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EPIDEMIOLOGICAL AND ETIOLOGICAL STUDIES OF *EUTYPA* DIEBACK OF GRAPE (*VITIS LABRUSCA* L.) CAUSED BY *EUTYPA ARMENIACAE*

By

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ABSTRACT

EPIDEMIOLOGICAL AND ETIOLOGICAL STUDIES OF *EUTYPA* DIEBACK OF GRAPE (*VITIS LABRUSCA* L.) CAUSED BY *EUTYPA ARMENIACAE*

By

Arthur Thomas Trese

The seasonal abundance of airborne *Eutypa armeniacae* ascospores was sampled over a two-year period at two 'Concord' vineyards in southern Michigan using Burkard volumetric spore traps. Ascospore octads were trapped only after a minimum of 2 mm of rainfall at temperatures above 0 C. The highest octad concentrations were evident in spring, followed by a decline in summer, and a rise again in the fall. No octads were trapped from mid-December through late February.

Ascospores remained viable after long periods of freezing and repeated cycles of freezing and thawing. Alternate wetting and drying greatly reduced ascospore viability.

Inoculation of field-grown and potted vines resulted in pruning wounds becoming infected in the fall, late winter, and spring. Greatest infection resulted from inoculations made on February 22, 1980. One-, two-, and three-year-old canes were equally susceptible to infection.

No infections were found in wounds caused by mechanical harvesters or frost injury.

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INTRODUCTION

Although recognized as a vascular pathogen of apricot (Prunus armeniaca L.) for many years (3, 5, 10, 27, 41) Eutypa armeniacae Hansf. & Carter has only recently been shown to be associated with a vascular dieback disease of grapes (Vitis labrusca L. and V. vinifera L.) (4, 14, 36). Moller and Kasimatis (35) have completed Koch's postulates, demonstrating that E. armeniacae is the causal pathogen of this disease.

Typical symptoms of Eutypa dieback of grape include stunting of spring shoot growth, yellowing and cupping of newly emerged leaves, shedding of blossom clusters, cankers around old pruning wounds, extending eliptically above and below the infected wound, vascular discoloration, and eventual death of one or more arms. Symptoms usually occur first on one arm and then spread to the main framework of the vine in succeeding years (36).

Eutypa dieback is of economic importance in Michigan due to a loss of production as the arms fail and, later, the cost of removal and replacement of the whole vine. In Michigan, the disease occurs in an average of approximately 10% of the vines, with older vineyards generally showing the greatest damage. In California, Moller et al. (36) found disease levels ranging from 6% in a 7-year-old vineyard

to 81% in a 15-year-old vineyard. This disease has also been found in Canada (28), Australia (4) and in several areas of Europe (1, 13, 25).

Extensive studies of the epidemiology of and infection by E. armeniacae in apricot have been done by Carter in Australia (2, 3, 5, 10, 11, 12) and Moller in California (33, 37). These studies have examined spore dispersal throughout the season (41), infection sites and parameters of infection, and also possible methods of control. However, in both cases the host species and the seasonal weather patterns differed greatly from those found in Michigan, where the pathogen is found in association with grapes and the season includes a very cold winter and a much wetter summer.

In California and Australia, fungicides have been tested for the control of Eutypa dieback in apricots but none has proven to be widely practical (17, 37). The control methods currently in use consist of a combination of sanitation and timed pruning (29).

The purpose of this study was to examine the spore dispersal patterns under Michigan conditions and to investigate the etiology of the disease in grapes. Experiments were designed to determine possible infection sites, seasonal susceptibility of the host, duration of pruning wound susceptibility, and several factors in relation to ascospore germination and mycelial growth under simulated winter and spring conditions. It was my purpose to use this information in attempting to design a program for control, either by cultural means or by fungicide application at suitable times.

LITERATURE REVIEW

The literature references to a "dead-arm," "dying-arm," or "dieback" of grape go back to those by D. Reddick in 1909 and 1914 (43, 44). Reddick described a disease having all the symptoms of Eutypa dieback as described presently, i.e., death of arms, vines dying during the growing season, but most commonly in the winter, a dry rot in the heartwood, extending to the bark in some places, dwarfed and cupped foliage of early spring growth plus yellowing and chlorosis of that growth in some instances. However, Reddick added other symptoms in describing this disease. He included reddish brown lesions on green growth, including petioles and peduncles, and a fruit rot, barely distinguishable from black rot. Reddick concluded that this disease was caused by the fungus Cryptosporella viticola, following Shear (45) who, in 1911, published a full description of the fungus believed to be responsible for these symptoms. Reddick named the disease "dead-arm."

In 1928 Coleman (18) published a paper dealing with "dead-arm" in Canada. Coleman found little evidence of leaf and cane infections but did find large numbers of dead arms in older vineyards, and many large lesions on these arms. He was often able to find conidia of Cryptosporella viticola near the lesions, but could not find the perfect stage of this fungus. Inoculation experiments

were done using conidia of C. viticola applied to pruning wounds and after two growing seasons 13 of 28 stubs had produced pycnidia of the same fungus, but only five of the wounds showed disease development beyond one inch from the inoculated surface. It was not clear whether these would form typical trunk cankers.

In 1937 Goidanich (21) renamed the pathogen after examining the cultural characteristics. This revision was based on the presence of two pycnidiospore types, α and β, which are typical of the genus Phomopsis. The name Phomopsis viticola was therefore adopted.

Pine, in 1958 (39), described dead arm as becoming increasingly important in California. He agreed with Goidanich's nomenclature, and gave a detailed description of the pycnidiospores (α) and scolecospores (β). Pine's description of the disease in California referred mainly to the leaf and cane symptoms such as necrotic spots and mentioned little about dead arms or cordons. He continued to use the name "dead arm," however, in his paper.

Willison et al., in 1965 (46), did inoculation studies of both green tissue and large pruning wounds. They found that infection by P. viticola occurred very rapidly on green growth, which is atypical of a "wound invader." Secondly, P. viticola was found to infect only 25% of the pruning wounds, and these infections did not become large lesions.

The first published attempt at separating the leaf and cane lesions from the more complete invasion and death of arms was done

by Dye and Carter in New Zealand in 1976 (19). They found that E. armeniacae could be isolated from canker borders on the main trunk of dying vines. Phomopsis viticola could also be isolated in some cases and sometimes both fungi were present. In the cases where E. armeniacae was most frequently isolated the symptoms were similar to those previously described in relation to a vascular disorder apparently caused by E. armeniacae in California (36). Eutypa armeniacae had also been previously isolated from cankered, dead grape wood in southern Australia by Carter (4, 7, 8) and Carter and Price (14). Dye and Carter (19) initiated inoculation experiments to test the pathogenicity on grapes but did not report any success.

Koch's postulates were completed in 1978 by Kasimatis and Moller in California (23). They were able to induce the cupped leaves, the stunted early growth and cankers two and a half years after inoculating three-year-old wood of cv. Grenache grapevines. The internal symptom of xylem invasion causing a dry rot was also observed, and finally death of the whole cordon occurred. Moller and Kasimatis clearly distinguished the green shoot lesions and fruit rot caused by Phomopsis viticola from the symptoms caused by E. armeniacae, and proposed that this latter disease be referred to as Eutypa dieback.

Eutypa armeniacae had been recognized as a vascular pathogen of apricot since 1955, when M.V. Carter published a report describing the perfect stage as present on "dieback" killed apricot wood (2). Since that time a large amount of research has been done regarding E. armeniacae as a pathogen of apricot, including spore dispersal and infection studies.

The ascosmycete Eutypa armeniacae Hansf. and Carter, is a member of the Class Pyrenomycetes, Order Sphaeriales and Family Diaporthaceae (38). Eutypa armeniacae does not produce a perfect stage in culture, but on dead grape or apricot wood a thin, rough, black stroma is formed. This stroma contains many perithecia at different stages of development. Asci are borne within the perithecia but there are no paraphyses. Eight allantoid spores, each non-septate, slightly bent, with rounded ends and a pale yellowish brown color, 7 to 11 by 1.5 to 2 μm, are produced in each ascus. These spores are forcibly discharged as an octad, all eight held together by the mucilaginous contents of the asci. Discharge occurs after a wetting period and the spores are ejected up to 1 mm above the stroma. An imperfect stage is also produced, both on infected wood and in culture. In early literature this has been referred to as Cytosporina sp. Pycnidia are formed and these produce scolecospores which are hyaline, bent to arcuate, and 18 to 25 by 1 μm (3). These scolecospores are quite similar to the scolecospores produced by Phomopsis viticola, which may have been partially responsible for the early "dead-arm" confusion.

To date E. armeniacae has been found infecting quite a number of plant species. These include, besides grape and apricot, Ceanothus spp. (26), almond, Tamarix spp. and apple (5), barberry (14), lemon (24) and western choke cherry (34).

In his detailed study of E. armeniacae as an apricot pathogen in Australia (3), Carter concluded that the scolecospores were non-infective. Since then studies of the disease and its spread

have dealt with the ascospores only. Several studies have been done concerning ascospore production and dispersal throughout the year. Carter's 1957 paper (3) described the requirement of stromatal wetting for ascospore discharge. Ingold (22) found this to be typical of Pyrenomycetes. Carter (3) found that free ascospores were discharged within 5 to 10 minutes after the stromata were immersed in water. He recorded spore counts as high as 9×10^8 following wetting of a 1 cm^2 piece of stroma. Carter also used a greased slide spore impacting unit operated at 10 liters of air flow per minute to measure spore counts in the field. He found that spores were released after a rainfall of at least 0.05 or 0.06 inches had occurred.

In 1965 Moller and Carter (30) published a detailed study of ascospore dispersal in Australia. They placed a Hirst spore trap in a commercial apricot orchard with a mean annual rainfall of 21 inches. A recording rain gauge was also used to monitor the daily rainfall. The results obtained showed an apparent seasonal periodicity. Over a two-year period spore counts were higher from August through May than in June and July, despite frequent and heavy rains in these latter months. In Australia, June and July are winter months with spring beginning in August or September. A second feature of the dispersal was that the decline in late fall, or May, was very abrupt, and the increase in late August was also abrupt. Daily catches dropped from over 5,000 to less than 50, and increased again in August to over 5,000 per day.

A more recent study of ascospore production and dispersal in California was published by Ramos et al. in 1975 (41). Perithecia were not found in areas that had less than 33 cm of rainfall per year, but areas with annual rainfall greater than 50.8 cm produced abundant perithecia on infected apricot wood. In recording seasonal spore release in the Suisun Valley, with an annual rainfall of 52.4 cm, they found spore release to be highest in September through mid-November, very low in late November through early January, when rainfall was heaviest, and then increasing in numbers through March, after which time the counts became very low again. Through the very dry months of May through August there was no rain and no spore release. It was suggested that the peak spore numbers in the fall may have been due to the absence of rain in the summer months. Ramos et al. (41) were also able to trap spores at a site near Tracy, California, where rainfall was scant. There were no perithecia present on Eutypa-cankered apricots. They concluded that the spores were being carried long distances by the wind from the rainier Suisun, California area. Both of the studies examined spore release patterns in areas where the seasons varied greatly from those found in Michigan.

That E. armeniacae needs exposed wounds to infect apricot was shown by Adam in 1938 (in Carter (3)). Since then several studies have been done concerning various aspects of the infection requirements of the host and pathogen. One of the first was reported by Carter (5). Apricot trees were pruned in July, the dormant season in Australia. Inoculations were made on some sites

the same day, and on other sites after an interval of from one to seven months. Wounds remained susceptible for at least two months. In 1970 Carter and Moller published the results of a further study (11). They found that if trees were pruned in early winter in Australia, trees exposed to natural microflora were no longer susceptible after two weeks. Trees kept under a rainproof shelter remained susceptible to infection after four weeks. Colonization and competition from resident microfloral organisms on the pruning site were hypothesized as the cause of reduced susceptibility in the unsheltered trees.

Price (40) examined this possibility further and found that microorganisms increased rapidly on pruning wounds until reaching peak populations 12 days after pruning. Bacteria were the major colonizers. In testing these organisms for antagonism against Eutypa armeniacae, 33 species of antagonistic fungi were found. Twenty-nine of the antagonistic fungi were species of the genus Fusarium.

Several studies have also been done with apricots to determine if there is a difference in infection relative to the time of the year pruning is done (10, 27, 42). Moller et al. (27) pruned trees on four dates from early winter through spring. It was found that wounds made during the winter lull in spore release were significantly less infected than those pruned in early October, after spores had reappeared in larger numbers. The lowest infection levels resulted from pruning in June, at the very beginning of the low inoculum period.

Carter and Moller repeated the experiment in a slightly different form from 1966 through 1967 (10). Pruning dates ranged from just before autumn leaf fall while ascospore levels were still in high numbers, until August, when the trees were beginning to bloom, and spores were in high numbers again. The month of June was again found to be the best time to prune, which coincided with a period immediately after leaf fall. Wounds of greater than 0.5 inch in diameter were significantly more susceptible than those smaller than 0.5 inch in diameter. Pruning in June was recommended as a control measure with emphasis on keeping the pruning wounds small wherever possible.

Ramos, Moller and English examined apricot wound susceptibility in California (42). They found a significant variation in relation to the time of year that pruning was done. Higher infection rates coincided with fall and winter pruning. They suggested that the susceptibility period of the wounds lasted longer if pruned during dry periods, and perhaps metabolic effects such as storage of sugar in the woody tissue in the winter could be a factor. Heartwood was shown to be non-infectable, but recently differentiated xylem, i.e., sapwood, was easily infected. English and Davis (20) have also shown that apricot heartwood is non-infectable. They found that the fungus first invades the young xylem, then the cambium and bark, causing cankers at that stage. Wounds going no deeper than the cambium did not become infected.

The general result of these investigations has shown that E. armeniacae ascospore dispersal followed a seasonal pattern of increase and decline. The lowest density was in the winter and the highest was in either the spring, as in Australia, or in the fall, as in California. Pruning at times of low spore density was tried as a means of controlling E. armeniacae caused dieback in apricots. In some cases (10, 27) this did decrease infection but did not give complete control. It has been shown (12) that as few as ten ascospores per wound in apricots will cause infection in 40% of the inoculated sites. One report (42) showed higher infection after fall and winter pruning than after early spring pruning, possibly due to extended susceptibility of the pruning wounds to infection. Because cultural methods did not promise complete control, several research groups began to research the effectiveness of chemical controls.

