

***Eutypa armeniaca* in Michigan Vineyards: Ascospore Production and Survival, Host Infection, and Fungal Growth at Low Temperatures**

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This research was supported in part by a line item appropriation for grape research from the Michigan State Legislature and reserve funds from the Director of AR/SEA-USDA.

The work reported in this publication is in partial fulfillment of the MSc. degree by the senior author.

Journal series Article 9022 of the Michigan State Agricultural Experiment Station.

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Accepted for publication 29 February 1980.

ABSTRACT

TRESE, A. T., C. L. BURTON, and D. C. RAMSDELL. 1980. *Eutypa armeniaca* in Michigan vineyards: ascospore production and survival, host infection, and fungal growth at low temperatures. *Phytopathology* 70:788-793.

The seasonal abundance of airborne *Eutypa armeniaca* ascospores was sampled over a 2-yr period at two *Vitis labrusca* 'Concord' vineyards in southern Michigan. Burkard volumetric recording spore traps placed within vineyards were encircled with a 2-m-diameter circle of diseased vine trunks bearing mature perithecial stromata. Airborne ascospore octads were found only after stromata had been exposed to a minimum of ~2 mm of rainfall at temperatures > 0 C. Free water (vine trunk wetness) maintained by prolonged rain resulted in continued dispersal of ascospore octads. The highest octad concentrations were evident in spring (late February through April); this peak was followed by a decline in the summer (June through August) and a rise in numbers of octads dispersed in September, October, and November. No octads were trapped from mid-

December through late February, because of subfreezing weather conditions during which no rain fell. Under laboratory conditions, ascospores maintained a high level of viability after subjection to long periods of freezing and to repeated cycles of freezing and thawing. Alternate wetting and drying had an adverse effect on spore germination. Such conditions are prevalent in the field during the periods of maximal inoculum dispersal, which coincide with the main part of seasonal vine pruning operations. Mycelium grown in vitro was capable of renewed growth after freezing. Controlled inoculations of 1- and 2-yr-old pruning cuts made on potted 4-yr-old vines held at -1 to 1 C resulted in a low level of infection during the winter months.

Additional key words: epidemiology, *Eutypa dieback*, *Vitis*, infection.

Although recognized as a vascular pathogen of apricot, *Prunus armeniaca* L., for many years (1-3,9,16) *Eutypa armeniaca* Hansf. & Carter has only recently been shown to be associated with a vascular dieback disease of grape (5,13). Moller and Kasimatis (12) completed Koch's postulates, demonstrating that *E. armeniaca* 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, vascular discoloration, and eventual death of one or more vine arms. These symptoms,

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collectively designated "deadarm disease" by earlier workers (8,17), were attributed entirely to invasion of the vine by the fungus *Phomopsis viticola* Sacc. It is now clear, however, that while these workers correctly attributed the leaf and cane spot disease symptoms on the annually produced parts of the vine to *P. viticola*, they were mistaken in believing that *P. viticola* was a primary invader and destroyer of the xylem, causing the well-known and spectacular "deadarm" symptoms that gave rise to the popular name. In fact, these researchers were observing a syndrome associated with the operation of two pathogens, one of which they diagnosed correctly (*P. viticola*) while they remained unaware of the presence of the other (*E. armeniaca*).

Eutypa armeniaca perithecia and ascospores recently have been

found in diseased grapevines in New York and in Ontario, Canada (10,18). The presence of *E. armeniaca* ascospores in perithecial stromata on old dead vine trunks was confirmed in Michigan during 1977 (W.J. Moller, D.C. Ramsdell, and A.T. Trese, unpublished).

Little is known about the epidemiology of *E. armeniaca* in grape, especially under the cold climatic conditions present in eastern North America. However, considerable work has been done in Australia and California on the epidemiology of the pathogen in apricot. The pattern of ascospore production has been recorded in southeastern Australia (1,11) and in northern California (16). In Australia, small amounts of rain (1.27 mm) caused the release of octads from a "ring source" of stromata-bearing diseased apricot wood surrounding an air spore trap. Ascospores were released abundantly during rains in the spring, summer, and autumn (September through May) and sparsely as a result of winter rains (June through August). In the rainy Suisun area of California, ascospore octads were trapped from air in large numbers after light rainfall during autumn (September and October), the main pruning period (15). Pruning wounds are known to provide the entry points in apricot (2-4,9,15). During the winter (November, December, and early January) far fewer octads were trapped, in spite of abundant rainfall.

