 days. These experiments were performed to identify environmental conditions which in the field would lead to infection and decay of fruit by C. gloeosporioides.

Field Location for Colletotrichum gloeosporioides Studies and Monitoring of Environmental Parameters in the Field

The C. gloeosporioides studies were carried out in the same field simultaneously with the A. alternata studies described in Section I.

Trapping of Rain Dispersed Conidia of Colletotrichum gloeosporioides

In April 1978, ten water traps were placed in bushes which contained dead fruiting wood similar to the kind from which C. gloeosporioides had been isolated previously. A water trap consisted of a 15-cm (6 inch) plastic funnel anchored in the bush

with epoxy glue and connected by a length of Tygon tubing to a plastic 3.79 liter (1 gallon) bottle anchored in the ground. Rainwater washing over the branches above the funnel carried any spores present into the bottles. Approximately 25 ml of a preservative solution containing 50% ethanol, 5% glacial acetic acid, 10% formaldehyde, and 35% water was added to each bottle each week. Experiments showed that conidia of C. gloeosporioides failed to germinate in this solution when it was diluted as much as 15:1.

In June of 1978 three additional water traps were installed in bushes which earlier had been inoculated with C. gloeosporioides and which had blighted blossoms. These traps collected spores from the blighted blossoms and in one case from rotted fruit.

In 1979 eight bushes which had been inoculated in 1978 with C. gloeosporioides had water traps installed in them. The pathogen had been reisolated from six of these bushes. In order to further increase the consistency of spore capture, the traps were "baited" with diseased plant material in 1979. Blighted twigs, flower parts and rooted fruits were wrapped in cheesecloth and placed in equal amounts in the funnels as the season progressed.

The bottles were emptied weekly in 1978 and early in 1979 and then after individual rains, starting on June 27, 1979. The concentration of C. gloeosporioides conidia in each bottle was estimated by taking a subsample of water from the bottle after recording the total volume of rainwater in the trap. Approximately 35 ml of this subsample was filtered through two layers of cheesecloth and centrifuged at 7000xg in a refrigerated centrifuge

(International Equipment Co., Needham Heights, Massachusetts 02194). The pellet containing spores was resuspended in one-fourth the original volume and the spores in the suspension were counted using a hemacytometer. Three counts were made on each subsample and averaged. The total number of conidia collected and the concentration of conidia in the rainwater was then estimated.

In Vitro and Field Testing of Fungicides for Control of Postharvest Decays of Blueberries Caused by C. gloeosporioides

All in vitro and field testing of fungicides for control of C. gloeosporioides was done simultaneously with and by the same methodology described in Section I for A. alternata.

Inoculation Studies of Mature Blueberry Bushes in the Field and at East Lansing

The inoculation studies using one single spore isolate of C. gloeosporioides were done simultaneously with and by the same methodology described in Section 1 for A. alternata. The only difference was that the concentration of C. gloeosporioides conidia used for inoculation studies was 10^6/ml sterile distilled water. The inoculation dates and phenotypic growth stages are given in Tables 2, 3 and 4.

Isolations from Host Tissues to Determine the Overwintering Site of the Pathogen

These isolations were done as described in Section I for A.. alternata. In addition to the isolations described in Section I, in the fall of 1978 and spring of 1979 isolations were made from

blighted twigs which resulted from inoculations made during the 1978 season. Isolations were made onto half strength PDA (½PDA) after surface disinfesting the twigs by soaking them in 1% sodium hypochlorite plus Tween-80 for 10 minutes with stirring.

RESULTS

Effect of Temperature on Radial Growth of Colletotrichum gloeosporioides

The optimal temperature tested for radial mycelial growth of C. gloeosporioides was 20C. Growth was slightly slower at 27C but almost no growth occurred at 30C (Figure 5). At 30C the colony morphology was greatly distorted and the characteristic pink/purple pigment was lacking. The fungus grew twice as fast at 20C than at 25C and almost no growth was found after ten days at 5 and 10C. The results indicate that anthracnose decays are favored by temperatures of 15 to 27C, but that higher temperatures would adversely affect the pathogen.

The Effects of Free Water 100% Relative Humidity, and Various Temperatures on the Germination of Colletotrichum gloeosporioides Conidia

Germination of C. gloeosporioides conidia in free water was slow at all temperatures tested (Table 1). Conidia failed to germinate at 5C and 10C after 40 hours in water. The optimal temperature tested for percent conidial germination in free water was 30C. However, germ tube elongation was much slower at this temperature than at 20C, which was consistent with the mycelial growth abnormalities observed at higher temperatures.

Conidia failed to germinate at any temperature in air at 100% relative humidity. The results indicate that free water

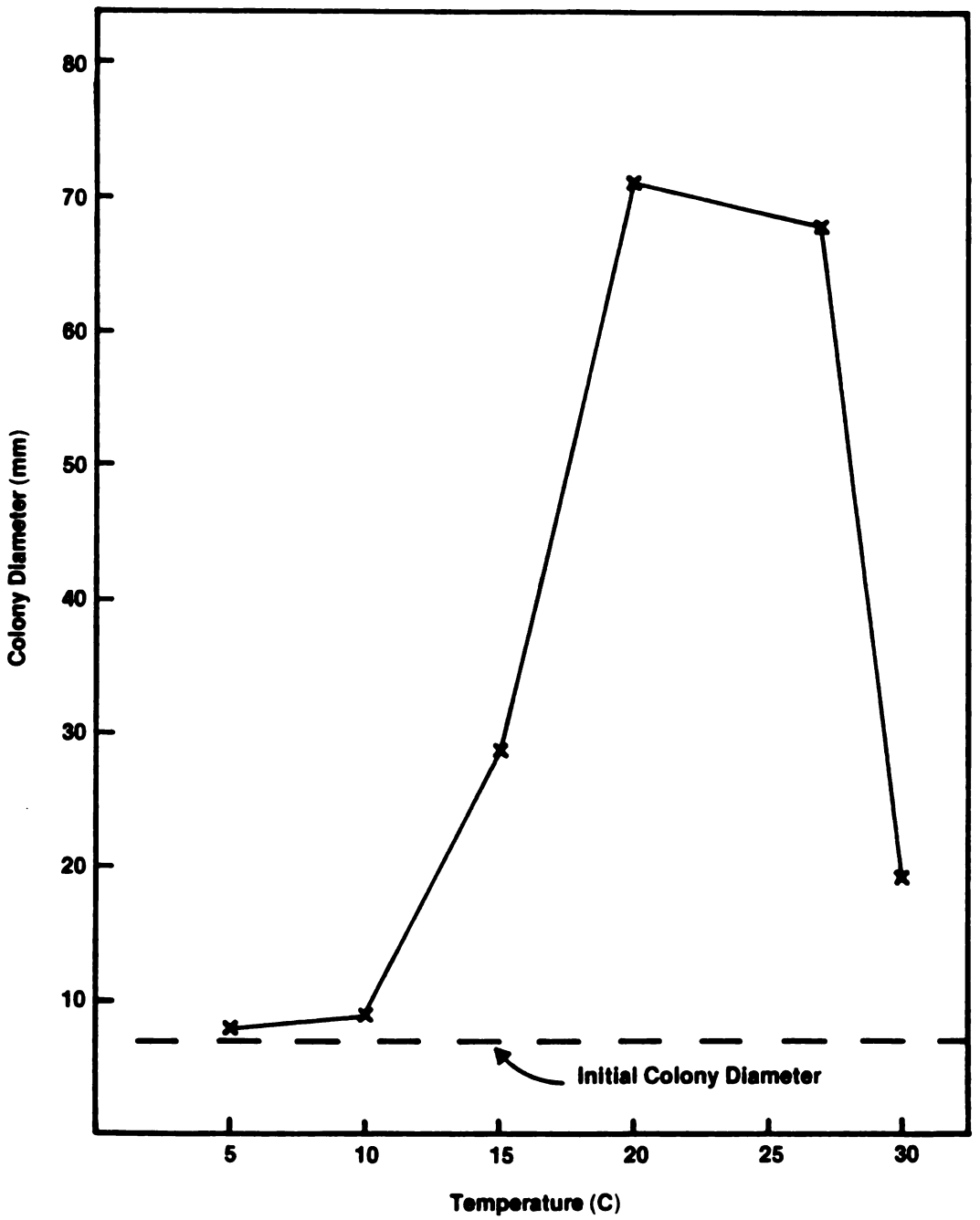


Figure 5.--Mycelial growth of Colletotrichum gloeosporioides on PDA at several temperatures after 10 days.