In 1969 Moller and Carter (31) published results of evaluations of 12 different fungicides applied to apricot pruning wounds. Only benomyl gave significantly better control than that afforded by the other chemicals. In 1970 Moller and Carter (32) reported a greater than 50% reduction of infection in inoculated wounds due to fungicide protection. Benomyl was applied by a hand-held sprayer until run-off occurred. When Carter and Price (17) used a turbomist sprayer they found no reduction in infection if wounds were inoculated three days after the application was made. Moller et al. in California (37) also found that commercially designed high volume orchard sprayers were not effective. The only

feasible method was hand application at high concentrations of fungicide (2.4% w/v) using a paint brush to apply the chemical. Carter (6) and Carter and Price (15, 16) have done extensive studies on biological control with Fusarium lateritium, or F. lateritium in combination with benomyl. They were quite successful in getting control but application was again by a hand-held sprayer.

To date there has been no study of the spore production and dispersal characteristics of E. armeniacae in grape. In addition, the seasonal pattern in Michigan is very different from that found in Australia and California. It is of great importance to know when spore release is prevalent under our conditions. Only recently have Moller and Kasimatis (35) been able to complete Koch's postulates, showing that E. armeniacae is the causal pathogen of Eutypa dieback of grape. There have been no further studies regarding infection sites, infection periods, would susceptibility, or possible control methods.

MATERIALS AND METHODS

Ascospore Dispersal Studies

In January, 1978, field stations were established at two Vitis labrusca L. 'Concord' vineyards. One station was on the Michigan State University campus at East Lansing, which is in the south central lower peninsula. The other station was located three miles south of Lawton, Michigan, in the southwest corner of the state, about 160 km southwest of East Lansing. The mean annual precipitation at East Lansing and Lawton is 77.5 and 82.5 cm, respectively. At East Lansing the station was situated in a 15-year-old 0.2-ha vineyard in which approximately 10% of the vines bore mature stroma of Eutypa armeniacae. The vineyard is remote from other established vineyards or apricot orchards. The Lawton vineyard was a commercial vineyard of about 10 ha, 25-years-old or older. In this vineyard 15-20% of the vines bore stromata during the 1978 growing season. Through the winter and spring of 1979 many of the most diseased vines were removed, so that the percentage of stromata-bearing vines was 10-15% during 1979. This vineyard was within 0.4 km of several other large vineyards.

Ascospores were trapped from the air at each site by a Burkard 7-day recording spore trap (Burkard Scientific (Sales) Ltd., Richmansworth, England). These traps were operated continuously for a full two years at each site. Spore traps were adjusted to

operate at an air sampling rate of 8 L/min. The trap at East Lansing was operated from a 12-volt DC car battery which was replaced weekly or more often. The trap at Lawton was operated from a 110 volt AC outlet. The traps were mounted and leveled so that the air intake orifice was 0.6 m above the ground. Each trap was encircled by a 2 m diameter ring formed of eight dead vine trunks bearing mature stromata.

Environmental data at each site were obtained with a seven-day recording rain gauge. Solar pyranograph, anemometer, (all from Weather Measure Corp., Sacramento, CA 95841), a sheltered hygro-thermograph (Bendix Corp., Baltimore, MD 21224), and a leaf wetness meter (M. De Wit, Hengelo, Holland). All instruments were positioned 1 m above the ground except the anemometer, which was 2 m above the ground. After one year the anemometer and solar pyranograph were removed from each site.

The spore trap tape and other recording charts were changed weekly. To count the ascospore octads (ascospores of E. armeniacae are released in groups of eight) the tapes were cut into seven 48 mm sections, each equal to a 24-hour segment. These sections were placed on a glass slide, three drops of cotton blue in lactophenol were added and a 50x24 mm cover slip applied. Sections were scanned transversely in 2 mm (1 hour) segments with 10x wide-field oculars and a 40x objective lens of a compound light micro-scope. After numerous trap tapes were examined to verify that spores were released only after sufficient rain had occurred to wet the stroma, counts were made starting three hours before a recorded

rain and were continued until three consecutive hours revealed no octads. Allantoid spores 1.5-2 x 7-11 μm, of a light brown color, non-staining in lactophenol-cotton blue and appearing in groups of eight were counted as octads as per the published description (5). A section of spore trap tape contianing E. armeniacae octads released in the laboratory was stained as above and used as a standard.

During July and August of 1979 stromata-bearing vine trunks were collected from the Lawton vineyard site and brought to the laboratory. Attempts were made to collect ascospores by soaking the trunks in distilled water for one hour and then placing them in a spore collection tower (Plas Labs Co., Lansing, MI 48956). Vacuum was applied and spores were collected onto glass slides. Examinations of the perithecia were made using a dissecting needle and light microscopy to determine the condition of the perithecia and asci during the summer season. In early September a total of eight trunk pieces 6 cm in diameter and 12 cm long and bearing mature stromata were soaked and placed in the spore tower. The number of ascospore octads collected was recorded. These pieces were returned to the field until late November, at which time they were again put in the spore tower and the total spore discharge was recorded.

Effect of Temperature Upon Eutypa armeniacae Ascospore Germination

Spores were collected as described earlier and suspended in distilled water. Several drops were added to each petri plate containing 2% potato dextrose agar (PDA) (Difco Laboratories, Detroit, MI 48232) and the plates were incubated in constant temperature

incubators kept at temperatures ranging from 0 to 30 C at 5 degree intervals. Each treatment consisted of three replicate plates. Percent germination was determined after 15, 24, 48, 72, and 110 hours.

Effect of Temperature on Mycelial Growth of Eutypa armeniacae

Seven mm plugs of an actively growing monoascospore culture were placed in the center of petri plates containing 2% PDA. These plates were incubated at a number of constant temperatures ranging from 5 to 35 C at 5 degree intervals for five days and each treatment was replicated with four plates. Radial growth was measured by subtracting the initial diameter of the plug from the final colony diameter.

Ascospore Survival at Low Temperatures

Two experiments were done to mimic the conditions that ascospores encounter during the fall, winter and early spring in Michigan. Ascospores were collected in the spore tower as previously described and suspensions were made in glass distilled water. Portions were placed in test tubes and kept in constant temperature incubators set at 0, -10, or -20 C for 1, 3, 7, 14, or 28 days. At the end of each period at each temperature, spores were streaked on 2% PDA and percent germination was determined after 3.5 days incubation at 10 C. Each treatment was replicated three times and consisted of at least 100 spores per replication. Other spore suspensions were frozen at -10 C for three days, then thawed for

six hours at 10 C, and then refrozen. This cycle was repeated from one to five times and the percent germination was then assayed as above, at the end of each freeze thaw cycle. Each treatment was replicated three times and consisted of at least 100 spores per replication.

Mycelial Survival and Growth at Low Temperatures

The ability of mycelium to survive freezing was examined by placing 8-mm-diameter plugs (from the edge of an expanding colony on PDA, started from a single ascospore) in the center of petri plates containing 2% PDA. Each plate was incubated at 0, -10, or 20 C for 1, 3, or 7 days. Plates were removed and placed in a constant temperature chamber kept at 10 C for eight days and the resulting radial colony growth was measured. Each treatment consisted of three replicate plates.

Effect of Alternate Wetting and Drying on Spore Germination

Ascospores were collected on clean glass slides using the spore collection tower. A spore suspension was made in glass distilled water. A portion was placed onto 2% PDA immediately and held at 25 C for 24 hours to determine baseline germination. Another portion was placed in glass depression slides and the water was allowed to evaporate to dryness over a period of eight hours. After three days the spores were re-suspended in distilled water and a portion was plated onto PDA for germination tests at 25 C for 24 hours, while another portion was allowed to evaporate to dryness

over an eight hour period. After a second cycle of dryness for three days the spores were re-suspended in distilled water and placed onto PDA for germination again. In all cases germination percentages were determined by averaging three replicate plates of 100 spores each.

Field Infection of Trap Plants

To examine whether pruning wounds would become infected in the field, four-year-old cv. Concord vines in 20 liter cans were taken to the Lawton field station on three dates in the spring of 1978. Five plants were used at each date and these were spaced around the spore trap and it's ring of E. armeniacae infected dead vines. Several one and two-year-old canes were pruned on the day they were placed in the field. Pruning cuts were made 1.5 cm above a healthy node. The first group of plants was put in the field on 19 January and left until 16 March. The first spring rain fell on 14 March, giving this set possible exposure to ascospores. The second set was left for seven weeks, from 16 March to 6 May, and the third set for six weeks, from 6 May to 15 June. All the plants were taken back to East Lansing after their field exposure period and kept in isolation from E. armeniacae ascospores for two growing seasons.

Reisolation of E. armeniacae from the pruning sites was attempted in the fall of 1979. Each cane was cut 8 to 10 cm below the original pruning wound. These sections of cane were then split lengthwise with a flamed knife blade to expose the center. From

the halves, ten small chips of wood were removed, using sterile technique, from areas below the old pruning wound downward wherever discoloration of the wood indicated the possibility of infection. These chips were placed on 2% PDA amended with 100 µg/ml streptomycin sulfate. The plates were examined after four days and all fungal colonies resembling E. armeniacae were transferred to fresh Potato Glucose Agar (an extract of 200 gm potatoes, 8 gm glucose, 20 gm agar in 1000 ml distilled water). These plates were placed under cool white fluorescent light (General Electric F 15T8CW) and soft black (General Electric F 30T8SB) with a 14 hour-day length. After about two weeks the presence of E. armeniacae cultures were confirmed by the presence of scolecospores.

Infection Studies with Potted Plants

An experiment was done to determine what factors may be involved in the infection process: (i) if pruning wounds are a site of infection on dormant and on growing plants; (ii) the effect of pruning date during the winter on the amount of infection; (iii) the relative susceptibility of one or two-year-old wood; and (iv) the effect of delay periods between making a pruning cut and inoculation of that site in both dormant and growing plants.

On each of three dates in the winter and spring of 1978, groups of 35 four-year-old cv. Concord vines in 20-liter cans were each given several pruning cuts on one and two-year-old canes. In groups of five these pruned vines were inoculated at a time interval

of either 0, 1, 3, 7, 14, 28, or 56 days after the pruning cuts were made.

The pruning cuts were made on 30 January, 8 March, and 17 May, 1978. Due to the severity of the winter weather the plants were kept in a cold room at -1 to 1 C until mid-April when they were placed out of doors. Thus, the third inoculation series was carried out completely outside while the vines were beginning to break dormancy and, later, when they were actively growing.

Inoculations consisted of 250 ascospores in 5 µl of distilled water applied to each pruning cut using a Hamilton microsyringe (Hamilton Co., Reno, Nevada 89510). Prior to inoculation each cut surface was moistened with sterile distilled water applied with a hand-held DeVilbiss #15 atomizer. The spores were obtained by the method described earlier, using a vacuum operated spore collection tower.

The inoculations were done under slightly warmer conditions than the cold room (about 5 C), and the vines were kept at this temperature for one week, after which time they were put back into the cold room until mid-April.

Reisolation of the pathogen was attempted 16 to 20 months after the inoculations, or two growing seasons later. The reisolation procedure was identical to the method used above.

Infection Studies with Mature Vines in the Field

Field infection studies were done in an attempt to determine the amount of infection relative to time of year pruning is

done, and again, the relative susceptibility of one, two, and three-year-old cane wood. A recently planted vineyard with vines of eight years of age or less, was located 0.4 km south of the vineyard containing the spore trapping equipment. Several trips were made through the vineyard in search of evidence of E. armeniacae. No symptoms or signs were found. On various dates during the winter and spring of 1978, and during the fall, winter and spring of 1978-1979, fresh pruning cuts on cv. Concord vines were inoculated. Seven vines were pruned and inoculated and seven more were pruned and left as controls. There usually were a total of 50 to 75 pruning cuts per treatment. The inoculation procedure was the same as above, and the control vines were also moistened and 5 µl sterile distilled water was applied to each pruning site. Inoculations were done on 9 and 30 March, 20 April, 18 May, 29 November, and 20 December in 1978, and 22 February, and 30 March of 1979. Reisolation of the pathogen was attempted from all treatments as described above. The isolations were done from September 1979 through January 1980, so that the first four inoculation periods had incubated through two growing seasons, while the last four had incubated for only one growing season.

In Vitro Evaluation of Fungicides for the Control of Eutypa armeniacae

Seven fungicides were tested using fungicide incorporation into PDA. The fungicides used were: Difolatan^{R} 4F (captafol) [cis-N-((1,1,2,2,-Tetrachloroethyl) thio)-4-cyclohexene-1,2-dicarboximide], Captan 50% WP [cis-N-((Trichloromethyl) thio)-

4-cyclohexene-1,2-dicarboximide], Bayleton^{R} 50% WP (triademifon) [1-(4-chloro-phenoxy)-3,3-dimethyl-1-1(1,2,4-triazol-1-yl)-butan-2-one], Bravo^{R} 75% WP (chlorothalonil) [Tetrachloroisiophthalonitrile], Phaltan^{R} 50% WP (folpet) [N-(Trichloromethythio) phthalimide], Ferbam 76% WP [Ferric dimethyldithiocarbamate] and Benlate^{R} 50% WP (benomyl) [Methyl-1-(butylcarbamoyl)-2-benzimidazolecarbamate]. These fungicides were mixed with 15 ml sterile liquified PDA on a Vortex mixer at various concentrations on an active ingredient basis, [0.1, 1, 10, and 100 µg/ml (ppm)]. Both ascospore germination and mycelial growth were examined. Ascospores were collected as previously described and suspended in sterile distilled water. Several drops of this suspension were added to each agar plate and spread with a sterile bent glass rod. Five replicate plates were used for each treatment, plus ten control plates with no fungicide. Percent germination was determined by counting 100 spores per plate after 3.5 days at 10 C. Percent inhibition was calculated as $(\frac{\text{control-treatment}}{\text{control}}) \times 100$.

To determine the effect of the fungicides on mycelial growth, 6 mm diameter plugs from an actively growing monoascospore culture were used to inoculate the fungicide-incorporated agar plates. Growth was determined after five days at 24 C by subtracting the initial diameter of the plug from the final diameter of the mycelial colony. Percent inhibition was calculated as above.

