EPIDEMIOLOGICAL STUDIES OF THE DISPERSAL OF AND INFECTION BY GUIGNARDIA BIDWELLII (ELLIS) VIALA AND RAVAZ, THE CAUSAL AGENT OF BLACK ROT DISEASE OF 'CONCORD' AND 'NIAGARA' GRAPES, VITIS LABRUSCA L.

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ABSTRACT

EPIDEMIOLOGICAL STUDIES OF THE DISPERSAL OF AND INFECTION BY GUIGNARDIA BIDWELLII (ELLIS) VIALA AND RAVAZ, THE CAUSAL AGENT OF BLACK ROT DISEASE OF 'CONCORD' AND 'NIAGARA' GRAPES, VITIS LABRUSCA L.

By

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To better understand the environmental factors affecting the life cycle of the black rot fungus, Guignardia bidwellii (Ellis) Viala and Ravaz, epidemiological studies were undertaken to study spore dispersal and infection of 'Concord' and 'Niagara' grapes, Vitis labursca L., in order to develop more economical control practices.

Ascospores of G. bidwellii were trapped from the air with a Burkard recording volumetric spore trap located in a 'Concord' vineyard near Paw Paw, MI. from May 9 to October 1, 1974, and in a 'Niagara' vineyard near Scottdale, MI. from May 17 to September 16, 1975. Ascospores were first trapped on May 14, 1974, and on May 20, 1975 when the shoots were 3.8 cm (1.5 inches) long. Ascospores were last trapped on September 21, 1974 and September 5, 1975. Ascospore discharge was associated with periods of

rainfall; as little as 0.3 cm (0.01 inches) caused their release. Duration of rainfall showed the highest correlation with ascospore discharge ($r = 0.6738$ with $P = 0.001$). Ascospores were trapped within one hour of the onset of rainfall, and continuously for up to eight hours after rainfall ceased. Free water in the form of dew did not cause ascospore discharge. Ascospore discharge was maximum during pre-bloom stage in late May when shoots were 15.3 cm (6 inches) long to mid-bloom stage in late June. Ascospore discharge decreased after petal fall.

Ascospore suspensions (5.0×10^4 spores/ml) were atomized at different times onto portions of vines in a field at East Lansing where inoculum was absent. Inoculated shoots were covered with polyethylene bags for 24 hours to maintain moisture. Leaf lesions resulted from inoculations made at pre-bloom stage when shoots were 10 to 20 cm (4 to 8 inches) long through early June when berries were pea-sized [ca. 1 cm (0.04 inches) in diameter]. Berry infection resulted from inoculations made at early bloom (ca. 5% bloom) through petal fall, with maximum berry infection resulting from mid-bloom inoculations. Inoculations made after August 8 did not cause infection.

Some ascospores germinated in free water on 'Concord' grape leaves in 6 hours at 10°C, 20°C, and 30°C. Temperature affected the rate of germination but

not the final per cent germination over a 24-hour period. The ability of ascospores to germinate after incubation on dry 'Concord' grape leaves decreased with time. No germination was obtained after incubation on dry leaves for 48 hours or longer.

Leaf infection of 'Concord' grapes by ascospores in free water was most rapid and greatest at 27°C (80°F). Infection was less rapid at 10°C (50°F), 16°C (60°F), and 21°C (70°F). No infection occurred at 32°C (90°F).

Infection of 'Concord' trap plants in the field occurred only during weeks with rain. Environmental factors with highest correlation to infection were the weekly temperature high ($r = 0.6113$ with $P = 0.004$) and the weekly duration of rainfall ($r = 0.3960$ with $P = 0.052$).

Conidia were trapped in rainwater run-off from infected leaves of 'Concord' and 'Niagara' vineyards from June 14 to October 3, 1974 and from June 11 to September 16, 1975, respectively. Peak conidial catches occurred during the week of July 10 to July 17 (4.9×10^5 conidia/ml) in 1974, and during the week of July 2 to July 9 (6.1×10^5 conidia/ml) in 1975. These peaks coincided with shoot lengths of 79 to 114 cm (31 to 45 inches) and a mean berry diameter of 1 cm (0.4 inches). Conidia were also trapped from newly rotted berries from July 30 to September 16, 1975, with a peak of 2.2×10^5 conidia/ml during the week of August 27 to September 3. Conidia

were trapped from April 23 to July 30, 1976 from rotted berries which had overwintered and were serving as the primary inoculum sources. A peak conidial catch of 4.4 x 10^5 conidia/ml was observed during the week of July 23 to July 30.