TABLE 1.--Conidial germination of Colletotrichum gloeosporioides on glass slides in glass distilled water at various temperatures.

Temperature (°C)	Time Interval									
	8 Hours		16 Hours		24 Hours		32 Hours		40 Hours	
	(%)[a]	(μm)[b]	(%)	(μm)	(%)	(μm)	(%)	(μm)	(%)	(μm)
5	0.0	---	0.0	---	0.0	---	0.0	---	0.0	---
10	0.0	---	0.0	---	0.0	---	0.0	---	0.0	---
15	1.3±1.0[c]	---	25±10	13± 7	22± 7	16± 9	37± 9	22±12	49± 3	39±36
20	0.0	---	33± 9	49±34	43±14	99±41	38± 8	110±42	46±13	99±68
30	16± 4	16± 9	50± 9	24±14	59± 9	32± 9	56±23	29±13	65±19	26±11

[a]Percent germination of first 100 conidia encountered in microscope field.

[b]Germ tube length in μm. Mean of 25 spores measured for germ tube length.

[c]Mean of four replicates ± standard deviation of the mean.

and temperatures of 15 to 25C may be required for about 12 or more hours in order for C. gloeosporioides to infect the fruit. Such conditions were provided after the inoculations which were successfully made in the present study.

Preliminary Screening of Fungicides for Activity Against Colletotrichum gloeosporioides

The compounds which showed the greatest activity against C. gloeosporioides were Funginex (triforine), Bayleton, captan, ferbam, and captafol (Difolatan). These compounds all caused 90% or greater inhibition of radial mycelial growth of C. gloeosporioides in this test at concentrations of 100 µg/ml (Figure 6). Bayleton and captafol (Difolatan) caused 80% or greater radial growth inhibition at a concentration of 10 µg/ml and benomyl was moderately effective against C. gloeosporioides at all concentrations tested in this experiment.

Fungicide Testing in the Field for Control of Colletotrichum gloeosporioides Decays of Blueberry Fruits

The incidence of C. gloeosporioides decay was too low in both 1978 and 1979 to give any meaningful data about fungicidal control of C. gloeosporioides decays. This was surprising since spore trapping data indicated that C. gloeosporioides conidia were being produced throughout both seasons in a portion of the same field in which the fungicides were tested. Decay incidence was determined by the same procedure which induced abundant sporulation of C. gloeosporioides on fruit harvested for evaluation in the

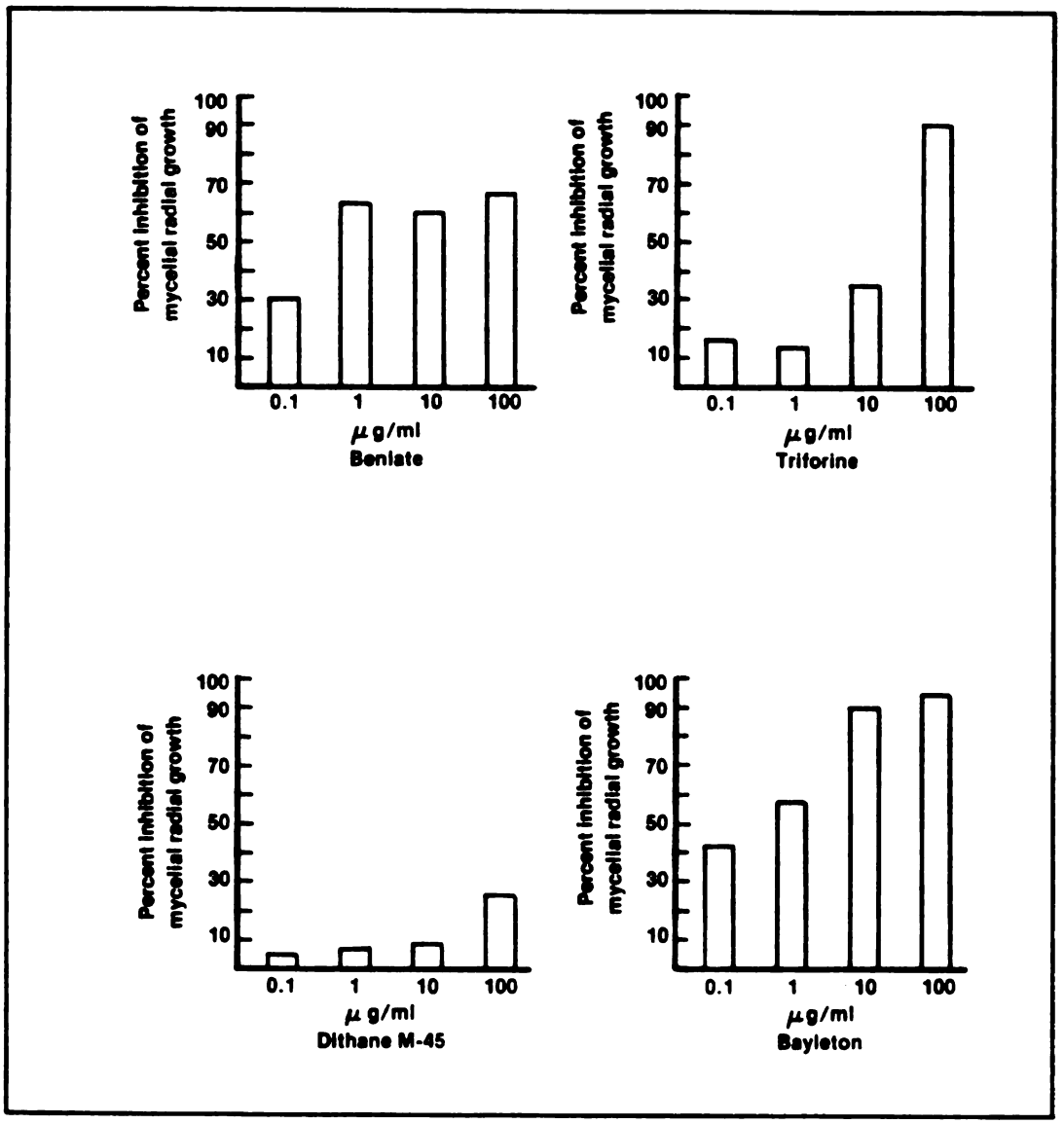


Figure 6.--Growth inhibition of Colletotrichum gloeosporioides mycelium at 96 hr by fungicides incorporated into PDA.