This paper reports the results of spore trapping in Michigan vineyards, where the winter season consists of 5 mo of freezing and subfreezing temperatures. The possibility that ascospore octads are released during rainy periods while temperatures are above freezing in late winter and early spring was investigated. In vitro and in vivo experiments were done to determine whether ascospores could survive severe freezing and thawing conditions and still cause infection of pruned vines.

MATERIALS AND METHODS

Spore trapping studies. In January 1978, field stations were established at two *Vitis labrusca* L. 'Concord' vineyards. One was on the Michigan State University campus at East Lansing, which is in the south central lower peninsula. The other station was at Lawton in the southwest corner of the state, about 160 km southwest of East Lansing. The mean annual rain precipitation at East Lansing and Lawton is 77.5 and 82.5 cm, respectively. At East Lansing the station was situated in a 0.2-ha vineyard, in which about 10% of the vines bore stromata of *E. armeniaca*. This vineyard was remote from other vineyards or apricot orchards. The Lawton vineyard was a commercial vineyard (about 10 ha) in which about 15% of the vines bore stromata. This vineyard was surrounded by other commercial vineyards.

A Burkard 7-day recording spore trap (Burkard Scientific [Sales] Ltd., Rickmansworth, England) was operated at each site. Spore traps were operated at an air-sampling rate of 8L/min with the intake orifice 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. Spore trap tape preparation and mounting was done according to the instructions for the Burkard sampler. Environmental data at each station were obtained with a 7-day recording leaf wetness meter (M. DeWit, Hengelo, Holland), a sheltered hygromograph (Bendix Corp., Baltimore, MD 21204), an anemometer, a rain gauge, and a solar pyranograph (all from Weather Measure Corp., Sacramento, CA 95841). All instruments were located 1 m above ground except the anemometer, which was 2 m above ground.

Ascospore octads (hereafter called octads) were counted by transversely scanning trap tapes in 1-hr segments (2 mm wide) with $\times 10$ wide-field oculars and a $\times 20$ objective lens of a compound light microscope. All spore trap tape pieces for a given 24-hr period were stained with cotton blue in lactophenol (11). After numerous trap tapes were examined to verify that spores were released only after sufficient rain had occurred to wet the stromata, counts were made starting 3 hr before a recorded rain and continuing until at least three consecutive hours revealed no octads. Allantoid spores $1.5\text{--}2.0 \times 7.0\text{--}11.0 \mu\text{m}$ were counted in groups of eight as octads per

the published description (7). A spore trap tape section containing *E. armeniaca* octads released in the laboratory and stained in the same manner was used as a reference standard.

Ascospore survival at low temperatures. Ascospores were obtained by immersing pieces of stromata-bearing vine trunk tissue in distilled water for 1 hr and then placing them in a spore collection tower (Plas Labs Co., Lansing, MI 48956). Vacuum was applied and ascospores were collected on glass slides. Spore suspensions were made in glass-distilled water and portions were placed in tubes in constant-temperature boxes 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 incubated for 3.5 days at 10 C on 2% potato dextrose agar (PDA) (Difco Laboratories, Detroit, MI 48232) and assayed for percent germination. Each treatment was replicated three times and consisted of at least 100 spores per replication. Other spore suspensions were frozen at -10 C for 3 days, thawed for 6 hr at 10 C, and then refrozen. This cycle was repeated from one to five times and percent germination was 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. The ability of growing mycelium to survive freezing was examined by placing 8-mm-diameter PDA plugs (from the edge of an expanding colony 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 to a chamber kept at 10 C for 8 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 in a spore settling tower. A spore suspension was made in glass-distilled water. A portion was plated on 2% PDA immediately at 25 C for 24 hr to determine a baseline of percent germination. Another portion was placed in glass depression slides and the water was allowed to evaporate to dryness over a period of 8 hr. After 3 days, the spores were resuspended in distilled water and a portion was plated onto PDA for germination tests at 25 C for 24 hr while another portion was allowed to evaporate to dryness over an 8-hr period. After a second cycle of dryness for 3 days the spores were resuspended in distilled water and placed on PDA for germination again. In all cases, germination percentages were determined by averaging counts of 100 spores from each of three replicate plates. This experiment was done twice.