Mechanical Harvester-Induced Injury as Possible Infection Sites

The possibility that mechanical injuries to canes, caused by mechanical harvesters, are infection sites was assessed. One hundred and four broken canes of one, two, or three-year-old wood were tagged after an over-the-row mechanical harvester had harvested the vineyard which contained the spore trapping field station at Lawton, MI. Injured vines were tagged on 25 October and 1 November 1978. Light rainfall occurred on the day of harvest and the day after, which caused some inoculum release. Isolations were made one year later onto PDA as previously described.

Frost Damage as Possible Infection Sites

Five potted cv. Concord vines were placed in a growth chamber (Sherer-Gilliett Co., Marshall, MI 49068) unit for five hours at 4 C followed by two hours at -3 C. The new spring growth on these canes was from 5 to 20 cm long. After the plants were freeze-injured they were inoculated with a distilled water spore suspension containing 10^4 ascospores/ml. This was done by misting with a DeVilbis No. 15 hand atomizer, applying 3 ml of the suspension to each plant. A plastic bag containing a moist paper towel was put over the plants for 24 hours to maintain free moisture and was then removed.

After two growing seasons, reisolation was attempted from these five plants by examining 25 sites per plant. The sites were selected to obtain samples from all ages of wood. Several chips

of wood were removed from each site using sterile technique and observed for E. armeniacae as previously described.

Five plants at the same growth stage, but not treated by freezing were also inoculated and incubated as above. These, too, were examined for the presence of the pathogen after two growing seasons.

RESULTS

Ascospore Dispersal Studies

Ascospore octads were released at both the East Lansing and Lawton vineyard sites after rainfall of at least 2 mm. Figure 1 shows an hourly summary of ascospore octads trapped from 20 March through 22 March, 1978, at East Lansing. This figure includes the presence of free water as measured by a leaf wetness meter, relative humidity, temperature C, rainfall and ascospore octads. Solar radiation and wind speed (data not shown) did not seem to affect spore release. During the period shown there was 0.5 m of snow on the ground. Small amounts of rain began at 1530 hours on 20 March and ended at 0400 hours on 21 March, 1978, resulting in wetness of the vine trunks and stromata (as measured by the leaf wetness meter and observations) until 1000 hours on 21 March. Octads were trapped from the air in low numbers, beginning at 1800 hours on 20 March. The numbers increased to more than 152/0.48 m^3 air/hour at 0800 hours on 21 March. Similar results were obtained at East Lansing in 1979 (Figure 2). Rainfall occurred between 1200 hours and 1300 hours on 29 March (3.25 mm) and octad release began shortly afterward. Peak numbers of 486 octads/0.48 m^3 air/hour were trapped at 1300 hours. A small amount of rain occurred from 1800 to 1900 hours. Spore release continued until 1300 hours on 30 March, or for a total

Figure 1.--Hourly levels of rainfall, temperature, leaf wetness, relative humidity and airborne Eutypa armeniacae ascospore octads trapped from the air (0.48 m^3/h) using a seven-day Burkard volumetric recording spore trap in a Eutypa dieback diseased vineyard. East Lansing, MI vineyard site, 1978.

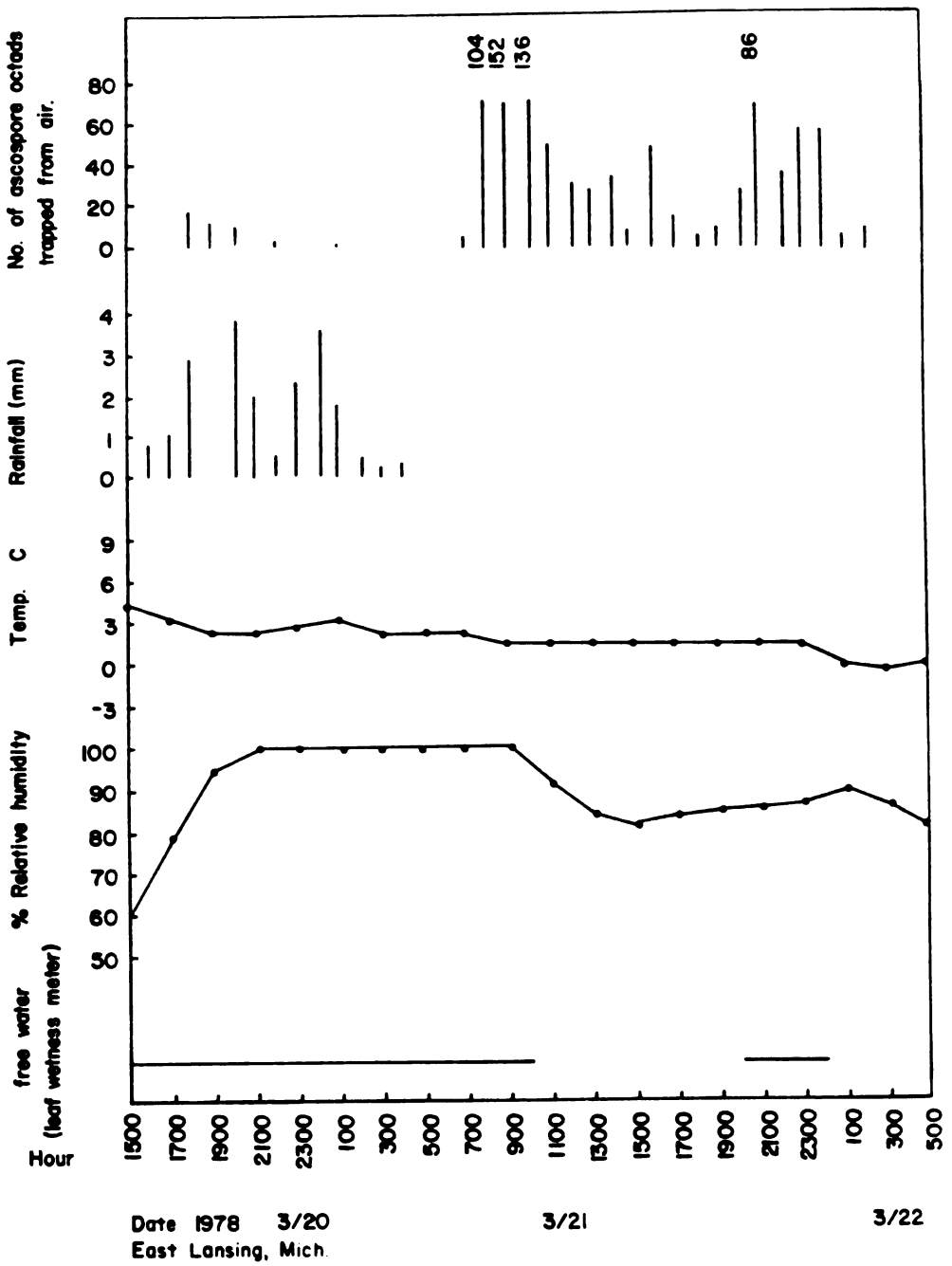
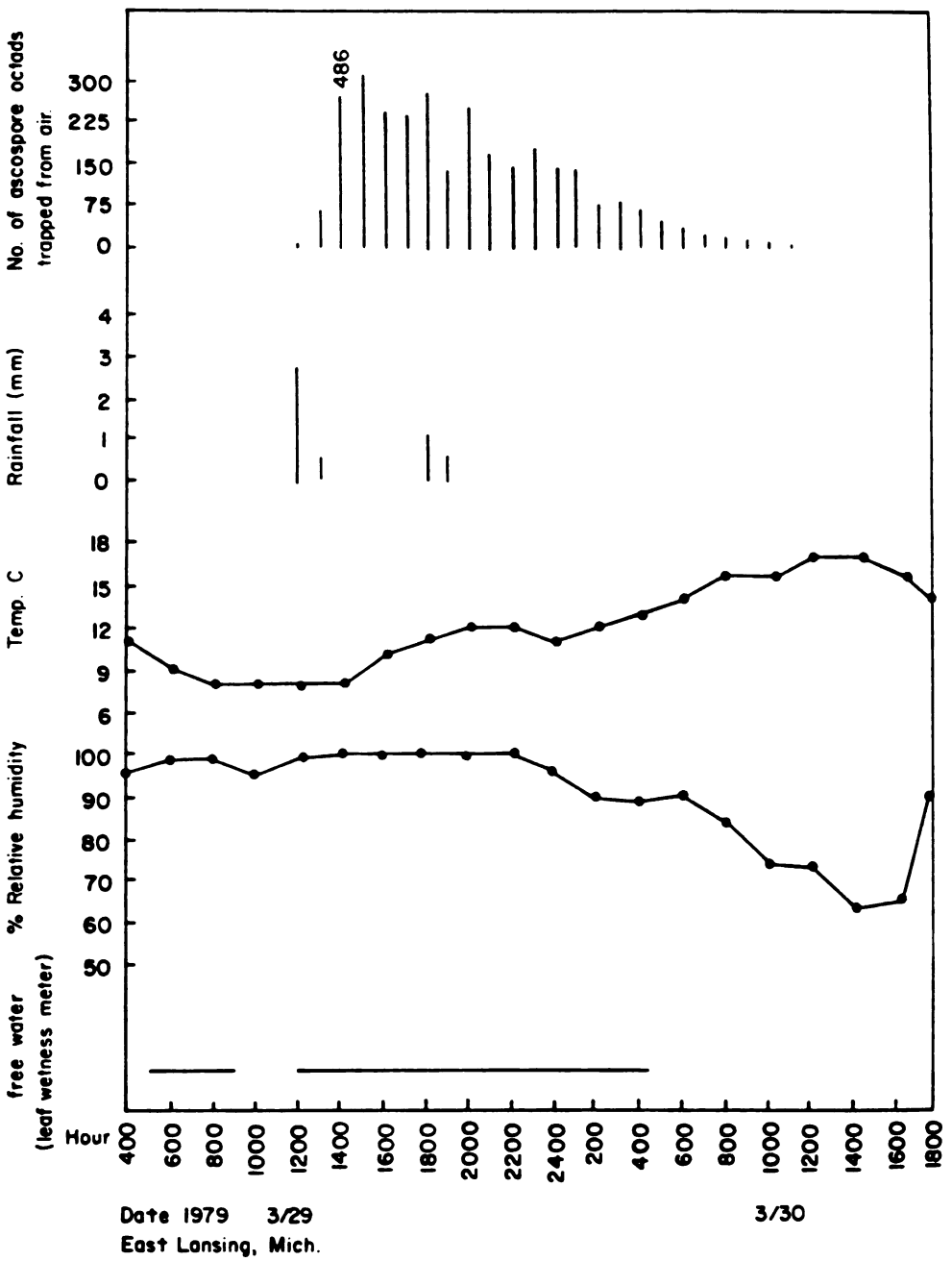

No. of ascospore octads trapped from air.
104
152
136
86
80
60
40
20
0
Rainfall (mm)
4
3
2
1
0
Temp. C
9
6
3
0
-3
% Relative humidity
100
90
80
70
60
50
free water (leaf wetness meter)
Hour
1500
1700
1900
2100
2300
100
300
500
700
900
1100
1300
1500
1700
1900
2100
2300
100
300
500
Date 1978 3/20
3/21
3/22
East Lansing, Mich.

Figure 2.--Hourly levels of rainfall, temperature, leaf wetness, relative humidity and airborne Eutypa armeniacae ascospore octads trapped from the air (0.48 m^3/h) using a seven-day Burkard volumetric recording spore trap in a Eutypa dieback diseased vineyard. East Lansing, MI vineyard site, 1979.


No. of ascospore octads trapped from air
300
225
150
75
0
486
Rainfall (mm)
4
3
2
1
0
Temp. C
18
15
12
9
6
% Relative humidity
100
90
80
70
60
50
free water (leaf wetness meter)
Hour
400
600
800
1000
1200
1400
1600
1800
2000
2200
2400
200
400
600
800
1000
1200
1400
1600
1800
Date 1979 3/29
3/30
East Lansing, Mich.

of 24 hours. Temperatures during the period of octad discharge ranged from 8 to 17 C.

Figure 3 shows an hourly summary of octads trapped at the Lawton vineyard in 1978. Rainfall totaled 5 mm over a four hour period on 27 March starting at 0800 hours. Octad release began at 1300 hours and continued until 2000 hours. At this time the temperature fell below freezing for six hours and octad release stopped. At 0200 hours on 28 March the temperature rose to 1 C and ascospore release resumed. A trace of rain (0.25 mm) fell at 0300 hours and 0800 hours on 28 March which may have aided further octad release.

Seasonal composite charts are shown for East Lansing, 1978 and 1979 (Figures 4 and 6) and Lawton, 1978 and 1979 (Figures 5 and 7). These represent ascospore octad catches and rainfall totals for selected rain periods throughout 1978 and 1979. At both locations in 1978 no octads were trapped during January, February, and the first half of March because of subfreezing temperatures and a lack of rain. Snowfall was considerable during this time, and at times the snow cover in the vineyards reached 1 m in depth. The first rain of the year occurred on 14 March at East Lansing. Due to equipment failure, this period was missed by the spore trap. On 20 March the temperatures were warmer at both locations and rain fell on this date.

At the East Lansing vineyard (Figure 4), each period of release of relatively high numbers of ascospore octads between 20 March and 15 April was associated with rainfall ranging from 2 to

Figure 3.--Hourly levels of rainfall, temperature, leaf wetness, relative humidity and airborne Eutypa armeniacae ascospore octads trapped from the air (0.48 m^3/h) using a seven-day Burkard volumetric recording spore trap in a Eutypa dieback diseased vineyard. Lawton, MI vineyard site, 1978.