Conidial suspensions (5.0 x 10^5 conidia/ml) were atomized onto portions of vines in a 'Concord' vineyard at East Lansing at various times during the growing season. Inoculated shoots were covered with polyethylene bags for 24 hours to maintain moisture. Leaf lesions resulted from inoculations made at the pre-bloom stage when shoots were 203 cm (80 inches) long. Inoculations made at 50% bloom resulted in maximum berry infection (21%). Post-bloom inoculations resulted in less rot and inoculations after August 22 did not cause berry infection.

Spray timing studies indicated that economic control could be achieved with fewer than the full program of seven sprays. The best control in 1975 was achieved with five sprays first applied when shoots were 1.3 to 3.8 cm (0.5 to 1.5 inches) long (May 17), and last applied at petal fall (June 18). These results were not significantly different (by Duncan's Multiple Range Test) from seven of the other spray schedules. Preliminary results from 1976 verified these findings.

EPIDEMIOLOGICAL STUDIES OF THE DISPERSAL OF AND INFECTION BY *GUIGNARDIA BIDWELLII* (ELLIS) VIALA AND RAVAZ, THE CAUSAL AGENT OF BLACK ROT DISEASE OF 'CONCORD' AND 'NIAGARA' GRAPES, *VITIS LABRUSCA* L.

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To my parents and my wife.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

INTRODUCTION

The disease of grapes known as black rot and caused by the fungus, Guignardia bidwellii (Ellis) Viala and Ravaz, is one of the most economically destructive diseases of the vine today. Prior to the development of protectant fungicides, even in years unfavorable to the development of the disease, a loss of only 25% of the crop was considered a good year (26). Although damage to the vines themselves is minimal, crop losses of from 70% to 100% were not uncommon in years favorable to the rot. Today black rot is found wherever grapes are grown, with the exception of California because of its unfavorably dry climate (14, 24, 26). This disease is of such importance that most spray recommmendations for grapes are aimed particularly at controlling it, especially in Michigan (20).

In spite of this, little attention was given to the disease until 1885, when it was first found in France (33), where much of the early work was done. The consequences of such a destructive disease going unchecked were even greater for the Europeans than for the Americans because the European grapes, Vitis vinifera L., were extremely susceptible, whereas the native American grapes

V. labrusca L., were somewhat more resistant (26). Early studies emphasized control practices and attempts to breed for resistance. It was not until the early 1900's that Donald Reddick at Cornell University attempted to clearly define the life cycle of the fungus in order to better understand and control it. His studies were basically observational, being of a qualitative, rather than a quantitative nature. With the advent of modern epidemiological methods, a more quantitative examination of the disease cycle is now in order.

The purpose of this paper is to provide such an examination, with particular emphasis on: (1) studying the dispersal of the fungal spores in relation to their numbers, the time of year the dispersal occurs, and the environmental factors involved; (2) determining the environmental conditions under which disease develops; (3) determining the time when the vines are most susceptible to infection; and (4) conducting spray timing trials to determine the most economical means of controlling this disease.

LITERATURE REVIEW

That black rot of grapes was indigenous to eastern North America is supported by these facts: (1) whereas the disease was first noted in France in 1885, it was so common in the United States at this time that it was known as the "rot," "Common Rot," or "Black Rot," the name which later came into universal usage; (2) the disease was observed virtually wherever grapes were grown in the United States and had been from the time of the colonists' first attempts at viticulture; (3) black rot was found on wild grapes throughout North America, even in isolated wooded areas; and (4) European grapes of Vitis vinifera L. stock were much more susceptible to the rot than American grapes of V. labrusca L. stock, as would be expected if it were of American origin (26).