Pruning wounds as a possible site of infection under cold conditions. An experiment was done to determine: If pruning wounds are the site of infection by *Eutypa armeniaca* ascospores under winter conditions; the effect of pruning date during the winter on the amount of infections; the relative susceptibility of 1- or 2-year-old wood; and the effect of delay periods between making a pruning cut and inoculation of that site.

Thirty-five 4-yr-old Concord grape vines in 20 L cans each were pruned on a given date. A number of pruning cuts were made on 1- and 2-yr-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 dates that pruning cuts were made were 30 January, 8 March, and 17 May 1978. Due to the severity of the winter weather, the plants were kept in a coldroom at -1 to 1 C until mid-April when they were placed out of doors.

Two hundred fifty ascospores in 5 μl of water were applied to each pruning cut with a Hamilton micro syringe (Hamilton Co., Reno, NV 89510). Reisolation of the pathogen was attempted 16-20 mo after inoculation, or two growing seasons later. Ten small chips of woody tissue were aseptically removed from the area below the inoculation site in the center of a longitudinally cut cane piece. The chips were plated out on Difco PDA containing 100 $\mu\text{g/ml}$ streptomycin sulfate. The plates were examined after 4 days and all colonies resembling *Eutypa* were transferred to fresh potato glucose agar (an extract of 200 gm potatoes, 8 gm glucose, 20 gm agar in 1,000 ml distilled water). These plates were placed under cool-white fluorescent light (General Electric F 15T8 CW) and soft black light (General Electric F 30T8 SB) with a 14-hr day length. After about 2 wk the identities of *Eutypa armeniaca* cultures were confirmed by the presence of scolecospores.

Mechanical harvester-induced vine injury as a possible infection site. The possibility that mechanical harvester injuries to canes might become infection sites was assessed. One hundred and four broken canes of 1-, 2-, or 3-yr-old wood were tagged immediately after an over-the-row mechanical harvester had harvested a row of vines at the Lawton, MI farm site. Injured vines were tagged in late October 1978. Rainfall, which probably caused some inoculum release, occurred on the day of harvest and a day later. Isolations were made 1 yr later onto PDA as previously described.