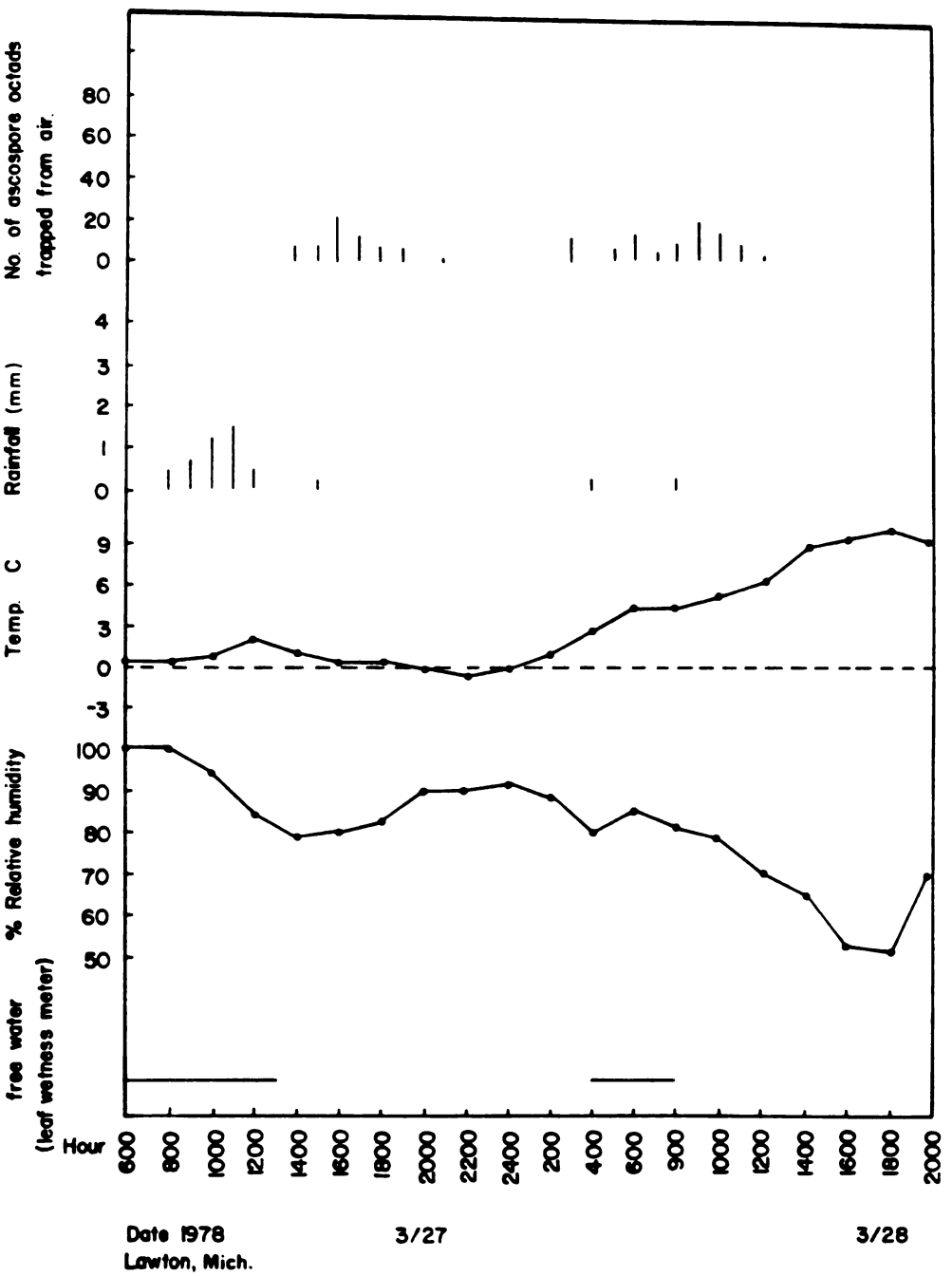

No. of ascospore octads trapped from air.
80
60
40
20
0
Rainfall (mm)
4
3
2
1
0
Temp. C
9
6
3
0
-3
% Relative humidity
100
90
80
70
60
50
free water (leaf wetness meter)
Hour
600
800
1000
1200
1400
1600
1800
2000
2200
2400
200
400
600
900
1000
1200
1400
1600
1800
2000
Date 1978
3/27
3/28
Lawton, Mich.

Figure 4.--Seasonal composite of Eutypa armeniacae ascospores trapped from the air (0.48 m^3/h) following rains using a Burkard volumetric spore trap. Daily 24 hour totals of rainfall and spore catch are shown. East Lansing, MI vineyard site, 1978.

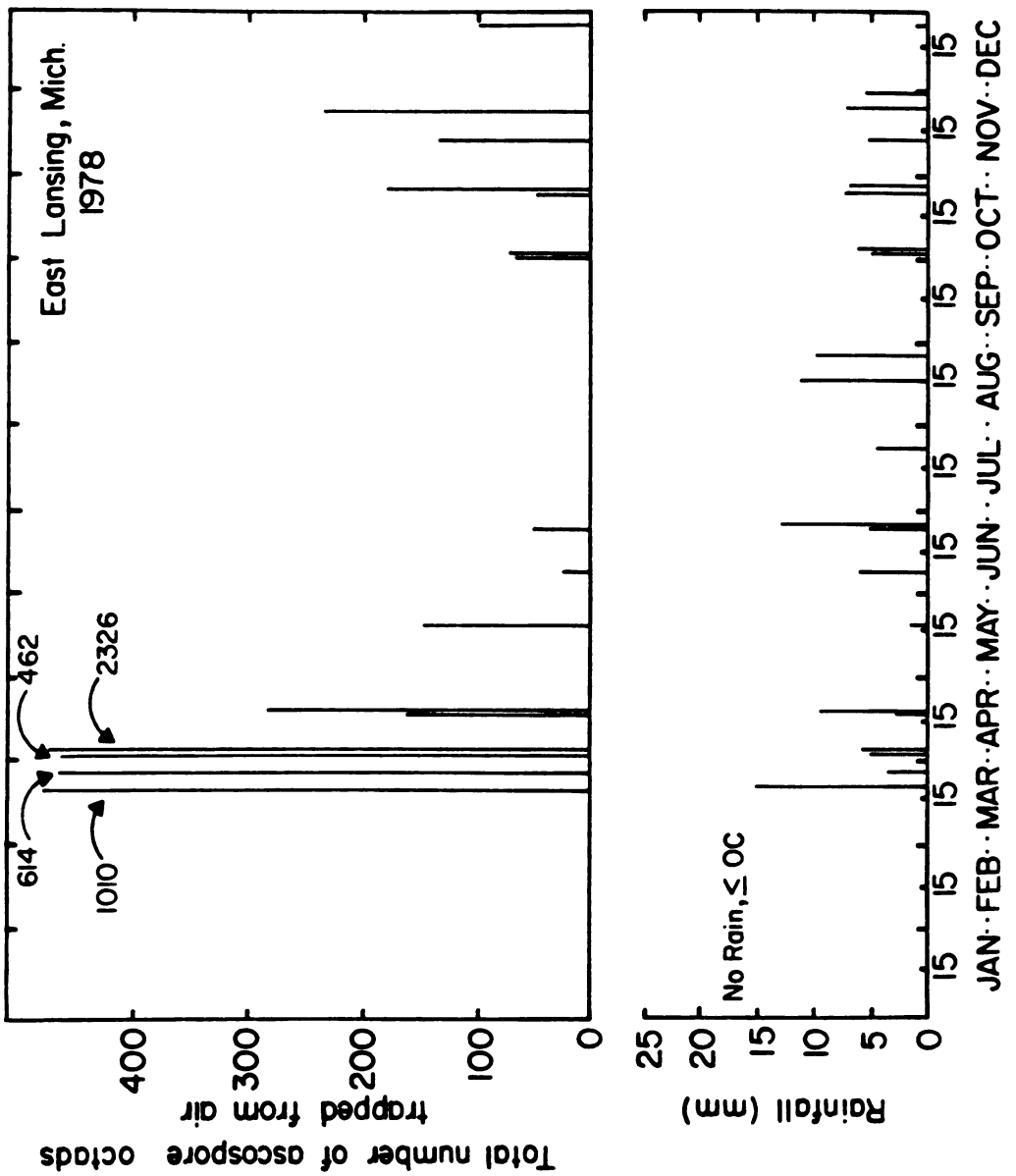

East Lansing, Mich.
1978
Total number of ascospore octads trapped from air
400
300
200
100
0
614
1010
462
2326
Rainfall (mm)
25
20
15
10
5
0
No Rain, ≤ 0C
15
JAN · FEB · MAR · APR · MAY · JUN · JUL · AUG · SEP · OCT · NOV · DEC

Figure 5.--Seasonal composite of Eutypa armeniacae ascospores trapped from the air (0.48 m^3/h) following rains using a Burkard volumetric spore trap. Daily 24 hour totals of rainfall and spore catch are shown. Lawton, MI vineyard site, 1978.

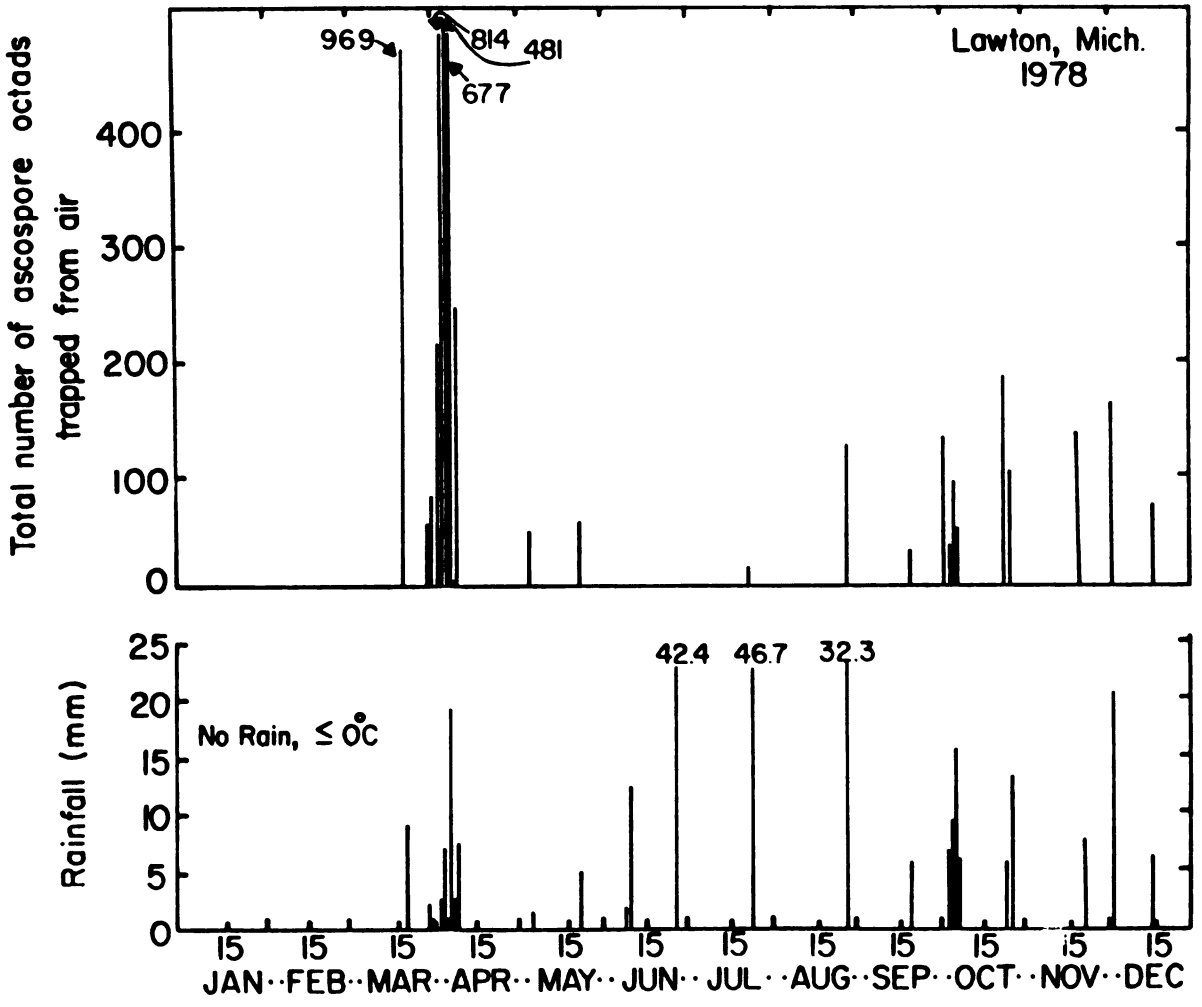

Total number of ascospore octads trapped from air
969
814
481
677
Lawton, Mich.
1978
400
300
200
100
0
Rainfall (mm)
25
20
15
10
5
0
No Rain, ≤ 0°C
42.4
46.7
32.3
15 15 15 15 15 15 15 15 15 15 15 15
JAN··FEB··MAR··APR··MAY··JUN··JUL··AUG··SEP··OCT··NOV··DEC

Figure 6.--Seasonal composite of Eutypa armeniacae ascospores trapped from the air (0.48 m^3/h) following rains using a Burkard volumetric spore trap. Daily 24 hour totals of rainfall and spore catch are shown. East Lansing, MI vineyard site, 1979. Asterisk denotes snow melt rather than rain measured.