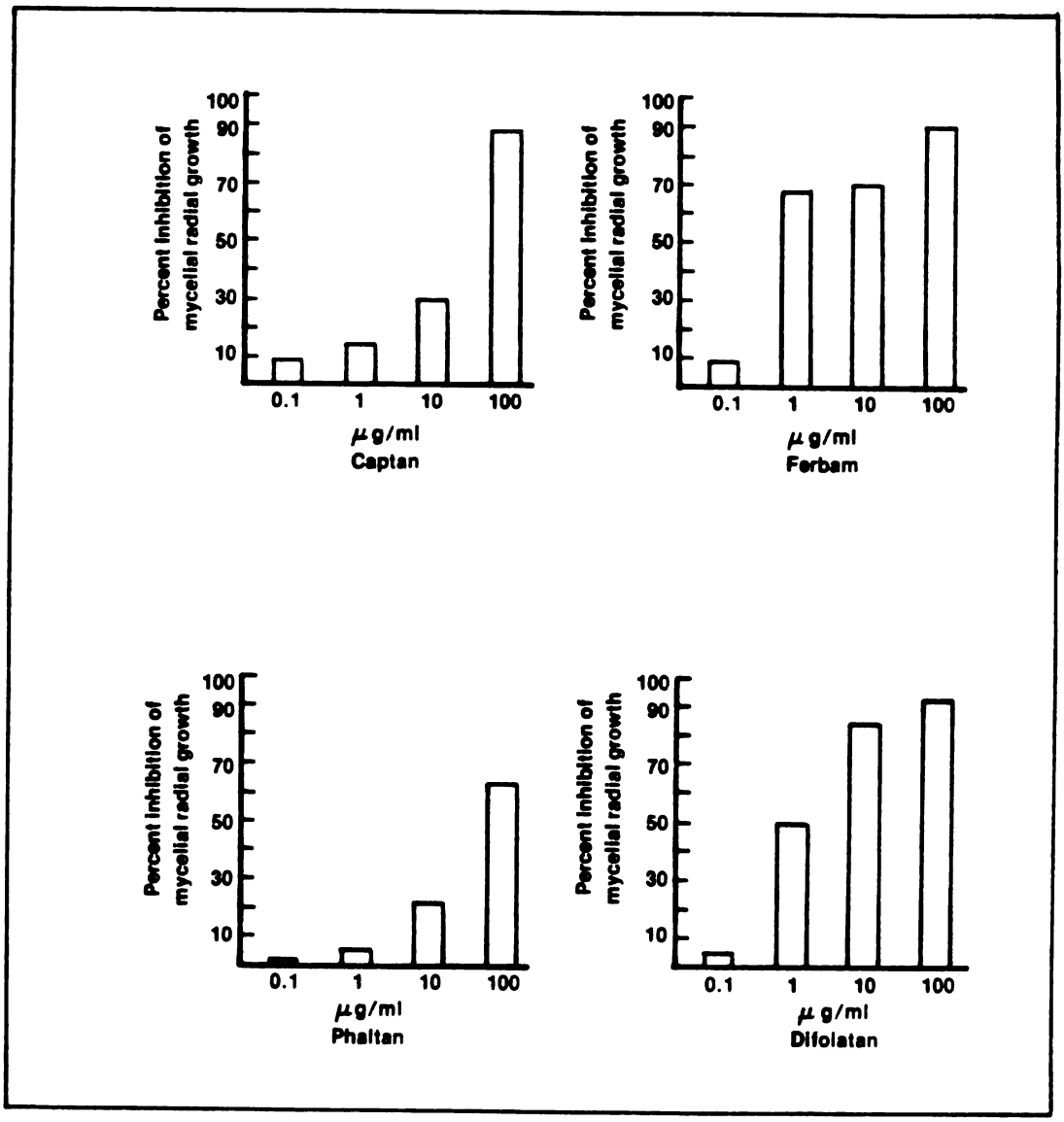


Figure 6.--Continued.

inoculation experiments (Tables 2, 3 and 4), so that this procedure was not responsible for the low observed decay incidence.

Workers in New Jersey found that the incidence of anthracnose decays increased greatly as the harvest season progressed (8). In both seasons of the present study, harvesting was limited to the early part of the harvest season. Perhaps if the harvesting of the fungicide treated plots had been extended into the late season a higher incidence of anthracnose decays would have been found.

Inoculation Studies in the Field and at East Lansing Using Colletotrichum gloeosporioides

The isolate of C. gloeosporioides used in this study was shown to cause a fruit decay after harvest in both seasons when inoculated during the growing season onto mature bushes in a commercial blueberry field (Tables 2 and 3). Fruit developed anthracnose decays postharvest when bushes were inoculated with the pathogen as early as the bud swell growth stage in both seasons. No differences in phenotypic growth stage susceptibility to C. gloeosporioides were apparent in either season. The data show that anthracnose decays after harvest can be the result of latent infections which are established during the growing season.

Colletotrichum gloeosporioides was also shown to cause a severe blossom blight when bushes were inoculated before or during the bloom period (Figure 2). This blossom blight can be distinguished from blossom blight caused by Monilinia vaccinii-corymbosi because C. gloeosporioides rapidly enters the fruiting wood (Figure 2)

TABLE 2.--Data from field inoculations of blueberry bushes with *Colletotrichum gloeosporioides* at Grand Junction, MI in 1978.[a,b]

Inoculation Date and Growth Stage	Mean Percent Fruit Rotted by *C. gloeosporioides*[c] (Inoculated)	Mean Percent Fruit Rotted by *C. gloeosporioides* (Control)
5/2/78 Bud Swell	16.6 ± 6.8	0.0
5/16/78 Bud Break	13.4 ± 9.1	0.2 ± 0.16
5/23/78 Pink Bud	33.4 ± 9.3	0.0
5/30/78 Full Bloom	5.5 ± 2.5	3.0 ± 2.0
6/6/78 Early Petal Fall	7.7 ± 6.4	0.0
6/13/78 Early Green Fruit	35.3 ± 16.2	0.0
6/22/78 Green Fruit	10.7 ± 2.2	0.5 ± .57
7/16/78 Early Blue Fruit	7.5 ± 4.2	0.0

[a]Bushes were inoculated on the dates given with a spore suspension of $1x10^6$ conidia/ml sterile distilled water or with sterile distilled water only.

[b]Fruit was hand harvested on 8/1/78 and held for 4 days at room temperature in moist chambers.

[c]Percent of fruit with visibly sporulating *C. gloeosporioides* on a count basis. Numbers given are the means of four replicates ± the standard deviations of the means.

TABLE 3.--Data from field inoculations of blueberry bushes with *Colletotrichum gloeosporioides* at Grand Junction, MI in 1979.[a,b]

Inoculation Date and Growth Stage	Mean Percent Fruit Rotted by *C. gloeosporioides*[c] (Inoculated)	Mean Percent Fruit Rotted by *C. gloeosporioides* (Control)
5/2/79 Bud Swell	31.0 ± 12.0	0.0
5/9/79 Bud Break	37.5 ± 9.2	---[d]
5/16/79 Pink Bud	1.5 ± 0.4	8.5 ± 5.2
5/23/79 50% Bloom	6.8 ± 1.9	14.5 ± 8.6
6/13/79 Early Green Fruit	18.8 ± 5.9	0.0
7/5/79 Late Green Fruit	29.0 ± 7.2	0.0
7/18/79 First Blue Fruit	12.6 ± 6.5	1.3 ± 0.6
8/1/79 First Ripe Fruit	23.0 ± 8.5	0.5 ± 0.2

[a]Bushes were inoculated on the dates given with a spore suspension of 1×10^6 conidia/ml sterile distilled water or with sterile distilled water only.