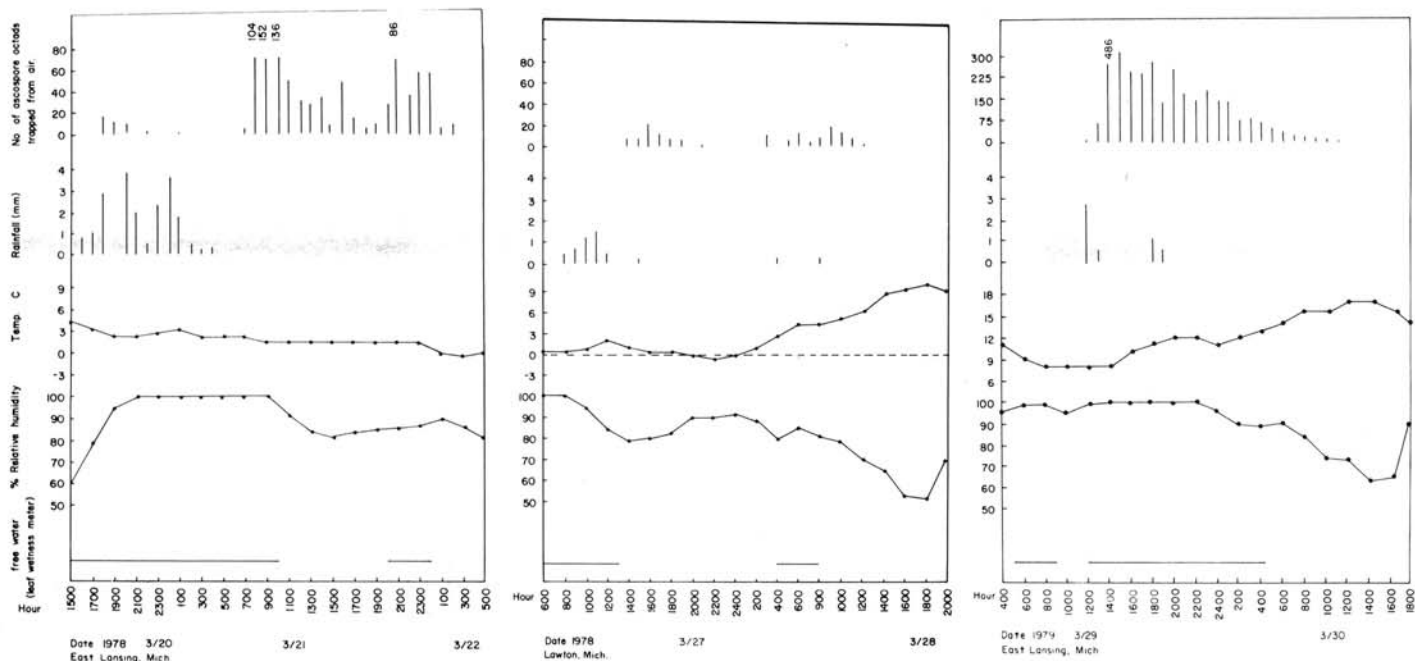
RESULTS

Environmental factors affecting ascospore octad release. Octads were released at both the East Lansing and Lawton vineyard sites after small amounts of rainfall (about 2 mm). Fig. 1 shows an hourly summary of octads trapped from 20 March through early 22 March 1978 at East Lansing. During this time there was about 0.5 m of snow on the ground and the temperature was above freezing. Small amounts of rain, which began at 1530 hours on 20 March and ended at 0400 hours on 21 March 1978, resulted in wetness of vine trunks (as measured by the leaf wetness meter and visual observations) until 1000 hours on 21 March. Octads were trapped in low numbers, beginning 1800 hours on 20 March; their numbers increased to more than 152/0.48 m³ air per hour at 0800 hours on 21 March and they continued to be trapped until 0200 hours on 22 March. Similar results were obtained at the Lawton vineyard in 1978 (Fig. 2), but numbers of octads were lower. It is interesting that at the Lawton site octads were released from 1300 to 2000 hours after a 4-mm rain which fell in 4 hr on 27 March, but no octads were released for a period of 6 hr when the temperature fell below freezing. At 0200 hours on 28 March the temperature rose to 1 C and octad release resumed. A trace of rain (0.5 mm) occurred at 0300 hours which may have aided further octad release. Fig. 3 shows an hourly summary of octads trapped at the East Lansing vineyard from noon 29 March to 1100 hours on 30 March 1979. A small amount of rain (3.25 mm) from 1200 to 1300 hours on 29 March triggered octad release shortly after the rain began. A peak number of 486 octads/0.48 m³ air was trapped at 1300 hours. A small amount of rain occurred from 1800 to 1900 hours. Spore discharge continued until 1300 hours on 30 March, a total of 24 hr. Temperatures during the period of octad discharge ranged from 8 to 17 C.

To determine the cause of the seasonal decline in octad discharge during the summer, in spite of rainfall, during July and August of 1979, stromata-bearing vine trunk pieces were collected from the Lawton vineyard. Attempts to collect octads by soaking trunk pieces and placing them in the spore settling tower produced no spores at that time. Examination of perithecia using a teasing needle and light microscopy revealed that the contents consisted of immature asci. In early September, a total of eight trunk pieces 6 cm in diameter by 12 cm long and bearing stromata were soaked and placed in the spore tower. A total of 106 octads were collected. These pieces were returned to the field until later November, when they again were put in the spore tower. A total of 13,630 octads were collected. Apparently, the reason for very low octad levels during the summer was the immaturity of the asci at that time. Relative humidity, solar radiation (author's, *unpublished*), and wind speed (author's, *unpublished*) did not seem to have any direct influence upon octad release.

Seasonal variation in release of ascospores. A seasonal composite chart is shown for the Lawton vineyard for 1978 (Fig. 4) and the East Lansing vineyard for 1978 and 1979 (Figs. 5 and 6, respectively). 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; at times the snow cover in the vineyards reached 1 m. On 20 March, temperatures were warmer at both locations, and the first rain fell. At the East Lansing vineyard (Fig. 5), each of six 24-hr periods of release of relatively high numbers of octads between 15 March and 15 April was associated with rainfall ranging from 2 to 15 mm. Another 2 mm of rain fell on 15 May after which about 150 octads were trapped. During the late spring-summer period of 16 May to 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 winter conditions set in. A similar pattern was observed in the Lawton vineyard (Fig. 4). 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.