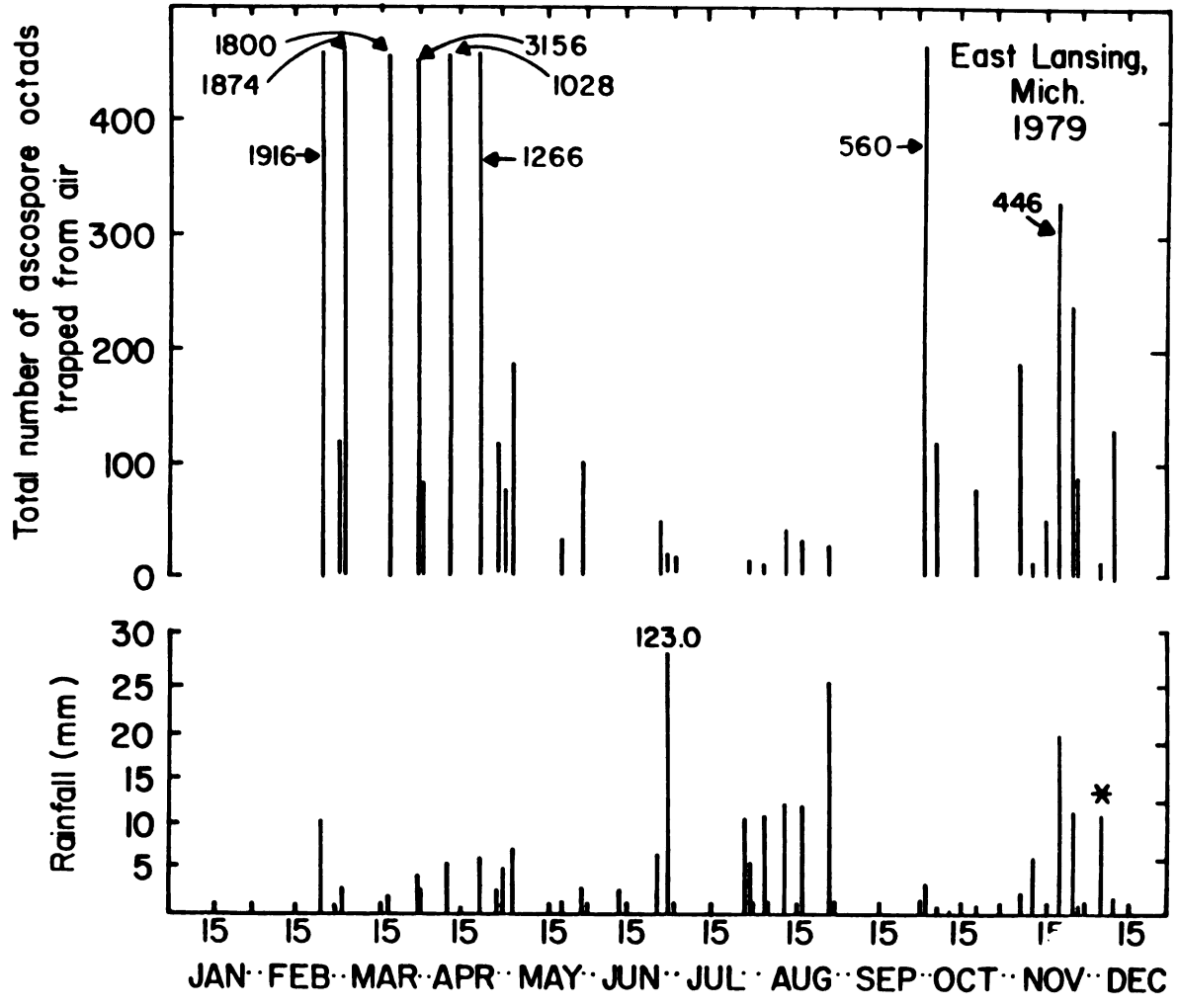
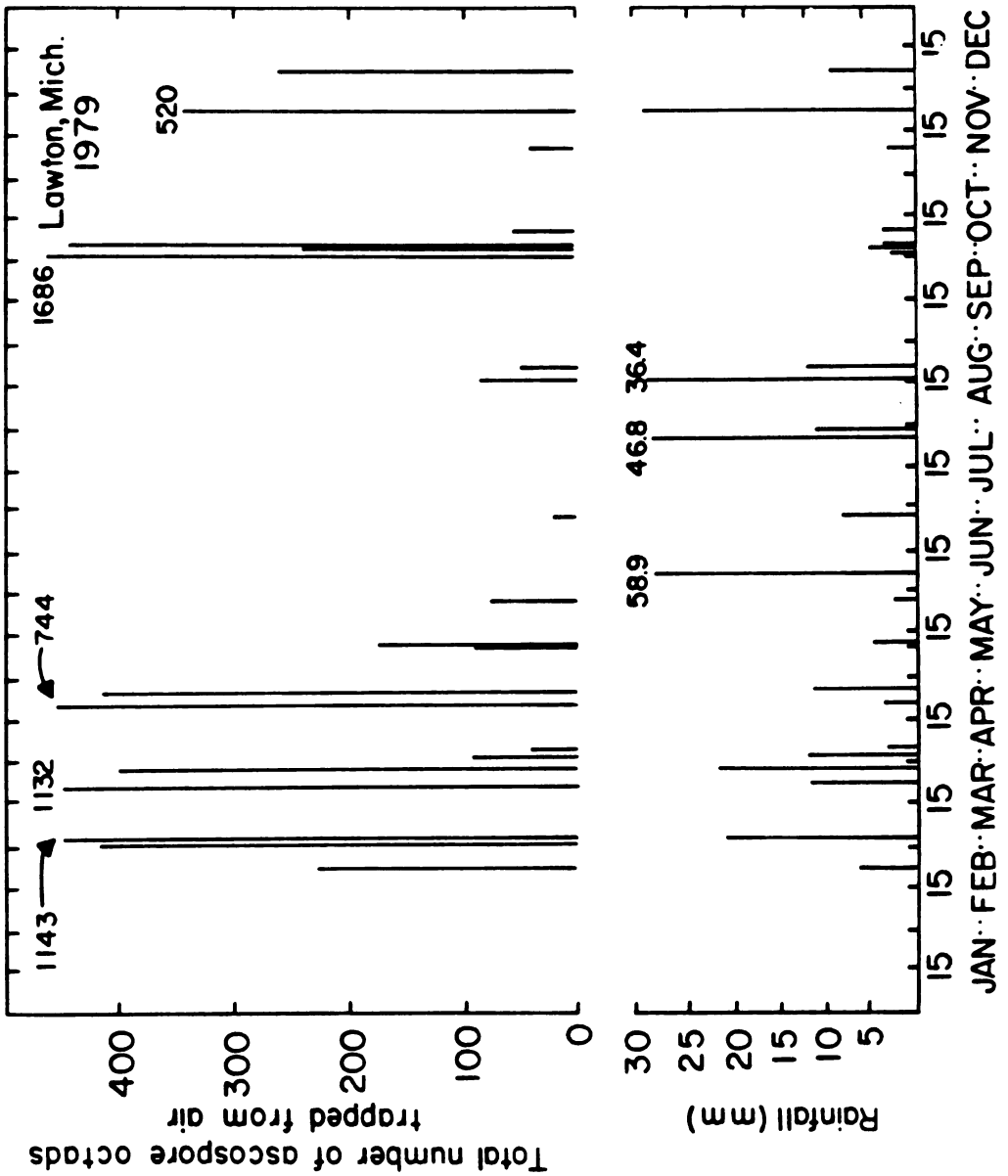

Total number of ascospore octads trapped from air
1800
1874
3156
1028
1916
1266
560
446
East Lansing, Mich. 1979
400
300
200
100
0
Rainfall (mm)
123.0
30
25
20
15
10
5
15 15 15 15 15 15 15 15 15 15 15 15
JAN FEB MAR APR MAY JUN JUL AUG SEP OCT NOV DEC

Figure 7.--Seasonal composite of Eutypa armeniacae ascospores trapped from the air (0.48 m^3/h) following rains using a Burkard volumetric spore trap. Daily 24 hour totals of rain and spore catch are shown. Lawton, MI vineyard site, 1979.


Lawton, Mich.
1979
1143
1132
744
1686
520
Total number of ascospore octads
trapped from air
0
100
200
300
400
Rainfall (mm)
5
10
15
20
25
30
58.9
46.8
36.4
15
JAN · FEB · MAR · APR · MAY · JUN · JUL · AUG · SEP · OCT · NOV · DEC

15 mm. On 16 May another 2 mm of rainfall occurred after which about 150 octads were trapped. During the late spring and summer, 17 May through late August, very few octads were trapped, despite numerous substantial rains. Beginning in early October, octads were trapped in increasingly large numbers through the end of November. The last catch was in late December before freezing conditions set in. A similar pattern was observed in the Lawton vineyard in 1978 (Figure 5). Again, abundant octads were trapped from mid-March to mid-April. There was a slack period in the summer, and the numbers of octads trapped increased during the fall, until winter freezing conditions began.

The first rain in 1979 was recorded on February 23 at both the East Lansing and Lawton vineyards due to subfreezing conditions in January and early February. At East Lansing (Figure 6) rains on 11 days between mid-February and mid-May caused the release of octads as a result of each rain. The octads trapped totaled 1,000 or more on six of these dates with a peak in late March of 3,156 octads from one rain period. Rainfall during May through August resulted in relatively low numbers of octads being trapped and in some cases no octads were trapped. No rain fell in September, and on 1 October, 3 mm of rain was followed by 560 octads trapped from the air. Octad levels averaged 150 per rain period through the remainder of autumn. On 7 December, 1979, at East Lansing, the rain gauge recorded 12 mm of precipitation due to snow melt. This resulted in 12 ascospore octads being trapped from the air. Several other

periods of melting snow at both locations were also examined but no spores were found on the tapes.

At Lawton in 1979 (Figure 7) a similar pattern was found. Spring rains resulted in the release of large numbers of octads but rainfall during the summer months triggered little or no discharge of ascospores. September was rain-free and the first rain of the autumn came on 1 October. Three millimeters of rain caused 1686 octads to be released on that date, which was the peak for that year. Other autumn rains also stimulated ascospore discharge but the totals were lower.

Stromata-bearing vine trunks were collected from the Lawton vineyard in July and August, 1979, and examined in order to determine the cause of the seasonal decline in octads. The spore settling tower was used and no E. armeniacae ascospores were collected. Examination of perithecia using a teasing needle and light microscope revealed that the contents consisted of immature asci. The eight sections of vine trunk that were collected from Lawton in early September released a total of 106 octads in the spore settling tower. These were returned to the field until late November, when they were again placed in the spore settling tower. This time the spore total was 13,630 octads collected. These results are in agreement with the figures showing seasonal patterns of ascospore discharge.

Effect of Temperature Upon Eutypa armeniacae Ascospore Germination

Figures 4 to 7 indicate that, in Michigan, E. armeniacae ascospore dispersal occurs primarily in the spring and fall. Weather conditions during these seasons include cycles of freezing temperatures alternating with warmer periods, and subfreezing temperatures for extended periods of time. An experiment was done to study the relationship between temperature and ascospore germination. The optimum temperature for ascospore germination was found to be approximately 25 C, with 81% germination after 24 hours (Table 1). Ascospores held at 5 C germinated 80% after five days.

Effect of Temperature on Mycelial Growth of Eutypa armeniacae

Mycelial growth was measured for colonies kept at various temperatures in order to determine the optimum conditions and the range of viability (Figure 8). Colony growth was found to occur most rapidly at approximately 25 C. Growth was observed at 5 C but not at 35 C.

Ascospore Survival at Low Temperatures

In the first experiment examining ascospore survival at low temperatures, suspensions of ascospores were exposed to freezing conditions for various lengths of time. Percent germination for these suspensions and their respective treatments are shown in Figure 9. From 50 to 53% of ascospores kept for 28 days at either 0, -10, or -20 C germinated after 3.5 days at 10 C on PDA. Sixty to 65% of

Table 1.--Germination of Eutypa armeniacae Ascospores on PDA at Various Temperatures.

Temperature C	Germination (%)[a]				
	15 h	24 h	48 h	72 h	110 h
5	0	0	0	3	80
10	0	0	9	25	--
15	0	7	50	67	--
20	2	33	55	--	--
25	47	81	--[b]	--	--
30	18	31	--	--	--

[a]One hundred spores were counted/replication, and each treatment was replicated three times.

[b]Mycelia had grown to the extent that germination counts were obscured.

Figure 8.--Radial mycelial growth of Eutypa armeniacae on PDA at several temperatures after five days, determined by final colony diameter minus the original diameter of the inoculum plug.

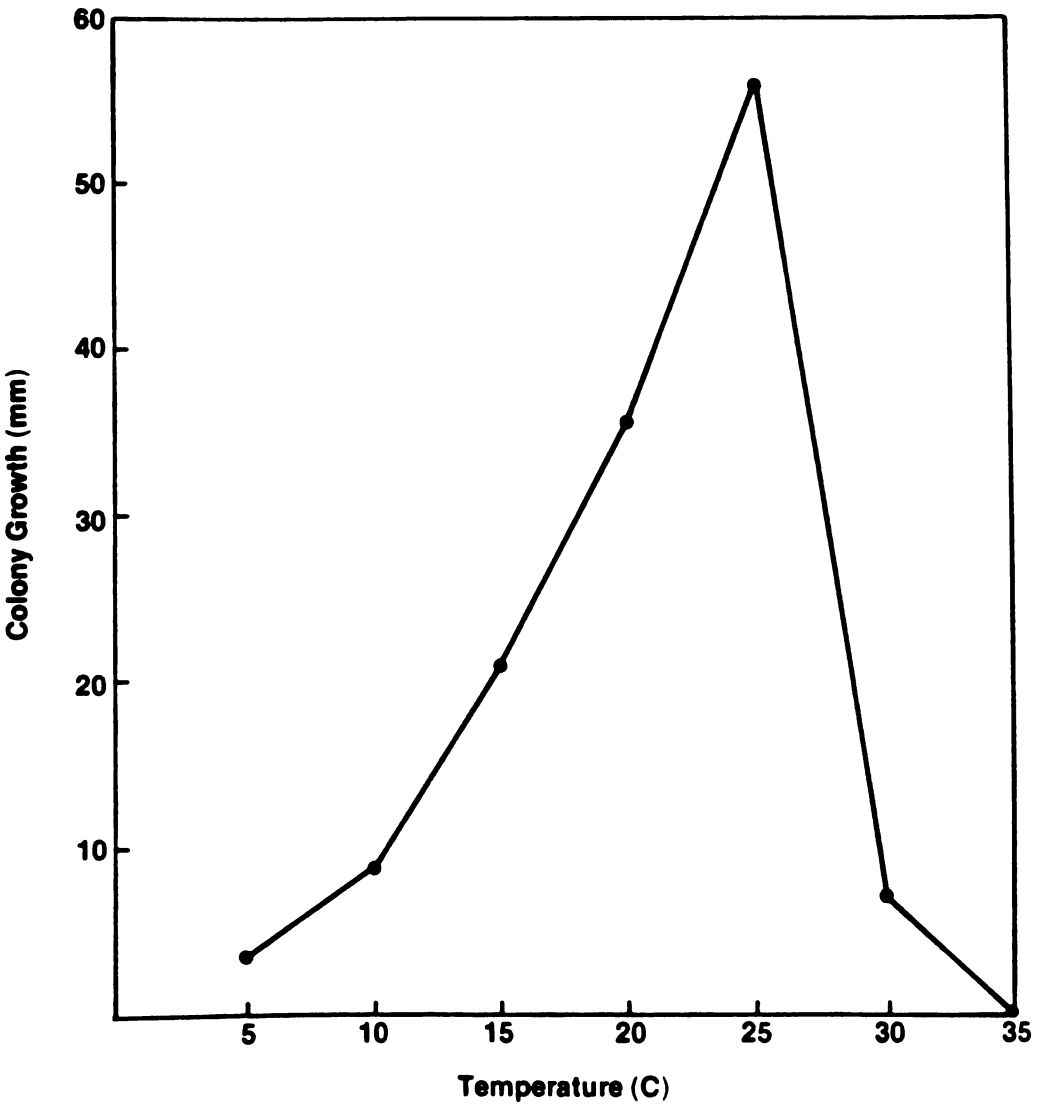
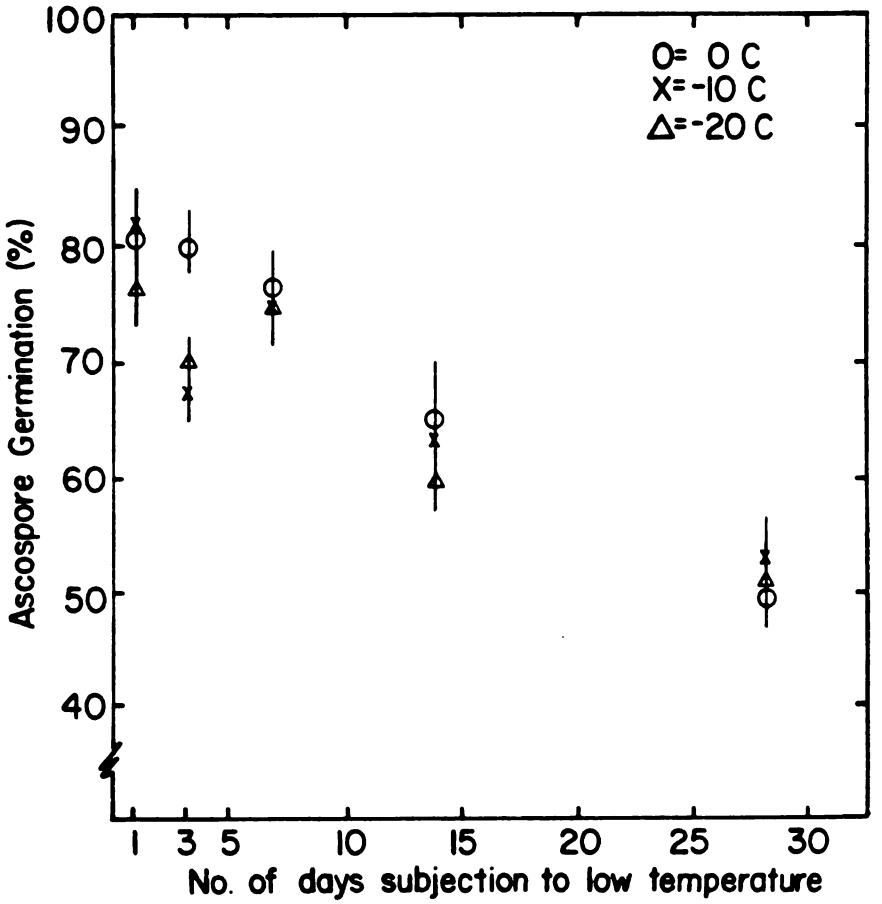