[b]Fruit was hand harvested on 8/15/79 and held for 4 days at room temperature in moist chambers.

[c]Percent of fruit with visibly sporulating *C. gloeosporioides* on a count basis. Numbers given are the means of four replicates ± the standard deviations of the means.

[d]Missing data.

TABLE 4.--Data from field inoculations of blueberry bushes with *Colletotrichum gloeosporioides* at East Lansing, MI, in 1979.[a,b]

Inoculation Date and Growth Stage	Mean Percent Fruit Rotted by *C. gloeosporioides*[c] (Inoculated)	Mean Percent Fruit Rotted by *C. gloeosporioides* (Control)
4/28/79 Bud Swell	5.1 ± 2.9	0.0
5/10/79 Bud Break	2.8 ± 1.1	0.0
5/17/79 Early Pink Bud	0.0	0.0
5/24/79 Full Bloom	7.1 ± 1.1	1.7 ± 1.1
6/14/79 Green Fruit	21.3 ± 3.5	0.0
7/19/79 First Blue Fruit	11.3 ± 4.8	0.0

[a]Potted 3 or 4-year-old bushes were inoculated on the dates given with a spore suspension of 1×10^6 conidia/ml sterile distilled water or with sterile distilled water only.

[b]Fruit was hand harvested on 7/31/79 and held for 6 days at room temperature in moist chambers.

[c]Percent of fruits with visibly sporulating *C. gloeosporioides* on a count basis. Numbers given are the means of four replicates ± the standard deviations of the means.

and M. vaccinii-corymbosi does not. Monilinia vaccinnii-corymbosi also produces characteristic cream-colored spore masses on the blighted tissue in the field. Colletotrichum gloeosporioides produces characteristic salmon colored spore masses in the field, and particularly if incubated in a moist chamber for 48 hours (Figure 3). Koch's postulates were completed for both the blossom blight and the postharvest fruit decay phase of blueberry anthracnose disease when the fungus reisolated from diseased tissue was indistinguishable in culture from the isolate used to inoculate the bushes.

The data from the potted bush experiment at East Lansing are in complete agreement with the field studies even though the cultivar Berkley was used at East Lansing in 1979 (Table 4). The results of the 1978 potted bush experiment at East Lansing were unuseable due to problems with insect and avian pests.

Trapping of Rain Dispersed Conidia of Colletotrichum gloeosporioides

The trends of the spore trapping data of 1978 and 1979 are very consistent (Figures 7 and 8). However, because of differences in the experimental procedures used in 1978 and 1979 the data in Figures 7 and 8 are qualitatively but not quantitatively comparable. The proportion of water traps which yielded C. gloeosporioides in 1979 was greater than in 1978 due to the relocation and "baiting" of the traps in 1979. High concentrations of conidia were found in rainwater collected during the bud swell and bud break growth stages of the blueberry bushes. Because no new growth was present

FIGURE 7.--Weekly trapping of Colletotrichum gloeosporioides conidia in a commercial blueberry planting at Grand Junction, MI in 1978.

a. Solid bars represent the number of conidia/ml rainwater collected (top portion) and the total number of conidia collected (bottom portion) in all the ten water traps placed in blueberry bushes in the spring of 1978. Only those traps which yielded c. gloeosporioides conidia were included in the calculations.

b. Cross hatched bars represent the number of conidia/ml rainwater collected (top portion) and the total number of conidia collected (bottom portion) in the three water traps placed in blueberry bushes in July of 1978. The bushes were previously inoculated with C. gloeosporioides and were showing symptoms when the traps were placed in the bushes. Only those traps which yielded C. gloeosporioides conidia were included in the calculations.

c. Open bars represent the total volume of rainwater collected in those water traps which yielded C. gloeosporioides conidia.

d. The numerator shows the number of water traps which yielded C. gloeosporioides conidia. The denominator shows the number of water traps which were sampled for that time period.

e. The numerator shows the number of water traps which had been placed in inoculated and symptom-bearing bushes and which yielded conidia in the time period specified. The denominator shows the number of such traps sampled in the specified time period.