At the East Lansing vineyard in 1979 (Fig. 6), no rains were recorded until late in February, due to subfreezing conditions prior to then. Rains on six different days between late February and



Figs. 1-3. Hourly levels of rainfall, temperature, leaf wetness, relative humidity, and *Eutypa armeniaca* ascospore octads trapped from the air (0.48 m³/hr) using a baited 7-day Burkard volumetric recording spore trap in a *Eutypa* dieback diseased vineyard during 2- or 3-day periods. 1, East Lansing, MI vineyard site, 1978. 2, Lawton, MI vineyard site, 1978. 3, East Lansing, MI vineyard site, 1979.

mid-April each caused large releases of octads. A peak octad release of 3,156/24 hr occurred at the end of March. The seasonal pattern of octad release followed that of 1978; ie, octad numbers declined after mid-April and levels were very low during the summer despite heavy amounts of rainfall. By early October numbers of octads released due to rain increased again. This followed the 1978 pattern also. The last spore trapping reported for 1979 was the end of October.

Ascospore survival at low temperatures. 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 (Fig. 7). Sixty to 65% of spores kept at these three temperatures for 14 days germinated as did 76-81% of spores held for only 1 day.

Germination of ascospores subjected to up to 5 cycles of freezing at -10 C for 3 days and thawing at 10 C for 6 hr decreased slightly with increasing numbers of freeze-thaw cycles (Fig. 8). One cycle of freezing and thawing permitted 93% germination, while five cycles permitted 85% germination.

Mycelial growth at 10 C after exposure to -10 or -20 C for 1, 3, or 7 days was reduced 52-62% compared with that after exposure to 0 C for the same period (Fig. 9). In control plates, mycelium not exposed to low temperatures completely covered the 9-cm diameter petri plate in 8 days. These experiments mimic the conditions that ascospores and mycelia encounter during winter and early spring in the Michigan vineyard.

Effect of alternate wetting and drying on spore germination. Ascospores germinated 83.5% when placed directly on PDA at 25 C. After one cycle of dryness for 3 days, only 18.4% of the resuspended spores germinated, and after a second cycle of dryness for 3 days, none germinated.