The fungal agent responsible for black rot was first described from the spermogonial stage found on rotted grapes by Engelmann in 1861, and was named Nemaspora ampelicida (12). In 1873, Berkeley named the imperfect stage Phoma uvicola as found on rotted grapes collected by Curtis in 1850 (4). Van Thümen gave the name Phyllosticta labruscae to the imperfect stage as

found on infected leaves of V. labrusca in 1878 (29). The first description of the perfect stage was given by Ellis in 1880 from rotted berries sent to him by Bidwell, and was called Sphaeria bidwellii in his honor (11). The confusion over the classification was at last settled in 1892 when Viala and Ravaz transferred the perfect stage to the genus, Guignardia (34). Thus, the current name Guignardia bidwellii (Ellis) Viala and Ravaz. These same authors had earlier shown that the imperfect stage on the leaves was identical to that on the berries (33). This fact had been missed by earlier investigators and had prevented the implementation of sound control practices (26). A more extensive list of the synonomy of the fungus is given by Reddick (24).

Much of the confusion regarding its classification was due to the polymorphic nature of the fungus. Four morphologically distinct forms are associated with this fungus: (1) the ascospore or perfect stage; (2) the pycinidiospores or imperfect stage (hereafter referred to as conidia); (3) the spermogonial stage; and (4) the pycnosclerotia (24). However, only the perfect and imperfect stages (the ascospores and conidia) are infective (21, 24, 25) and will be discussed here.

G. Bidwellii belongs to the class Loculoascomycetes, the order Dothidiales, and the family Dothidiaceae (19). The asci are produced in the spring from the base

of the small locules of the mummified berries, provided there is sufficient moisture (24). Asci are clavate, short-stalked, and borne in fascicles within separate, ostiolate, erumpent pseudothecia, with no paraphyses being present. Each hyaline, bitunicate ascus contains eight hyaline ascospores. The ascospore contents appear granular and usually contain two guttules (19). Ascospores appear one-celled, but at one end can be found an inflated hyaline vesicle which is believed to be of a muscilaginous nature and probably aids in the adhering of the ascospores to the leaves, etc. (24). The ascospores measure 5-7 x 12-17 μm and are subovoid to elliptical and slightly flattened on one side. The ascospores are discharged forcibly from the ascus as a result of wetting. Scribner and Viala demonstrated that ascospore discharge does not exceed 4 cm, the greatest number being found at 2 cm or less (26).

Conidia are produced abundantly within the globose pycnidia on infected leaves and berries. Under dry conditions the conidia will ooze out of the pycnidia in a cirrhus which dissolves in water and liberates the conidia. The conidia are globose, ovoid, or sometimes oblong, measuring 6.5-8.5 x 8.5-11-5 μm. The conidia are hyaline and granular in appearance, like the ascospores (24).

Unlike the ascospores, which Reddick was not able to germinate in less than 36 hours under any conditions, the conidia germinate readily in water in as short as 3 to 4 hours (24, 26). In addition, conidia may be kept dry for long periods without affecting subsequent germination (24). Germinated ascospores and conidia produce dark appressoria delimited from the germ tube by a septum. Infection is believed to occur by direct penetration of the cuticle (24).

Following infection, an incubation period of from 10 to 21 days is required for lesions to develop on leaves, and from 8 to 18 days on berries, both depending on weather conditions (24).

The fungus has been observed to infect only young, green, growing tissue of the vine and will infect all vine parts. Following the maturation of the leaves, infection will no longer occur (24).

Symptoms on leaves are characterized by circular, reddish-brown lesions (ca. 0.7 cm in diameter) within which a concentric ring of black pycnidia are formed. Only the second, third, and fourth basal leaves become infected (24); however, limited infection is observed to occur on later growth. Infection of shoots and tendrils results in the formation of black, sunken, irregular lesions, also forming the dark pycnidia. Berry infection is first seen as a minute white spot which rapidly

enlarges and turns gray. Pycnidia then develop and the whole berry soon shrivels, hardens, and becomes gray to black (24).

Except for observations concerning the etiology, symptomology, and generalized life cycle of the fungus, most early work with the disease was confined to studies relating to disease control, either with chemicals or resistant varieties.

Chemical control of black rot was obtained with both Bordeaux mixture and later, fermate (Ferbam). Control was obtained with sprays applied immediately preceding and immediately following bloom with an additional spray applied 7 to 14 days later. This latter spray proved the most important. No additional benefit was obtained from additional pre- or post-bloom sprays (5, 6, 7, 16, 21, 35). Sprays were also found to be effective only if applied prior to periods of rain (23).