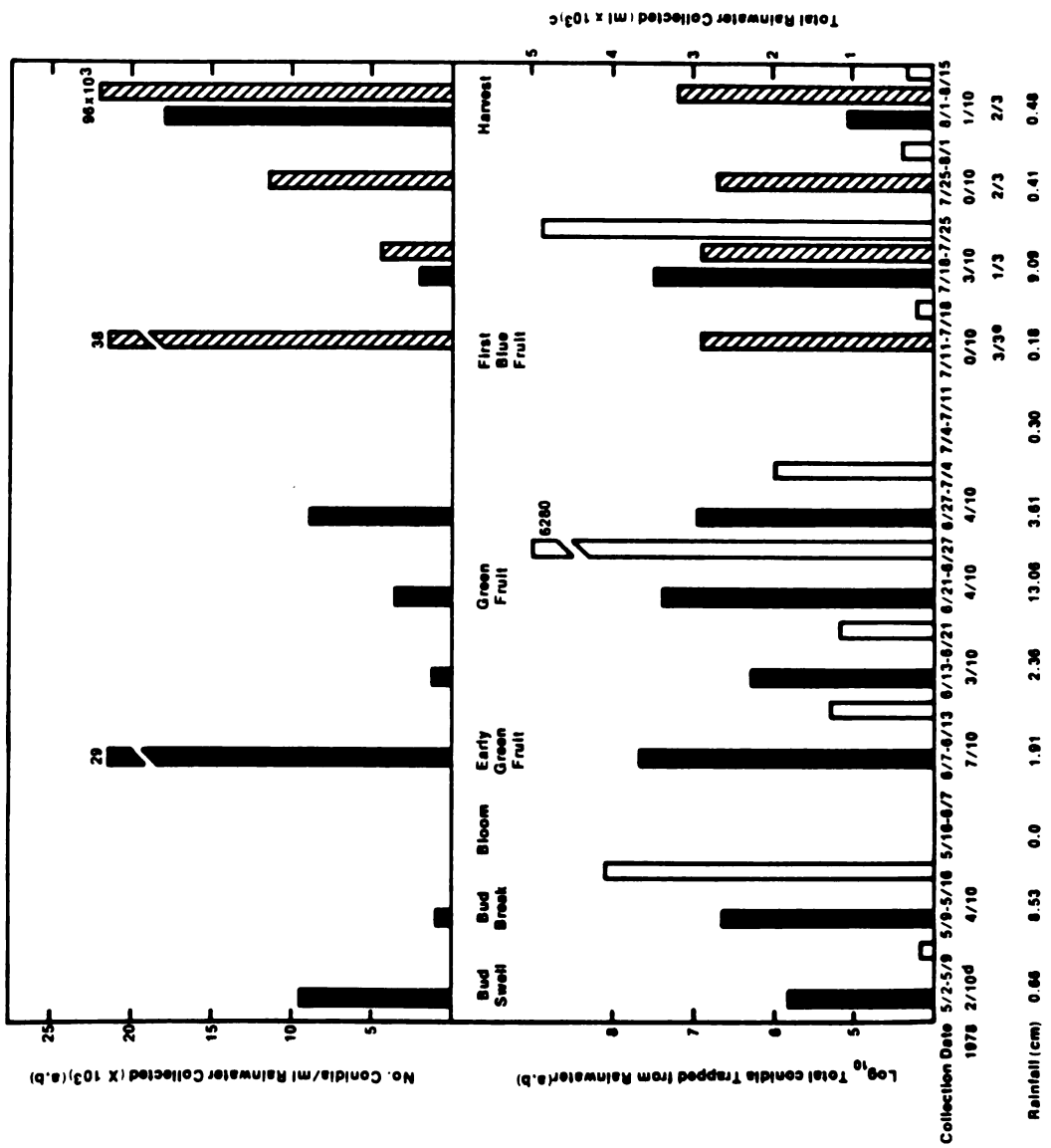
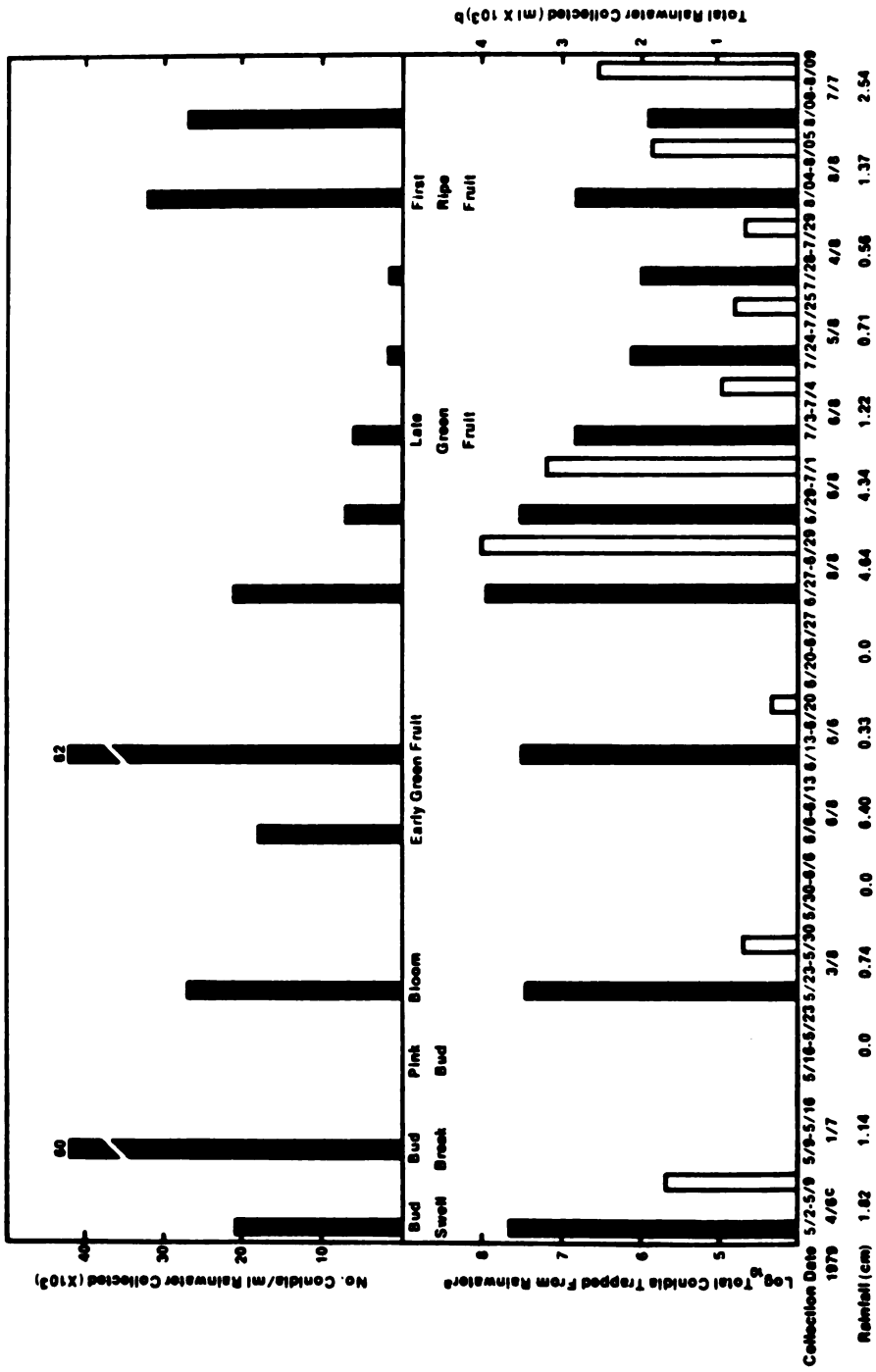

No. Conidia/ml Rainwater Collected (X 10³)(a,b)
Log10 Total conidia Trapped from Rainwater(a,b)
Total Rainwater Collected (ml x 10³)c
Bud Swell
Bud Break
Bloom
Early Green Fruit
Green Fruit
First Blue Fruit
Harvest
96±10³
38
29
6280
Collection Date
Rainfall (cm)
1978
5/2-5/9
5/9-5/16
5/16-6/7
6/7-6/13
6/13-6/21
6/21-6/27
6/27-7/4
7/4-7/11
7/11-7/18
7/18-7/25
7/25-8/1
8/1-8/15
2/10d
4/10
7/10
3/10
4/10
4/10
0/10
3/10
0/10
1/10
0.66
8.53
0.0
1.91
2.36
13.06
3.81
0.30
0.18
9.09
0.41
0.48
3/3e
1/3
2/3
2/3

FIGURE 8.--Weekly trapping of Colletotrichum gloeosporioides conida in a commercial blueberry planting at Grand Junction, MI in 1979.

a. Solid bars represent the number of conidia/ml rainwater collected (top portion) and the total number of conidia collected (bottom portion) in all of the eight water traps placed in blueberry bushes in the spring of 1979. All of the eight bushes which had water traps in 1979 has been inoculated with C. gloeosporioides in 1978 and the pathogen had been reisolated from six of them in the spring of 1979. Water traps were "baited" by placing diseased fruiting wood, blossoms, or fruit in the funnel mount as the season progressed. Only those water traps which yielded C. gloeosporioides conidia were included in the calculations.

b. Total rainwater collected in those water traps which yielded C. gloeosporioides conidia.

c. The numerator shows the number of water traps which yielded C. gloeosporioides conidia in the specified time period. The denominator shows the total number of traps sampled in the specified period.


No. Conidia/ml Rainwater Collected (X10³)
Log10 Total Conidia Trapped From Rainwater[a]
Total Rainwater Collected (ml x 10³)[b]
Bud Swell
Bud Break
Pink Bud
Bloom
Early Green Fruit
Late Green Fruit
First Ripe Fruit
82
50
Collection Date
1979
Rainfall (cm)
5/2-5/9 5/9-5/16 5/16-5/23 5/23-5/30 5/30-6/6 6/6-6/13 6/13-6/20 6/20-6/27 6/27-6/29 6/29-7/1 7/3-7/4 7/24-7/25 7/26-7/29 8/04-8/05 8/06-8/09
4/8c 1/7 3/8 6/8 6/6 8/8 6/8 6/8 6/8 6/8 5/8 4/8 8/8 7/7
1.82 1.14 0.0 0.74 0.0 0.40 0.33 0.0 4.64 4.34 1.22 0.71 0.56 1.37 2.54

on the bushes at this time, these conidia were being produced on blighted fruiting wood from which the pathogen was readily isolated. Large numbers of conidia were trapped during the bloom period in 1979 although there was no rain at all during this period in 1978 from which to trap spores. Particularly large numbers of conidia were trapped during the early green fruit stage in both seasons in nearly all of the traps in the field. Following the green fruit stage the number of conidia per ml of rainwater collected and the number of traps which collected C. gloeosporioides decreased in 1978 in the non-inoculated bushes. This phenomenon was observed to a lesser extent using the "baited" traps in 1979. Conidial production in inoculated symptom-bearing bushes was high as the fruit ripened in 1978. Large spore catches were made with the "baited" traps in 1979 at this stage. Conidial concentrations greater than 30,000/ml were observed during the blue fruit stages of both seasons.