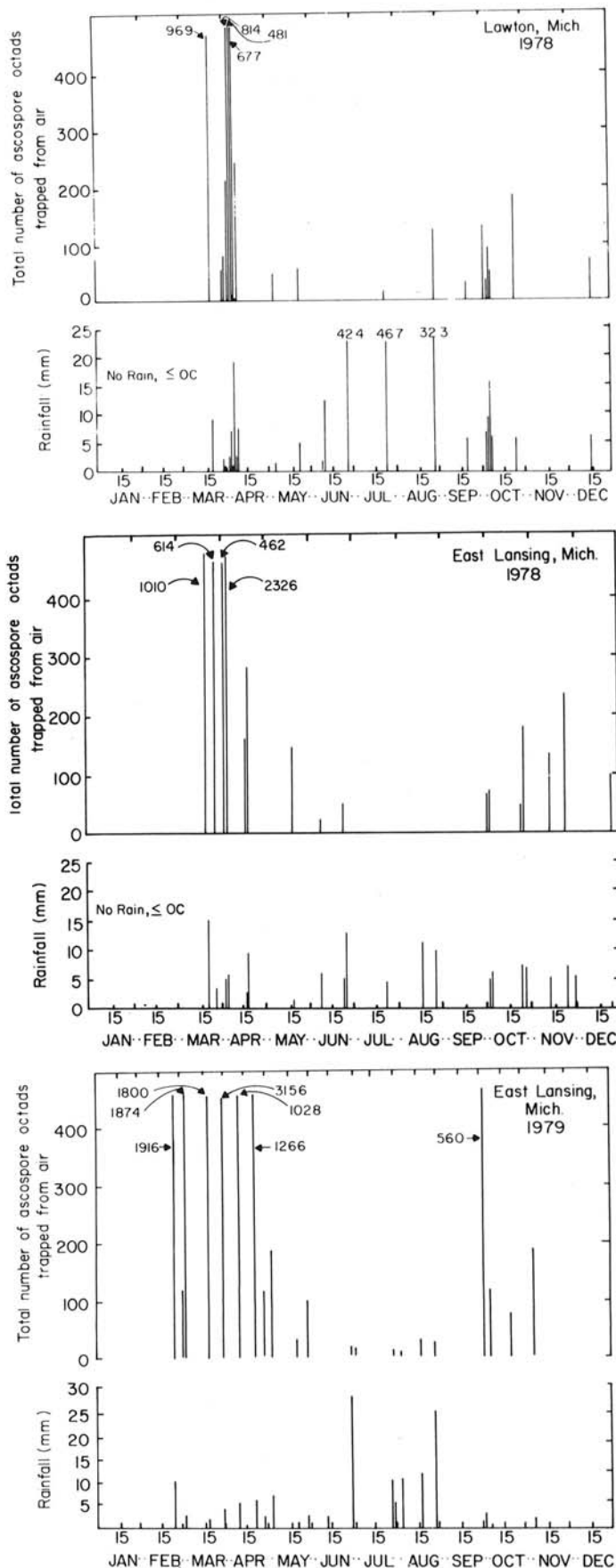
Infection of pruning wounds by ascospores. Table 1 summarizes the results of the dormant season controlled inoculation experiments. Chi square analysis was done to test three null hypotheses: H_0 : Infection is independent of the age of wood pruned (1- versus 2-yr-old); H_0 : Infection is independent of the winter pruning date; 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 was refuted. Under the conditions of this experiment about 8.6% (grand mean of all inoculation dates) of 1- or 2-yr-old pruning wounds were infected as a result of inoculum being applied to a pruning cut. Even as long as 56 days after a pruning cut is made if a pruning wound is inoculated, a low percentage of infection can result. 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 *Eutypa armeniacae* colonies reisolated.

Mechanical harvester-induced cane damage as a possible infection site. None of the 104 damaged canes produced *Eutypa armeniacae* cultures upon isolation from these damaged areas onto PDA 1 yr later. This indicates that mechanical harvester-damaged canes were not a major source of infection court, possibly due to low inoculum levels in fall 1978. Further work needs to be done, including inoculation of broken canes at harvest and continuing to isolate from mechanical harvester-damaged canes.

DISCUSSION

Our data regarding the effects of small amounts of rainfall on ascospore octad release are in general agreement with those of studies from Australia and from the Suisun area in California.

However, the Michigan seasonal octad-release data differ from the Australian apricot-orchard study (11) and the California Suisun area apricot-orchard study (16) in the time of release within the growing season. Under Michigan conditions, maximum numbers of octads were trapped during the spring months of February, March, and April and the fall months of September, October, and November. In contrast, large numbers of octads were trapped in the Australian study during all periods except winter (June, July, and August). In the California study at Suisun, the maximum numbers of octads were trapped during early fall (October), while very few were trapped in late fall (after 15



Figs. 4-6. Seasonal daily 24-hr amounts of *Eutypa armeniacae* ascospore octads trapped from the air ($0.48 \text{ m}^3/\text{hr}$) using a baited 7-day Burkard volumetric recording spore trap in a *Eutypa* dieback-diseased vineyard. Daily 24-hour rainfall totals at the vineyard site are shown. 4, Lawton, MI vineyard site, 1978. 5, East Lansing, MI vineyard site, 1978. 6, East Lansing, MI vineyard site, 1979.

November). Octad levels increased in January, February, and March and then diminished to almost none through the spring and dry summer. Because Moller and Kasimatis (12) have shown that *Eutypa armeniacae* is a pruning-wound pathogen of grape in California and since we have shown that under controlled conditions at -1 to 1 C it can infect pruning wounds in Concord grape, it is probable that ascospore inoculum liberated during the late fall, winter, and spring in Michigan vineyards can cause infection of pruning wounds. Michigan growers begin pruning in late November or early December and continue to prune until early May. Some growers delay pruning until mid-March when the weather is less cold. Our results from ascospore freezing and

thawing tests suggest that inoculum can germinate even during winter and early spring conditions of alternating above-freezing temperatures and very low freezing temperatures (ie, -20 C). If a pruning site became infected during even a 1- or 2-day period above freezing, a sudden plunge to -20 C would simply delay the continuation of the infection process, which would resume when temperatures rose above 0 C. After April 1, daytime temperatures range 5-25 C. During the fall pruning time, daily temperatures range 10-20 C, which would permit infection of pruning wounds. If spores became alternately wet and dry several times before conditions were favorable for infection, spore viability would be impaired, and it is likely that little infection would result. It was