60
50
40
30
20
10
Colony Growth (mm)
5
10
15
20
25
30
35
Temperature (C)

Figure 9.--Effect of subjecting aqueous suspensions of Eutypa armeniacae ascospores to various time periods of freezing or below-freezing temperatures, prior to thawing and germinating the spores on 2% PDA at 10 C for 3.5 days. Vertical lines indicate ± 1 standard deviation.


100
90
80
70
60
50
40
Ascospore Germination (%)
O= 0 C
X=-10 C
Δ=-20 C
1 3 5 10 15 20 25 30
No. of days subjection to low temperature

spores kept at these three temperatures for 14 days germinated while spores held for only one day germinated from 76 to 81%.

In a second experiment, ascospores were exposed to cycles of freezing for three days at -10 C and thawing at 10 C for six hours. Suspensions were exposed to from one to five cycles before germinating the spores at 10 C for 3.5 days. Germination of ascospores decreased slightly with increasing numbers of freeze-thaw cycles (Figure 10). One cycle of freeze-thaw permitted 93% germination, while five cycles permitted 85% germination.

Mycelial Survival and Growth at Low Temperatures

Mycelial growth at 10 C after exposure to -10 to -20 C for one, three, or seven days was reduced 52 to 62% compared with that after exposure to 0 C for the same period (Figure 11). The length of exposure at each temperature did not affect the growth rate. These growth rates were measured after eight days at 10 C on PDA.

Effect of Alternate Wetting and Drying on Spore Germination

Ascospores deposited upon pruning wounds in the fall and spring may be exposed to periods of dryness alternating with periods of free water. Eutypa armeniacae ascospores were placed on PDA at 25 C for 24 hours after varying numbers of cycles of suspension in water followed by three days of dryness. Initial germination of ascospores placed directly on PDA was 83.5%. After one cycle of dryness for three days, resuspended spores germinated at only 18.4% and after a second cycle of dryness for three days germination was zero.

Figure 10.--Effect of alternate cycles of freezing and thawing on germination of Eutypa armeniacae ascospores in an aqueous suspension. Vertical lines indicate ± 1 standard deviation.

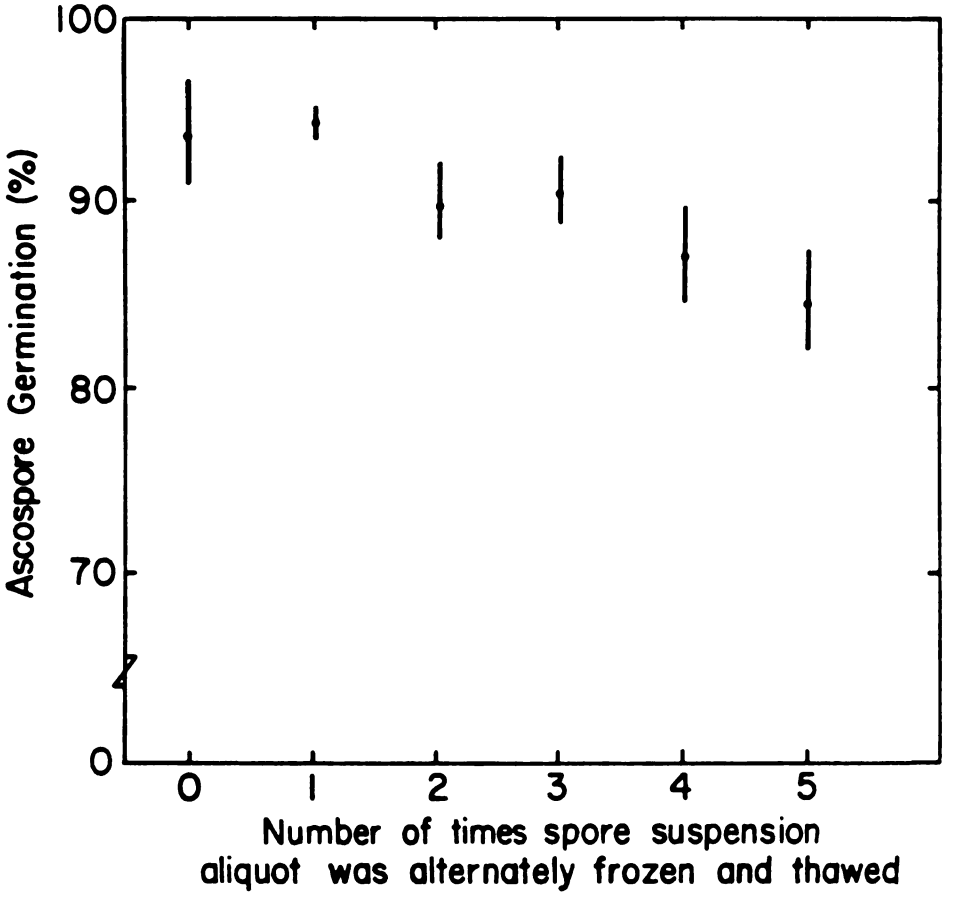
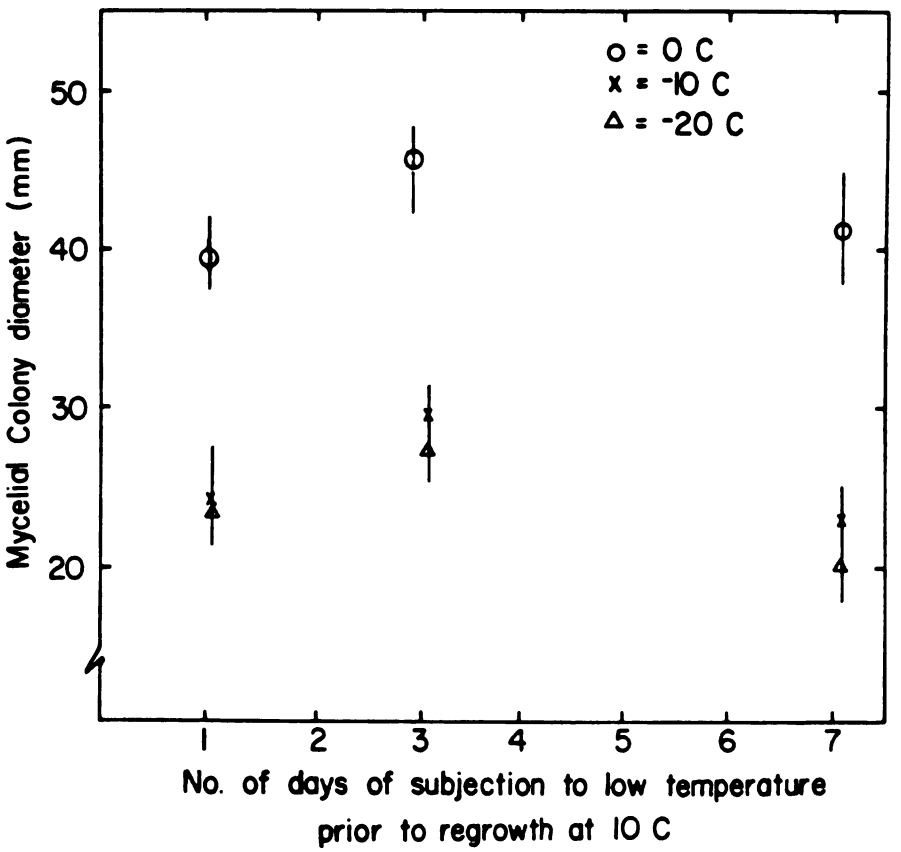

100
90
80
70
0
Ascospore Germination (%)
0
1
2
3
4
5
Number of times spore suspension
aliquot was alternately frozen and thawed

Figure 11.--Effect of freezing and below freezing temperature upon survival and growth of Eutypa armeniacae mycelium. Petri plates containing 2% PDA and 8 mm agar plugs of mycelium were subjected to freezing or below-freezing temperatures for one, three or seven days prior to thawing and regrowth for eight days at 10 C. Vertical lines indicate ± 1 standard deviation.


o = 0 C
x = -10 C
Δ = -20 C
Mycelial Colony diameter (mm)
50
40
30
20
1
2
3
4
5
6
7
No. of days of subjection to low temperature
prior to regrowth at 10 C

Field Infection of Trap Plants

The results of isolating from pruning wounds made on potted vines kept at the Lawton field station in the spring of 1978 are given in Table 2. Five vines were kept at Lawton for the interval 19 January to 16 March 1978. The first rain of 1978 fell on 14 March and presumably exposed these wounds to E. armeniacae ascospores. One E. armeniacae infection was found in six two-year-old pruning stubs examined for this period. Eight wounds of two-year-old canes were examined from vines exposed through the 16 March to 6 May, 1978 interval and one positive was found. Numerous rains had occurred during this time and these wounds had been exposed to ascospores repeatedly. From the vines exposed from 6 May to 15 June 1978 no E. armeniacae infections were found upon isolation from a total of 18 pruning wounds. Spore discharge diminished substantially after 15 April 1978 as seen in Figure 6, indicating that these wounds were not exposed to large numbers of ascospores.

Infection Studies With Potted Plants

Table 3 summarizes the results of the dormant season controlled inoculation experiment. Chi-square analysis was done to test the three null hypotheses: i. H_0: Infection is independent of the age of wood pruned (one versus two-year-old); ii. H_0: Infection is independent of the winter pruning date; iii. H_0: Infection is independent of the time interval in days between pruning and application of inoculum to the pruning wound. At $P = 0.05$, none of the null hypotheses were refuted.

Table 2.--Natural Infection of Pruned Potted Vines in a Vineyard at Lawton, Michigan, in 1978.[a]

Interval in the field[b]	Age of Wood Pruned	
	One Year	Two Years
1/19-3/16	0/7[c]	1/6
3/16-5/6	0/9	1/8
5/6-6/15	0/10	0/8

[a]Five four-year-old plants were used per interval in the field.

[b]On each date the plants in the field were removed and five new ones replaced them.

[c]The numerator denotes the number of pruning sites infected. The denominator denotes the number of pruning cuts made on five vines (one or two-year old wood).

Table 3.--Effect of Age of 'Concord' Grape Cane Wood (Years) and Pruning Wounds (Days) Upon Infection by Eutypa armeniacae Ascospores.[a]

Pruning Date 1978[c]	Age of Cane Wood Pruned (Years)	Number of days elapsed between making pruning cut and inoculation[b]							
		0	1	3	7	14	28	56	Totals[d]
10 January	1	2/9[e]	0/5	2/5	0/4	0/5	0/4	0/4	4/36 (11.1%)
	2	0/6	0/6	2/4	0/6	1/5	0/6	0/5	3/38 (7.9%)
8 March	1	0/5	0/5	1/5	0/4	0/5	0/3	0/4	1/32 (3.1%)
	2	0/7	0/7	0/5	1/6	1/6	2/7	1/11	4/49 (8.2%)
17 May	1	0/3	2/12	2/13	3/7	0/8	0/8	0/17	7/68 (10.3%)
	2	1/13	2/12	2/11	3/11	1/8	0/10	0/17	9/82 (11.0%)
Totals[f]	1	2/17 (11.8%)	2/22 (9.1%)	5/23 (21.7%)	3/15 (20.0%)	0/18 (0.0%)	0/15 0.0%)	0/16 (0.0%)	
	2	1/26 (3.8%)	2/25 (8.0%)	4/20 (20.0%)	4/23 (17.4%)	3/19 (15.8%)	2/23 (8.7%)	1/27 (3.7%)	

[a]Four-year-old 'Concord' grapevines in 20-liter cans were inoculated and held in a cold storage room at a temperature of -1 to 1 C. The vines were stored outside during the growing season.