Isolation from Host Tissues to Determine the Overwintering Site of the Pathogen

Among the blighted fruiting twigs from which tissue isolations were made in November 1977, only 1/35 (3%) yielded Colletotrichum gloeosporioides. Many more yielded Alternaria spp. None of the 129 healthy flower buds from which tissue transfers were made yielded C. gloeosporioides on PDA.

Among the blighted twigs from which tissue isolations were made after surface disinfestation in the spring of 1978, 3/36 (12%)

yielded C. gloeosporioides; only 1/32 of the non-surface disinfested twigs yielded C. gloeosporioides.

Of ten blighted twigs originating from C. gloeosporioides inoculations made in 1978, six yielded C. gloeosporioides in the spring of 1979 upon isolation onto PDA. This comparatively high rate of overwintering in these twigs implies that the pathogen overwinters in such wood. The lower rates of C. gloeosporioides isolated from other blighted twigs may imply that those twigs were killed by severe winter weather and not by C. gloeosporioides or that Alternaria spp. can replace C. gloeosporioides in some wood since such twigs yielded predominantly Alternaria spp. (Section I).

Other fungi isolated from blighted twigs in these experiments included Trichoderma spp. and Penicillium spp.

DISCUSSION

In contrast to Alternaria alternata which was shown in this study to be a problem for blueberry growers mainly at harvest time, Colletotrichum gloeosporioides was shown to be a problem for blueberry growers from the bud swell stage in the spring through the end of the harvest season. In both seasons large numbers of C. gloeosporioides conidia were trapped in rainwater runoff from bushes from the bud swell stage of the blueberry bush through harvest time. Inoculations of fruit buds, blossoms, and fruits with C. gloeosporioides caused high rates of anthracnose decay postharvest. Fruit which was apparently healthy rapidly developed anthracnose decay postharvest even if the inoculation with C. gloeosporioides had been made during the pre-bloom stages of the host.

The fact that C. gloeosporioides is both present and destructive to blueberries from the bud swell stage through the harvest season dictates that a control program should be begun at budbreak and continued through harvest. Because C. gloeosporioides overwinters in fruiting wood killed by the pathogen, such material should ideally be pruned out of the bushes and removed from the field.

The fungicide testing experiments for control of anthracnose decays carried out in 1978 and 1979 were disappointing because they revealed very low disease pressure at harvest time in 1978 and 1979.

The disease pressure was so low that no useful information about the relative efficacy of the various fungicides for control of anthracnose decays was gained.

The verylow postharvest anthracnose decay incidence was surprising since spore trapping data indicated that the pathogen was present and active in the field throughout the season. Anthracnose blossom blight was observed in the field in both seasons in non-inoculated bushes. The incubation method for the fruit in the fungicide trials was the same as that used in the inoculation trials, and so cannot be blamed for preventing detection of C. gloeosporioides infection.

The incidence of anthracnose decays of blueberries has been reported to increase dramatically late in the harvest season (12). Since in both seasons our fruit was harvested relatively early in the season at the growers' request, the period of maximum disease pressure was probably missed. Nonetheless, it is still curious that the observed decay incidence was so low when the pathogen was present and active in the field.

Colletotrichum gloeosporioides has been shown to cause latent infections on citrus, avocado, and papaya fruit (3, 4, 31). The results of this study clearly show a similar phenomenon with blueberry fruit since inoculation of blueberry bushes throughout the season resulted in apparently healthy fruit which developed anthracnose decays postharvest.

With citrus as a host, C. gloeosporioides developed appresoria on the surface of the fruit. Infection hyphae remained dormant in the cuticle and in the outer three or four cell layers of the flavedo. Growth of these dormant infection hyphae was very rapid in cv. Robinson tangerines in response to degreening with 50 ppm ethylene (4). The amount of anthracnose decay in inoculated cv. Robinson tangerines was also proportional to the amount of ethylene added to the incubation chambers (5).

The number of dormant appressoria and infection hyphae/mm^2 of fruit surface was also shown to be proportional to subsequent antracnose decay. If 99 or fewer appressoria/mm^2 of fruit surface were present, no anthracnose decay developed even in response to 50 ppm ethylene for five days (5).

The concept of latent infection, and the requirement of a certain minimum density of latent infections to cause disease can explain why C. gloeosporioides is more prevalent as a cause of decay late in the harvest season. Only late in the harvest season have the blueberry fruits acquired a large enough number of latent infections of C. gloeosporioides that ethylene produced as a part of the senescence process activates enough of the latent infections to cause anthracnose decays. Fruits harvested earlier in the season, as for example those used to evaluate fungicides in the course of this research, have not accumulated enough latent infections to develop postharvest anthracnose decays.

The fact that flower buds and blossoms inoculated with C. gloeosporioides before and during the bloom period produced apparently healthy fruit which rapidly decayed postharvest, may imply that these latent infections remained viable even after an entire growing season. It would be dangerous to assert, however, that the fruit rot observed postharvest on fruit obtained from bushes inoculated before or during bloom was entirely due to latent infections, all of which were established on the date of inoculation. Secondary spread of the pathogen from blighted blossoms to fruit was probably observed, as shown in Figure 4. However, inoculations made after petal fall consistently caused latent infections which resulted in decay postharvest without causing any other visible symptoms in the bushes. The latent infections were therefore viable for at least six to eight weeks, and quite likely for the full twelve weeks of the growing season.

The accumulation of viable latent infections throughout the course of the growing season is an interesting phenomenon which could lend itself to further studies. Knowledge of the precise requirements for fruit infection and the critical number of appressoria/mm^2 of blueberry fruit surface required for anthracnose decay development could lead to the development of mathematical models to guide fungicide applications. The use of fungicides with eradicant action for control of anthracnose decays is a very attractive possibility which deserves study. The nature of the interaction between the fruit, ethylene, and the latent infection

also deserves further study to determine if the ethylene acts directly on the dormant appressoria and hyphae to revive them, or if this response is mediated by other physiological changes in the host. The use of hot water dips after harvesting fruit may be effective in controlling decay caused by C. gloeosporioides (6, 7) because the high water temperature is capable of eradicating superficial, latent infections which otherwise develop into anthracnose decays.

APPENDIX

EXPERIMENTS TO EVALUATE THE ROLE OF MECHANICAL HARVESTING AND SORTING METHODS IN THE EPIDEMIOLOGY OF POSTHARVEST DECAYS OF BLUEBERRY FRUIT

MATERIALS AND METHODS

Purpose and Location of Harvesting Experiments

It has been observed that the recent increase in relative importance of Alternaria and Colletotrichum (Gloeosporium) post-harvest decays of blueberry fruits coincided with the mechanization of the blueberry harvest (10), but no attempt to document the effects of mechanical harvesting and sorting methods on blueberry decay has been reported. This was attempted in the present study. All harvesting experiments were carried out in the commercial field previously described during the first harvest of the field in each season.