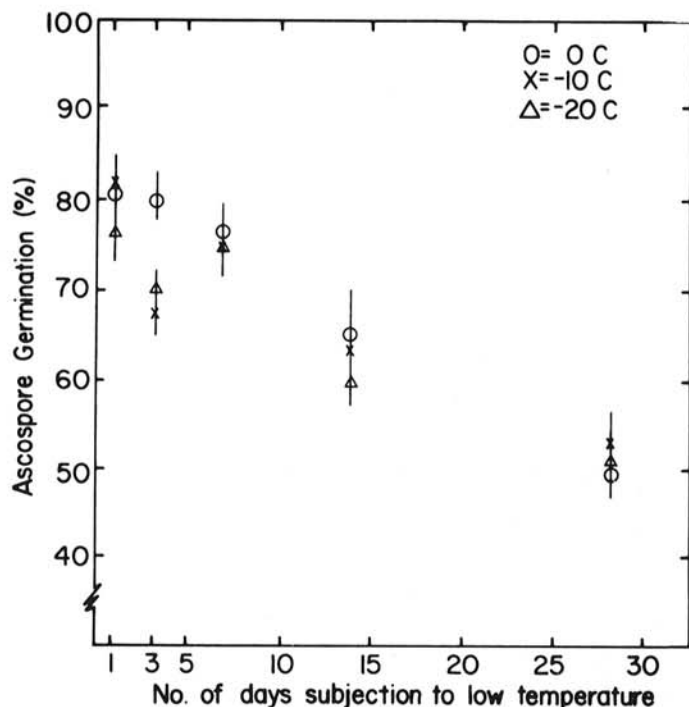


Fig. 7. Effect of subjecting aqueous suspensions of *Eutypa armeniacae* ascospores to 0, -10, or -20 C for various time periods prior to thawing and germinating the spores on 2% PDA at 10 C for 3.5 days. All data points are averages of three replications of at least 100 spores each. Vertical lines indicate ± 1 SD.

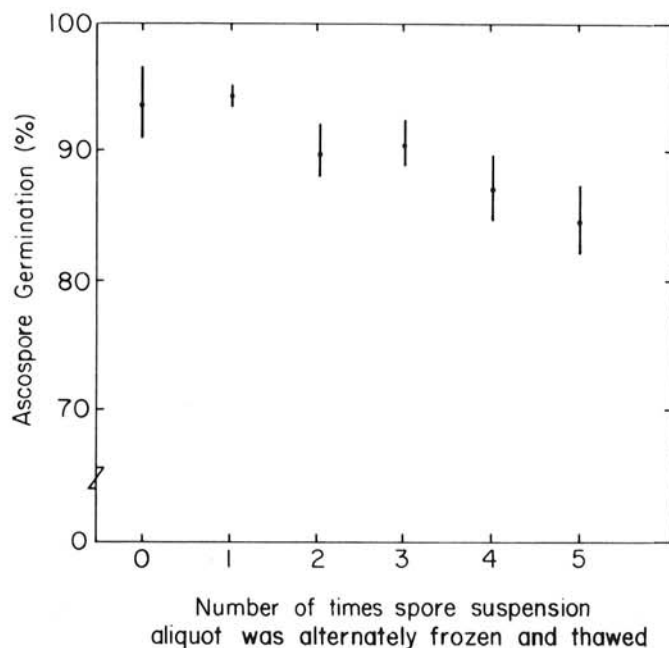


Fig. 8. Effect of alternate cycles of freezing and thawing on germination of *Eutypa armeniacae* ascospores in aqueous suspensions. Aliquots of spore suspensions were frozen at -10 C for 3 days, thawed at 10 C for 6 hr and then were germinated for 3.5 days on 2% PDA at 10 C, or given up to five cycles of alternate freezing and thawing prior to germination. All data points are averages of three replications of at least 100 spores each. Vertical lines indicate ± 1 SD.