[b]Inoculation consisted of applying a 5 µl drop of water containing 250 ascospores of Eutypa armeniacae on each pruning wound at the indicated time in days after pruning. Sets of 35 vines were pruned on each of three dates and pruning cuts on five vines were inoculated at each interval after pruning.

[c]Chi-square tests were run to test the following null hypotheses: i. H_0: Infection is independent of age of wood. ii. H_0: Infection is independent of winter pruning date. iii. H_0: Infection is independent of the time interval in days between pruning and application of inoculum to the pruning wound. At $P = 0.05$ none of the hypotheses are refuted.

[d]Total infections (irrespective of number of days elapsed between making pruning cuts and inoculation) versus age of wood and pruning date.

[e]The numerator signifies the number of pruning wounds that were infected as determined by reisolation of the pathogen onto PDA. The denominator signifies the number of pruning wounds that were inoculated on a given date.

[f]Total infections versus number of days elapsed time between making a pruning cut and inoculation, irrespective of the pruning date (time of year).

The data also show that inoculations made as long as 56 days after a pruning cut was made resulted in a low percentage of infection. Under the conditions of this experiment 8.6% (grand mean of all inoculation dates) of one and two-year-old pruning wounds became infected as a result of inoculum being applied to a pruning cut. During reisolation procedures a large number of saprophytic fungi were isolated onto PDA from inoculated pruning stubs, along with E. armeniacae. Their presence may have reduced the percentage of E. armeniacae colonies successfully isolated.

Infection Studies with Mature Vines in the Field

Results of the field site inoculations done at Lawton are summarized in Table 4. Chi-square analysis was done to test the three null hypotheses: i. H_0: Infection is independent of inoculation; ii. H_0: Infection is independent of age of wood inoculated; iii. H_0: Infection is independent of the date on which pruning and inoculation is done. At $P = 0.05$ i and iii were refuted. These data agree with those obtained using potted plants in showing that infection levels were the same for all ages of wood tested, but differ from them in that the late February and late March, 1979, inoculations resulted in significantly higher levels of infection than other inoculation dates.

The inoculation procedure used was shown to be effective in increasing the amount of infections established in the pruning wounds. There was a low level of "background" infection in the

Table 4.--Effect of Age of 'Concord' Grape Wood and Date of Pruning Upon Infection by Eutypa armeniacae Ascospores in a Mature Vineyard. Lawton, Michigan, 1978-79.

Pruning Date 1978/79	Inoculated Pruning Sites[a,g] Age of Wood Pruned 1 yr.	2 yrs.	3 yrs.	Overall % Infection[b]	Non-Inoculated Pruning Sites[c,g] Age of Wood Pruned 1 yr.	2 yrs.	3 yrs.
3/9/78	0/14[d]	1/29	0/3	2%	1/17	0/31	0/7
3/30/78	1/26	7/40	0/5	11%	0/16	1/29	1/4
4/20/78	2/19	5/40	0/3	11%	0/15	0/28	0/4
5/18/78	5/25	2/37	0/3	11%	0/22	0/31	0/2
11/29/78	--[e]	2/26	1/23	6%	--	0/26	1/23
12/20/78	--	0/23	1/24	2%	--	0/19	0/27
2/22/79	5/16	12/24	10/24	42%	0/16	0/23	0/19
3/30/79	4/20	6/26	5/27	20%	0/18	0/24	0/25
Totals[f]	17/128 (13.3%)	35/245 (14.3%)	17/112 (15.2%)		1/104 (1.0%)	1/211 (0.5%)	2/111 (2.0%)

[a]Pruning cuts were made and the sites immediately inoculated with a spore suspension of 250 ascospores of Eutypa armeniacae in 5 µl of water.

[b]The overall infection by date includes all three ages of wood that were pruned and inoculated.

[c]Five µl of water was applied to each pruning wound on non-inoculated sites.

[d]Numerator denotes the number of inoculated sites infected with E. armeniacae and the denominator denotes the number of pruning wounds, either inoculated or non-inoculated.

[e]No inoculations were made or controls marked for one year canes on these dates.

[f]Total number of infected pruning sites per age category of wood pruned.

[g]Chi-square tests were run to test the following null hypotheses: i. H_0: Infection is independent of inoculation; ii. H_0: Infection is independent of the age of wood inoculated; iii. H_0: Infection is independent of date on which pruning and inoculating is done. At $P = 0.05$ i and iii were refuted.

non-inoculated pruning stubs. The nearest known source of inoculum was 0.4 km distant. Infection in these control sites averaged 1.0%. Saprophytic fungi were commonly isolated from both the inoculated and the control pruning stubs in this experiment. Their presence may have reduced the number of E. armeniacae colonies isolated.

On all dates examined some infection did result from the inoculations. February 22, 1979, was one inoculation date that resulted in significantly higher infection than the other dates. This date corresponded to the first period of above freezing temperatures at the Lawton field station in 1979.

In Vitro Evaluation of Fungicides for the Control of Eutypa armeniacae

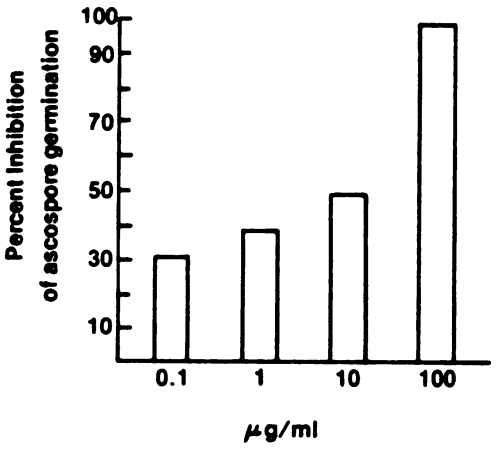
Of the seven fungicides tested against Eutypa armeniacae ascospore germination by the poison agar method four were 100% effective in preventing germination at all concentrations (Figure 12). These fungicides were Difolatan^{R} (captafol), Bravo^{R} (chlorothalonil), captan and Phaltan^{R} (folpet).

Of the same seven tested for inhibition of mycelial growth by the poison agar method only Benlate^{R} (benomyl) was 100% effective at all concentrations (Figure 13). Difolatan^{R} was the second most effective.

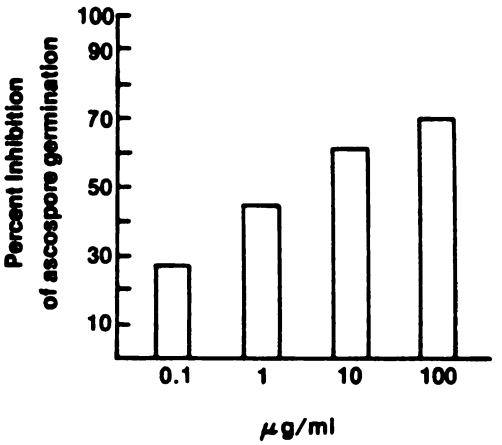
Mechanical Harvester-Induced Injury as Possible Infection Sites

To assess the possible role of mechanical harvesting in the etiology of Eutypa dieback, damaged and broken canes in the Lawton

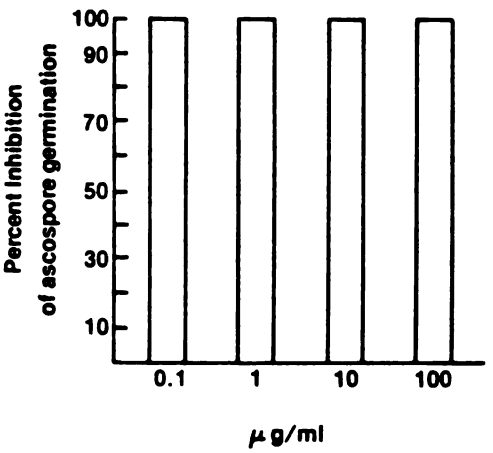
Figure 12.--Inhibition of Eutypa armeniacae ascospore germination by fungicides incorporated in PDA. Measured after 3.5 days at 10 C.



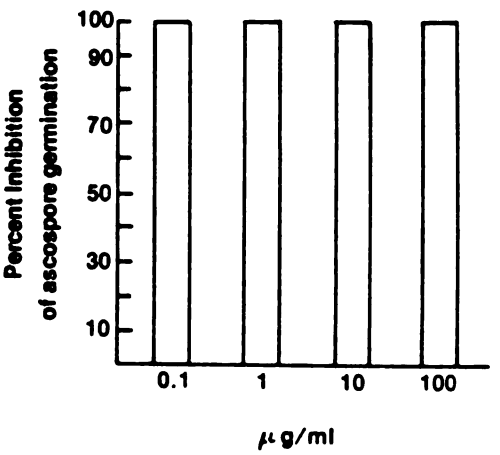
Baymeb



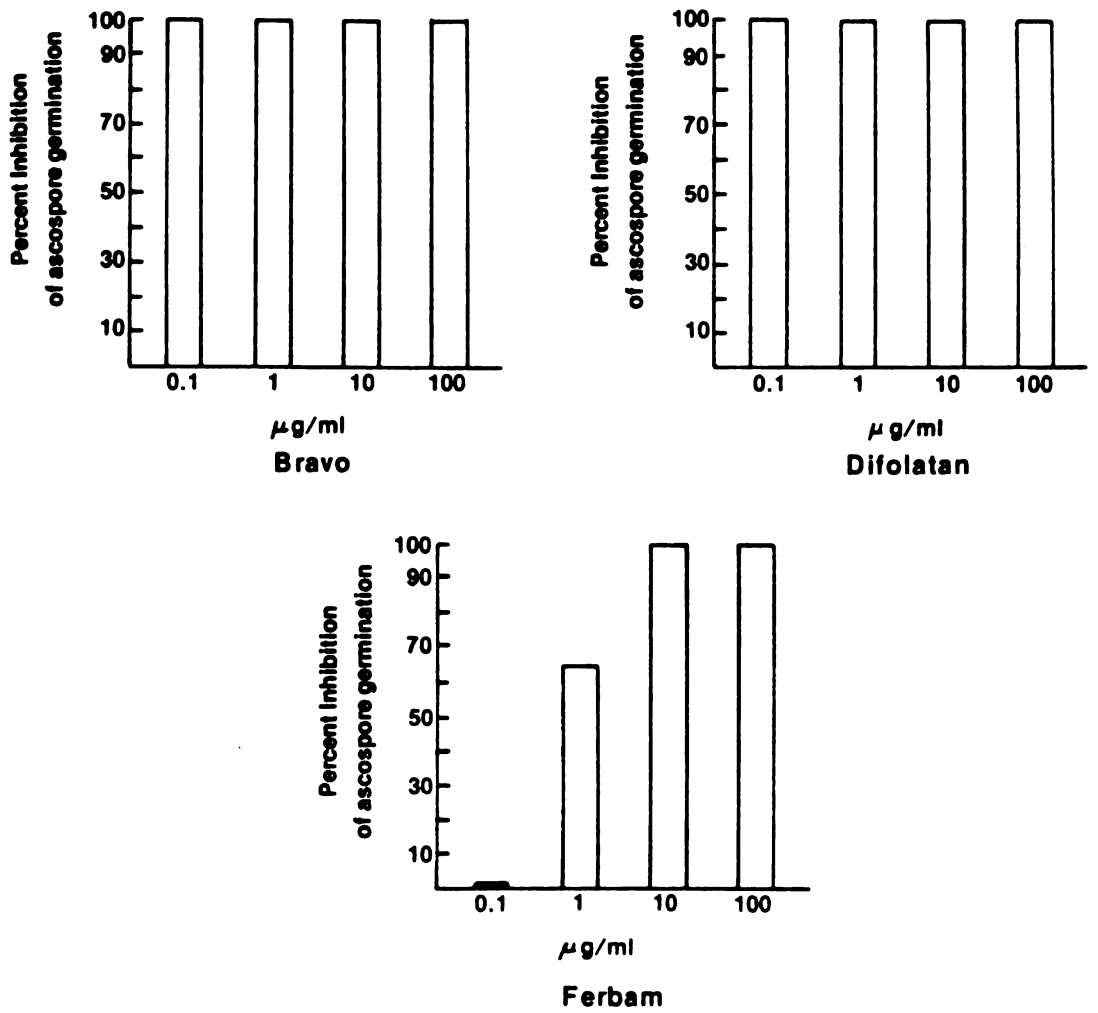
Benlate



Captan

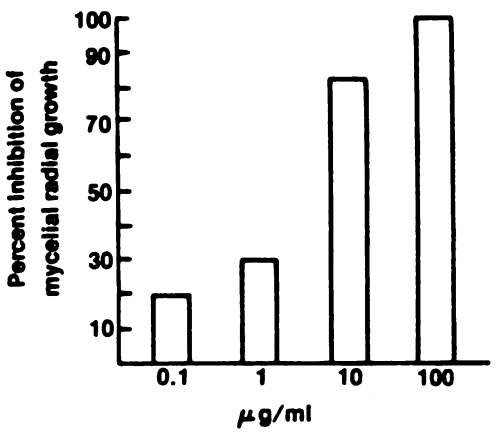


Phaltan

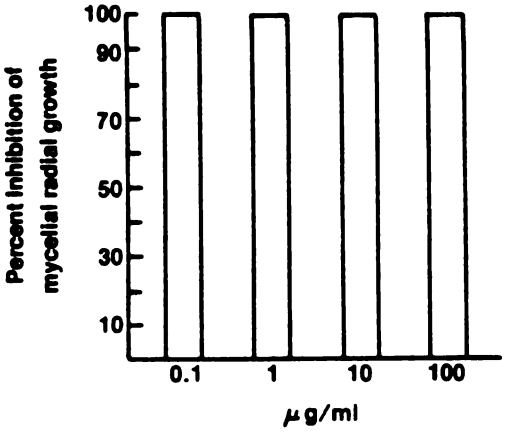


(Figure 12 continued)

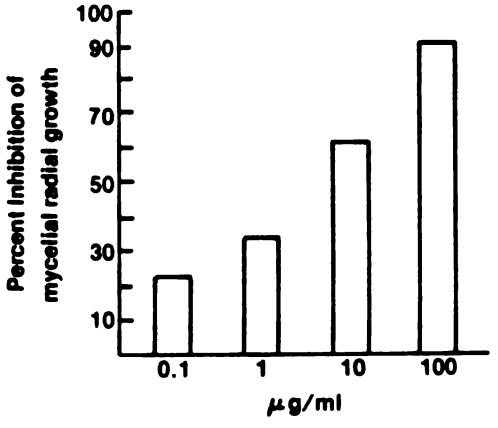
Figure 13.--Growth inhibition of Eutypa armeniacae mycelium after five days by fungicides incorporated into PDA.