The Effects of Harvesting Methods and Bruising on Postharvest Decay Incidence

An experiment was carried out in 1978 to determine if bruising of hand harvested fruit would cause a decay incidence pattern different from that of hand harvested fruit and comparable to that of mechanically harvested fruit. Some bushes in the field were expected to have a higher decay incidence than others, due to higher pathogen populations (particularly of C. gloeosporioides) in those bushes than in others, so the experiment was carried out with a factorial design of three harvesting treatments at each of five locations in the field with four replicates per treatment-location combination. This gave a total of sixty experimental units.

Five groups of three bushes each were marked with colored ribbon and eight cups of fruit were harvested by hand at each location. Four cups of fruit from each location received no further treatment; the other four cups were bruised by dropping them two times from a height of one meter into a plastic container lined with paper towels. Disposable plastic gloves were used to pick the fruit at each location and the paper towels lining the plastic container were changed after the fruit from each location had been bruised in order to avoid cross contaminating fruit from different locations in the field.

Immediately after the fruit was hand picked at each location, four cups of fruit were collected from the same bushes from an over-the-row mechanical blueberry harvester (Blueberry Equipment Co., South Haven, Michigan 49090). The three harvesting treatments were: mechanical harvesting only, hand harvesting only, and hand harvesting followed by bruising.

Each replicate of fruit weighing approximately 200 gm, was incubated for four days in ventilated plastic containers at room temperature. The replicates were then scored for the presence of visibly sporulating Alternaria, Colletotrichum, or other fungi. The data were recorded as the percent rotted fruit in each category on a fresh weight basis. The same experiment was repeated in 1979 except that four instead of five locations in the field were used in 1979 giving only 48 experimental units.

Mechanical vs. Hand Harvesting and Sorting Methods of Commercially Raised Fruit

An experiment was done in 1978 and repeated in 1979 in order to determine what effects, if any, hand vs. mechanical harvesting, hand vs. mechanical sorting, and the location of origin of the fruit in the field had on the incidence of postharvest decay. The treatments were arranged in a 2 by 2 by 4 factorial design, with four replicates per treatment combination, giving a total of 64 experimental units.

The experiment was carried out by marking with ribbons four groups of five bushes each from which the fruit was to be harvested. These were located at different places in the field and were considered as the location treatment. Eight one-half liter (1 pint) lots of fruit were harvested by hand at each location using disposable plastic gloves to avoid cross contamination of the replicates. Eight one-half liter (1 pint) lots of fruit were taken from the mechanical harvester at the same locations immediately after it harvested the bushes. Four of the hand harvested lots from each location, and four of the mechanically harvested lots from each location were sorted mechanically using a commercial pneumatic sorter (Blueberry Equipment Co., South Haven, Michigan 49090) and the remaining lots were sorted by hand. After receiving their various harvesting and sorting treatments, the fruits were incubated by replicates of about 200 gm in ventilated plastic containers for four days at room temperature. The fruits were then scored as healthy (firm marketable fruit), rotted by C. gloeosporioides,

rotted by Alternaria spp. or rotted by other fungi (visible sporulation), or as "leakers" (soft, watery, unusable fruit without visible sporulation). The results were recorded as the percent of each replicate in each category on a fresh weight basis.

Isolation of Pathogens from Blueberry Branches Broken by a Mechanical Harvester

The possible role of the commercial over the row mechanical blueberry harvester in providing overwintering sites for decay causing fungi was investigated. Immediately after the commercial blueberry field had been harvested in 1978, numerous twigs which had been broken by the harvester were tagged with ribbon so that they could be found at a later date. In November of 1978, three months later, 79 of these broken twigs were collected and tissue transfers were made onto ½PDA after surface disinfestation in 1% sodium hypochlorite plus Tween-80 for 10 minutes. At the same time, 54 apparently healthy, unbroken twigs were brought to the lab and tissue isolations were made from them in the same manner. In each case four isolations were made from each branch onto each plate. The percentage of branches of each type yielding Alternaria spp. of C. gloeosporioides was recorded.

RESULTS

The Effects of Harvesting Methods and Bruising on Decay Incidence Postharvest

The results of these experiments carried out in 1978 and 1979 are shown in Tables 1 and 2, respectively.

In 1978, the incidence of visibly sporulating C. gloeosporioides decays was significantly increased by both mechanical harvesting and bruising. Colletotrichum gloeosporioides decay incidence was also dependent on the location in the field from which the fruit originated in 1978. These effects were not reproduced in 1979.

In 1978 and 1979 the incidence of visibly sporulating Alternaria decays was significantly increased by bruising the fruit prior to incubation. Mechanical harvesting also increased Alternaria decays slightly in 1978, but decreased them in 1979. This was probably due to the high incidence of "leakers" (watery, unusable fruit whose decay was of unknown etiology) in 1979 which was not recorded for this experiment.

The total decay incidence (Alternaria + Colletotrichum) was increased in the 1978 season by bruising the fruit as compared to either hand harvesting only and mechanical harvesting only. In 1979 mechanical harvesting appeared to reduce total decay incidence. This observation is very misleading, however, since the number of "leakers" was not recorded for this experiment.

TABLE 1.--Effect of harvesting method and bruising on blueberry fruit rot at Grand Junction, MI in 1978.[a,b]

	Mean % *Colletotrichum*[c]	Level of Significance of F Value	Mean % *Alternaria*[d]	Level of Significance of F Value	Rotted Fruit Mean % Total	Level of Significance of F Value
Treatment						
Hand-harvested	0.67	0.018	8.2	0.001	8.82	0.001
Mechanically-harvested	2.5		9.2		10.8	
Hand harvested and bruised[e]	2.3		14.5		16.7	
Location						
1	2.8	0.033	10.2	0.001	13.0	0.001
2	0.18		7.3		7.3	
3	0.94		13.4		14.3	
4	1.7		8.2		9.9	
5	3.4		14.1		15.9	

[a]Factorial design: Treatment by location in the field where fruit originated, with four replicates per treatment-location combination.

[b]Fruit was held for 4 days in ventilated plastic containers at room temperature after treatment. Each replicate weighed about 200 gm.

[c,d]Percent of rotten fruit with visibly sporulating *Colletotrichum gloeosporioides* or *Alternaria* spp. Percent rot was calculated on a fresh weight basis.

[e]Hand-harvested fruit was dropped two times from 1 meter height into a plastic pan in order to bruise fruit.

TABLE 2.--Effect of harvesting method and bruising on blueberry fruit rot at Grand Junction, MI in 1979.[a]

	Mean % *Colletotrichum*[b]	Level of Significance of F Value	Mean % *Alternaria*[c]	Level of Significance of F Value	Rotted Fruit Mean % Total	Level of Significance of F Value
Treatment						
Hand-harvested	5.8	0.803	10.5	0.001	17.2	0.002
Mechanically-harvested	3.8		3.9		8.1	
Hand harvested and bruised[d]	5.5		13.4		19.8	
Location						
1	6.6	0.264	9.6	0.008	16.5	0.127
2	6.2		12.1		19.0	
3	0.5		9.7		10.5	
4	6.8		5.8		14.1	

[a]Treatments arranged in a factorial design: Treatment by location in field where fruit originated, with four replicates per treatment-location combination. Fruit was held for 4 days in ventilated plastic containers at room temperature after treatment. Each replicate weighed about 200 gms.

[b,c]Percent of rotten fruit with visibly sporulating *Colletotrichum gloeosporioides* or *Alternaria* spp. Percent rot was calculated on a fresh weight basis.

[d]Hand-harvested fruit was dropped two times from 1 meter height into a plastic pan in order to bruise fruit.