TABLE 1. Effect of age on Concord grape cane wood (years) and pruning wounds (days) upon infection by *Eutypa armeniacae* ascospores^a

Pruning Date 1978 ^b	Age of cane wood pruned (years)	No. of days elapsed between making pruning cut and inoculation ^c							Totals ^d
		0	1	3	7	14	28	56	
30 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	0/5	1/32(3.1%)
	2	0/7	0/7	0/5	1/6	1/6	2/7	1/11	5/49(10.2%)
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	2/22	5/23	3/15	0/18	0/15	0/26	
	2	(11.8%)	(9.1%)	(21.7%)	(20.0%)	(0.0%)	(0.0%)	(0.0%)	
		1/26	2/25	4/20	4/23	3/19	2/23	1/33	
		(3.8%)	(8.0%)	(20.0%)	(17.4%)	(15.8%)	(8.7%)	(3.0%)	

^a Four-year-old Concord grapevines in 20 L cans were inoculated and held in a cold storage room at a temperature of -1 to 1 C until mid-April. The vines were stored outside during the growing season.

^b Chi-square tests were run to test the following null hypotheses: H₀: Infection is independent of age of wood; H₀: Infection is independent of winter pruning date; H₀: 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.

^c Inoculation consisted of applying a 5- μ l 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.

^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).

shown under Australian conditions (3,9) 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. Similarly, 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.

According to our data, there are only two periods when pruning might be done to coincide with a very low level of inoculum. These are during January, and the summer months. However, pruning during January is usually very difficult because of extremely low temperatures and deep snow cover. If a January thaw and a rain occurred, infection could result from pruning at that time. During the summer, pruning is not practical because vines consist of a tangled mass of leaves and canes. Since our data show the 1- and 2-yr-old pruned canes can become infected at about the same frequency, this means that 30–50 potentially infectable sites could be present on most mature pruned vines. Winter pruning and inoculation tests in progress in a mature vineyard near Lawton, Michigan should yield information about infection under field

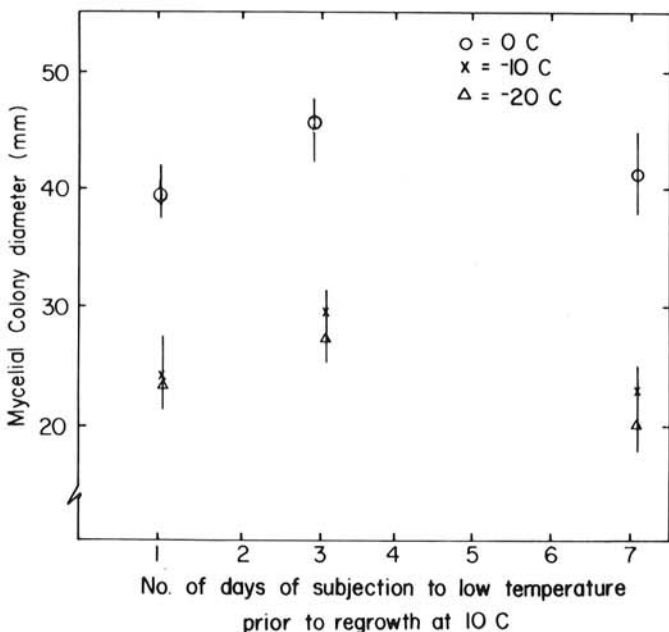


Fig. 9. Effect of freezing and below-freezing temperatures upon survival and growth of *Eutypa armeniaca* mycelium. Agar plugs 8 mm in diameter were taken from the edge of an actively growing colony and centered in a petri plate containing 2% PDA. These were subjected to 0, -10, or -20 C for 1, 3, or 7 days prior to thawing and assay for resumed growth after 8 days at 10 C. Data points are averages of three replicates. Vertical lines indicate ± 1 SD.

conditions in 1–2 yr.

If it is not possible to control the disease by a change in pruning time, it may be feasible to spray a fungicide directly on the wounds at the time of pruning. This procedure has shown promise with apricots in both Australia and California (6,14). Another possibility is the use of biological control with or without chemicals (6), as has been demonstrated in apricots. We have begun field tests of fungicidal spray applications to pruning wounds.

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