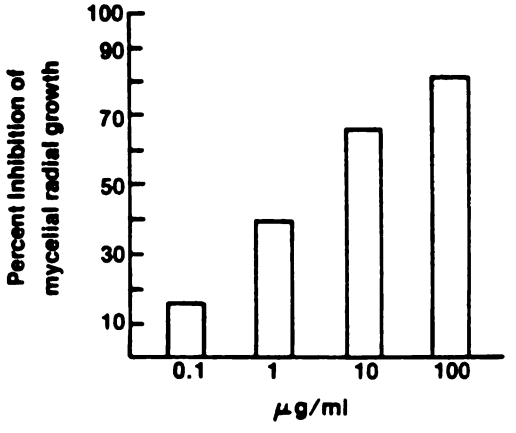
Baymeb



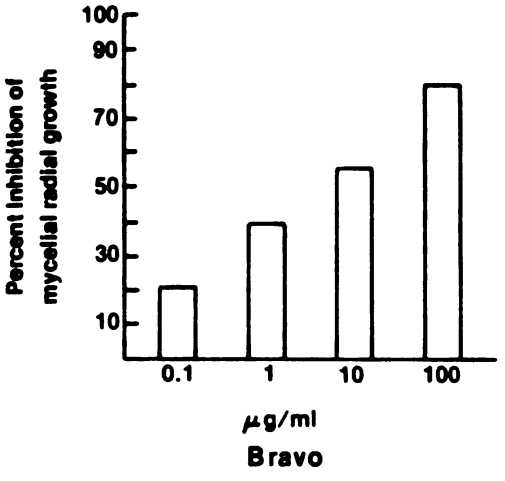
Benlate

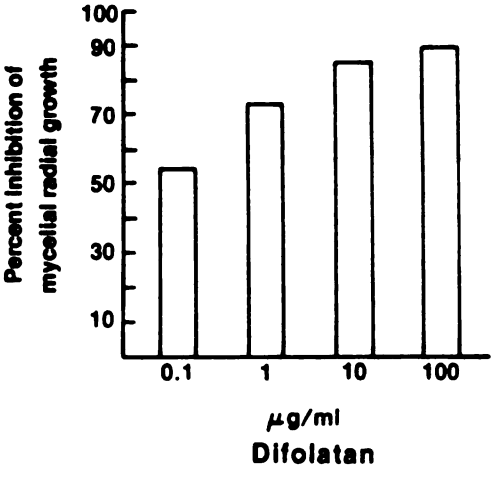


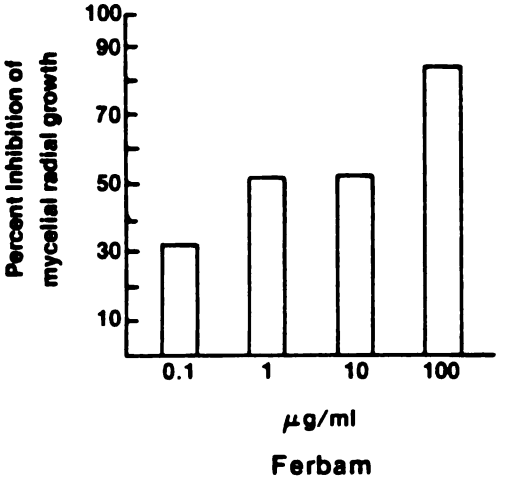
Captan



Phaltan







(Figure 13 continued)

vineyard were tagged following the passage of the mechanical harvester in 1978. Eutypa armeniacae was not isolated from any of the 104 one, two, or three-year-old damaged or broken canes that were examined.

Frost Damage as Possible Infection Sites

Late frost injury to fresh spring growth was considered to be a possible infection site. From the five vines treated by freezing and inoculating, a total of 125 sites were examined. None of these were found to contain E. armeniacae. No E. armeniacae was found in the 125 sites examined from the green growth plants not subjected to freezing.

DISCUSSION

Ascospore octads of Eutypa armeniacae were found to be dispersed following sufficient wetting of the stromata, which requires at least 2 mm of rain or melting snow. This agrees with results published in Australia (3) and California (42) where small amounts of rain caused ascospore octad release. Solar radiation, wind speed, and relative humidity had minimal effect on spore liberation in this study. Temperature became a controlling factor only when it fell below freezing. At other times the temperature was not a decisive factor in determining patterns of spore release.

The seasonal pattern of spore release in Michigan was found to include peak numbers of octads in the late winter or early spring, beginning with the first rains and lasting until late April or early May. Numbers as high as 3,156 octads were recorded during several hours following one rain period during this peak. Numbers of spores trapped from the air during rain periods were very low during the summer months and were often zero. In September and October, spore numbers began to rise again and continued at an intermediate level until mid-winter, when freezing conditions set in, terminating rain periods. These fall spore release periods normally produced lower spore quantities than did rainy periods in the spring.

The seasonal pattern found by Carter in Australia consisted of an autumn peak, with an abrupt drop in May (early winter), very low numbers through June and July (winter), and an abrupt increase in August (spring), but not reaching the high numbers trapped in the fall. The winter season in Australia includes frequent rains but not extended periods of sub-freezing temperatures. The pattern found in California, at Suisun, showed maximum numbers of octads trapped during the early fall (October), while very few were trapped in the late fall (after 15 November). Octad levels increased in January, February, and March and then diminished to almost none through the spring and dry summer. The pattern found in Michigan differs in that the peak of ascospore production for the year is in the spring and there are two seasons with little or no spore liberation, i.e. the winter when constant freezing conditions prevail and in the summer.

The most significant aspect of the seasonal dispersal pattern is that pruning in Michigan is done in the late fall, after the vines have become dormant, or in late winter and early spring. Pruning is seldom done in mid-winter because very cold temperatures and deep snow make it very difficult. In years when the winter is very mild and pruning can be done in January it is probable that some rain periods and spore release will occur at this time also, resulting in infection of pruning wounds. Pruning cannot be done in the summer months because the vines consist of a tangled mass of leaves and canes. The two periods of spore liberation that were

identified coincide with the only practical seasons for pruning.

Ascospore dispersal in the late fall, winter, and early spring and pruning wound production at this time suggests that these may be major sites of infection and that these may be the times of the year that infection takes place. Environmental conditions during these seasons include days as warm as 10 C followed by nights as low as -10 C, variations between warm and cold trends that last for several days each, and long periods of subfreezing conditions, as low as -20 C. These conditions do not appear to be conducive to ascospore germination or mycelial growth. The optimum for ascospore germination was found to be 25 C, as was the optimum for mycelial growth. Spores did germinate at 5 C after four or five days and some mycelial growth did occur at 5 C.

Experiments designed to test the capability of E. armeniacae ascospores to withstand extended periods of cold temperatures indicated that the spores can tolerate low temperatures (-20 C), in a water suspension, for as long as 28 days and still germinate. Spores were also able to germinate after as many as five cycles of freezing and thawing, from -10 to 10 C, in a water suspension. Mycelial plugs resumed growth at 10 C after being held for periods of up to seven days at -20 C. These results indicate that E. armeniacae ascospores may be able to infect freshly made pruning wounds in the fall, early winter, or spring, during short warm periods, and that the infection could continue to develop during

later warm periods. After April 1, daytime temperatures range between 5 C and 25 C. During the fall pruning time, daily temperatures range between 5 C and 20 C, which would permit infection. It is possible that if the spores became alternately wet and dry several times before conditions were favorable for infection, spore viability would be impaired and little infection would result.

Eutypa armeniacae has been known as a pruning wound invader of apricots since 1955 (2). More recently, Moller and Kasimatis (35) have shown that E. armeniacae is capable of actively invading grape vine pruning wounds when applied as a mycelial plug. In the spring of 1978 potted grape vines were set out in the Lawton vineyard for intervals and then brought back to East Lansing and kept in relative isolation from E. armeniacae ascospores. One positive infection was found in each of the first two sets of plants, from a two-year-old cane in each case. The first set of five plants was exposed to only one rain and spore release period, 14 March. The second interval contained at least 15 rain periods with high levels of spore release. The third interval began on 6 May, after the spring peak in numbers had passed, plants were thus exposed to fewer spores, despite the frequent rain periods. These results show that natural infection of pruning cuts on two-year-old canes does occur in the field at a measurable level during the late winter and early spring season, when the environment is far from the optimum for ascospore germination and growth.

Pruning wounds are made in the fall and early spring in Michigan, coinciding with the highest levels of ascospore release.

The overlapping patterns suggest that these pruning wounds may be major sites of infection. It was shown under Australian conditions (10, 27) that pruning apricot trees in winter at the completion of leaf drop substantially reduced the amount of infection compared to that after pruning at other times of the season when ascospore inoculum was much more abundant. Similarily, in a relatively dry apricot growing area near Tracy, California (15), it was found that pruning in February, March or April resulted in a significant reduction in pruning wound infection. This timing coincided with a marked reduction in ascospore inoculum.

The data from inoculations of pruning wounds on potted vines was analyzed using the chi-square test. At $\underline{P} = 0.05$, there was no significant difference among the three dates on which pruning was done. There was also no significant difference in infection levels of one and two-year-old canes. The third analysis concerned the effects of elapsed time between pruning and inoculation upon infection levels. This was done to find if pruning wounds became less susceptible to infection with the passage of time. There was no significant difference between the wounds inoculated soon after pruning and the wounds inoculated after 56 days. This may have been due to small sample size and low levels of infection. The plants used were necessarily kept indoors for the first two inoculation series and this may have obscured a decline in susceptibility that would have occurred if the plants had been kept outdoors, exposed to the natural airborne microflora. Carter and Moller (11) found that apricot wounds became resistant to infection within 15 days

if the wounds were unsheltered from the weather. Wounds on sheltered trees remained susceptible for longer periods of time.

Chi-square analysis of the data from the field inoculation of mature vines revealed some useful information. In comparing percent infection totals for the eight dates of pruning there was, at $\underline{P} = 0.05$, a significant difference. The highest total level of infection was recorded for the 22 February, 1979 inoculation. The second highest was from the inoculation on 30 March, 1979, five weeks later. The pruning dates that produced the lowest level of infection were 20 December and 9 March, 1978. On the calendar, the second lowest date for 1978, 9 March, falls directly between with the greatest amount of infection dates one year later. This indicates that there is a great amount of yearly variation and that the immediate environmental conditions may be more influential in determining infection levels than the calendar date. These results demonstrate that there is no time during the possible pruning range when the vines are clearly less susceptible to infection. There is some indication that the fall pruning time may be better than the spring, but it is not certain that this is the case every year. It is possible that 250 ascospores on each wound was excessive and that the method of inoculation obscured some natural differences in relative susceptibility at different times of the year by using artificially high inoculum levels.

The results of the inoculation experiment, as expressed in percent infection, are influenced by the specific weather conditions prevalent immediately following the inoculations and for several

weeks afterward. These environmental parameters probably play a substantial role in determining the resulting infection levels. However, there is no clear understanding of the optimal environmental conditions necessary for initiation of infection, nor what conditions will prevent or deter infection. Therefore, it is not appropriate to examine, arbitrarily, the weather parameters measured during the period following the inoculations on these eight dates because no valid conclusions can be made.

Inoculations made on 22 February, 1979, resulted in 42% infection, double the second highest level. It is difficult to determine what factors were important in influencing these results, but one feasible explanation is that this was the first period of above freezing temperatures during the winter of 1979. The long period of very cold weather and the very deep snow covering most of the plant litter suggests that there would be reduced levels of airborne microorganisms at this time. It is possible that the ability of E. armeniacae to initiate infection was enhanced by the relative absence of competing microorganisms at the pruning wound site.

The control vines had a total of four positive Eutypa armeniacae infections out of 426 pruning sites examined (<1%) and apparently represents the natural, background infection level in this vineyard. However, the vineyard used for this experiment was relatively young and no stromata of E. armeniacae were known to be present on the vines. The nearest known source of inoculum was 0.4 km north, the site of the spore trapping station. A more accurate

estimate of the natural infection levels might be obtained by isolating from a large number of pruning wounds made throughout the year at an older vineyard that has stromata present on some of the vines.

The field plot experiment should be repeated for another year, possibly using fewer spores per inoculation site. An improvement would be to prune, inoculate, and cover each individual site with an aluminum foil cap, doing an equal number of covered and uncovered sites on each date. This might help reduce the inconsistencies in the infection data caused by variable weather conditions and competition from other microorganisms.

The preliminary fungicide tests demonstrated that benomyl was the most effective in preventing mycelial growth of the pathogen in vitro. Captafol was second most effective in reducing mycelial growth and was 100% effective in preventing ascospore germination at all concentrations tested. These two fungicides were chosen for evaluation in field plots.

No E. armeniacae was isolated from 104 harvester-damaged canes that were tagged during the 1978 harvest at the Lawton vineyard containing the spore trapping equipment. Rain fell the day of harvest and the day after, exposing the wounds to inoculum. There is no evidence that mechanical harvesters increase the incidence of Eutypa dieback through damaging the vines during harvest. This experiment should be repeated to obtain another year of data. It should be noted that many of the damaged canes in a vineyard would be removed during the winter pruning operations.

No E. armeniacae was isolated from the five vines that were subjected to a simulated frost and inoculated. There was also no E. armeniacae isolated from the five vines that were inoculated at the same growth stage (canes six to eight inches long) without prior freezing damage. These results, and those from the mechanical harvester experiment, imply that pruning wounds are the major source of infection sites on grapes in Michigan.

The results of this research show that there is a seasonal variation in airborne spore numbers following a rain. Ascospores appear to be capable of initiating an infection in either the fall, late winter, or early spring. Mechanical harvesters and late spring frosts do not seem to be important infection sites, leaving pruning wounds as the major sites. Under some conditions pruning wounds remained susceptible for at least eight weeks. Pruning is presently done in the fall and early spring. Changing the pruning time to mid-winter to avoid ascospore discharge is often impractical and is not certain to reduce infection levels. Fungicide and biological control alternatives should be investigated.

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