Mechanical vs. Hand Harvesting and Sorting Methods of Commercially Raised Fruit

The results of these experiments carried out in 1978 and 1979 are shown in Tables 3 and 4, respectively.

The incidence of Alternaria decay was not significantly effected by harvesting method, sorting method, or location in the field from which the fruit originated in 1978. The results were similar in 1979 except that the incidence of Alternaria decays was actually reduced by mechanical as compared to hand harvesting of the fruit. These results are misleading because they do not take into account the incidence of "leakers" which was not recorded in 1978 but which was probably very high.

The incidence of C. gloeosporioides decays was significantly increased by mechanical as compared to hand harvesting and was dependent on the location in the field from which the fruit originated in 1978. These results were not reproduced in 1979. In both seasons, however, hand vs. pneumatic sorting of blueberry fruit had no influence on subsequent decay incidence.

The incidence of "leakers" was very high in 1979, and comprised 75% of all unusable fruit in the experiment in which it was measured. The incidence of "leakers" was significantly increased by both mechanical harvesting and sorting as compared to hand methods, and was also dependent on the location of origin of the fruit in the field.

Because "leaker" incidence was not recorded in 1978, the percent total decay in 1978 is not directly comparable to the

TABLE 3.--Effect of harvest and sorting methods on blueberry fruit rots at Grand Junction, MI, in 1978.[a,b]

	Mean % *Alternaria*[c]	Level of Significance of F Value	Mean % *Colletotrichum*[d]	Level of Significance of F Value	Rotten Fruit Mean % Total	Level of Significance of F Value
Harvesting Method						
Hand	8.3	0.341	2.39	0.001	10.7	0.001
Mechanical	7.3		9.3		16.5	
Sorting Method						
Hand	8.0	0.695	5.4	0.469	13.4	0.784
Pneumatic	7.5		6.2		13.8	
Location						
1	7.5	0.182	1.1	0.001	8.5	0.001
2	6.8		3.4		10.1	
3	9.1		12.9		22.0	

[a]Factorial design: Harvesting method x Sorting method x Location in field where fruit originated with four replicates per treatment combination.

[b]Fruit was harvested, sorted and held in ventilated plastic containers at room temperature for four days. Each replicate weighed about 200 gms.

[c,d]Percent of rotten fruit with visibly sporulation *Colletotrichum gloeosporioides* or *Alternaria* spp. Mean percent rot was calculated on a fresh weight basis.

TABLE 4.--Effect of harvest and sorting methods on blueberry fruit rots at Grand Junction, MI, in 1979.[a,b]

	Mean % *Alternaria*[c]	Level of Signif. of F Value	Mean % *Colletotrichum*[d]	Level of Signif. of F Value	Mean "Leakers"[e]	Level of Signif. of F Value	Rotted Fruit Mean % Total	Level of Signif. of F Value
Harvesting Method								
Hand	7.3	0.001	5.7	0.197	12.1	0.001	24.7	0.001
Mechanical	2.1		3.1		33.0		38.2	
Sorting Method								
Hand	5.1	0.446	4.1	0.824	17.8	0.001	26.6	0.001
Pneumatic	4.0		4.9		28.9		37.4	
Location								
1	4.0	0.775	4.1	0.410	15.0	0.001	23.1	0.001
2	5.7		7.0		28.9		41.3	
3	4.3		3.4		24.2		31.8	
4	4.3		2.4		24.9		30.6	

[a]Factorial design: Harvesting method x Sorting Method x Location in field where fruit originated with four replicates per treatment combination.

[b]Fruit harvested, sorted, and held for four days in ventilated plastic containers. Each replicate weighed about 200 gms.

[c,d]Rotten fruit with visible, sporulating *Colletotrichum gloeosporioides* or *Alternaria* spp. Percent rot on a fresh weight basis.

[e]Soft, water, unusable fruit of unknown etiology. Percent rot was calculated on a fresh weight basis.

[f]Total of all unusable fruit.

percent total decay in 1979. In both years, however, total decay incidence was increased by mechanical harvesting and was dependent on the location in the field from which the fruit originated. The sorting methods used did not significantly affect Alternaria or Colletotrichum decay incidence in either year.

Isolation of Pathogens from Blueberry Twigs Broken by a Mechanical Harvester

Three months after being broken by the mechanical blueberry harvester 34/79 (43%) of the blueberry twigs yielded coolonies of Alternaria spp. after tissue isolations were made onto PDA as compared to 12/54 (22%) from unbroken twigs. Usually all four of the individual tissue isolations made from a broken twig on to PDA yielded Alternaria spp. if one of them did, while often only one of the tissue transfers from unbroken twigs yielded Alternaria spp. on PDA. The pathogenicity of these Alternaria spp. isolates from wood was not determined. However, it was determined that the isolates were not the same Alternaria alternata which was isolated from decayed blueberry fruit, although the wood isolates were members of the "Alternaria alternata group" (30). Other members of this group have been reported to cause decays of blueberry fruit, so it is possible that these isolates could cause decay as well (10, 18). It is clear that Alternaria spp. invaded twigs broken by the harvester, and damage to bushes caused by the mechanical harvester may provide additional overwintering sites for Alternaria spp. in the blueberry field.

Colletotrichum gloeosporioides was not found in the unwounded stems, and in only 1/79 (1.2%) of the harvester broken twigs.

DISCUSSION

The role of mechanical harvesting and sorting methods in the incidence of postharvest decays of blueberry fruit is complex. In 1978 and in 1979 bruising of fruit increased the incidence of visibly sporulating Alternaria decays as compared to unbruised hand harvested fruit. Mechanical harvesting had little effect on sporulating Alternaria decays in 1978 and decreased their incidence in 1979. This reduction in sporulating Alternaria decay incidence in mechanically harvested fruit is misleading since the proportion of "leakers" was not recorded in either year in this experiment but they comprised 75% of all unusable fruit in the 1979 harvest and sorting methods experiment. The effect which bruising had on Colletotrichum decay incidence was also obscured by the incidence of leakers in the experiment.

The incidence of visibly sporulating Alternaria decays was little effected by either methanical harvesting or sorting as compared to hand methods in 1978 or in 1979. The incidence of "leakers" was greatly increased by both mechanical harvesting and sorting in 1979. The incidence of "leakers" was not determined in 1978, but "leakers" were present.

Mechanical harvesting and sorting methods favor a non-sporulating form of deterioration, the magnitude of which far exceeds that of Alternaria and Colletotrichum decays. This

deterioration may be caused by bacteria or yeasts or may be of fungal etiology since the common blueberry rotting fungi have been isolated from such fruit (10). The etiology of these "leaker" decays needs to be determined, as well as the mechanism by which mechanical harvesting and sorting methods increase their incidence.

The shift in pathogen expression from sporulating decay on hand harvested fruit to non-sporulating decay on mechanically harvested fruit cannot be attributed to simple bruising of the fruit, since in both seasons simple bruising increased the amount of sporulating Alternaria decays, while mechanical harvesting had no effect or decreased the incidence of such decays.

This study documents an increase in unusable fruit after harvesting by machine as compared to hand harvesting. In several of the experiments it was shown that the location in the field from which the fruit originated had an effect on the decay incidence of the fruit after harvest, implying that fungicidal spray programs in the field before harvest could contribute to postharvest decay control. Further work should be done to modify harvesting and sorting methods to reduce decay incidence. The use of hot water and fungicide dips in processing mechanically harvested fruits would be of value in controlling decays caused by damage to the fruits as has been demonstrated (6, 7).

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