Eradicant sprays using Elgetol applied in the spring prior to bud break proved effective in reducing ascospore inoculum and disease incidence. Elgetol alone gave control comparable to that obtained using foliar sprays only (27).

Cultivation between rows to cover the rotted berries on the ground with soil prevented ascospore formation in the spring. This fact, in addition to

strict sanitation measures, also proved beneficial in controlling the disease (23, 24, 25).

Studies concerning varietal resistance have been carried out with no definite advances being made (3, 9). However, it was found that the sap concentration within a variety was found to decrease from the resistant, mature leaves, to the susceptible, immature leaves. This was not found to be true between resistant and suceptible varieties though, and its significance has never been established (32).

G. bidwellii was also found to infect Vitis rotundifolia Michx. (the muscadine grape), Parthenocissus quinquefolia L. (Virginia creeper), and P. tricuspidata (Sieb. and Zucc.) Planch. (Boston ivy), all of the family Vitiaceae. It was found that three distinct physiological races of the fungus were involved, each attacking its own host, but not the others. The race associated with V. labrusca L. was designated G. bidwellii f. envitis n. The races were distinguishable not only by their host specialization, but by the mean lengths of their ascospores and their characteristics in culture (G. bidwellii f. envitis nf. forms white, rapidly growing mycelium which turns gray to black with age) (17, 18).

Characteristics of the fungus in culture were found to be affected by various environmental factors. The optimum temperature for growth and production of

pycnidia was 25°C. Germination of conidia in yeast-extract broth was greatest (50% germination) at 30°C for 36 hours, whereas in distilled water after 36 hours, germination was only 10% (8). In addition, the fungus was observed to lose pathogenicity in culture after a period of time (3).

Time temperature infection studies with conidia showed that infection occurred with a 6 hour period of leaf wetness at 26.5°C, but 24 and 12 hour periods of leaf wetness were necessary for infection to occur at 10°C and 32°C, respectively. The fact that fluctuations in temperature caused less leaf infection than when a constant temperature was maintained was also shown (28).

This then was the state of our knowledge concerning black rot prior to this report.

MATERIALS AND METHODS

Ascospore Germination

Ascospores of *Guignardia bidwellii* were discharged onto the lower surface of one-cm-square pieces of 'Concord' grape leaves placed on glass slides by soaking mummified berries in glass distilled water for several minutes before placing them on the leaf pieces. Spore discharge was allowed to proceed for an hour at which time the berries were removed and glass distilled water was added to the leaf pieces to insure the presence of free water throughout the incubation period. The slides with the leaf pieces were then placed on top of a stack of six or seven clean glass slides in covered glass Petri plates and glass distilled water was added to the bottom of the plates to prevent evaporation from the leaf surface. Three leaf pieces were placed in each Petri plate so as to provide three replications at each time-temperature regime to be studied. The Petri plates were then placed in appropriate controlled temperature boxes at 10°C, 20°C, and 30°C and allowed to incubate for 6, 12, or 24 hours. At the end of each incubation period, Petri plates were removed and the three leaf pieces were cleared following Tuite's

method (30) by immersing in a glacial acetic acid-ethanol mixture (1:1) for 24 hours, followed by a second immersion in 85% lactic acid for an additional 24 hours. The ascospores were stained by placing the leaf pieces on glass slides and adding a drop of lactophenol - cotton blue for six hours. The excess stain was removed by washing with lactophenol and the leaf pieces were mounted in lactophenol. Per cent germination was obtained by counting at random one hundred spores per replication and observing for the presence of germ tubes. The final per cent germination was obtained by averaging the data of the three replications.

Ascospore Longevity

To determine the effects of various periods of dryness following the arrival of the ascospores at the infection court, an ascospore suspension was atomized onto rooted 'Concord' grape cuttings and the leaves were allowed to remain dry for various periods of time prior to rewetting the leaves to permit germination of the ascospores.

Ascospores were discharged onto glass slides for an hour and the ascospores were collected by rinsing the slides with glass distilled water into a beaker. The surface of the slides was then rubbed with a sterile glass rod and again rinsed into the beaker. The resultant ascospore suspension was determined to contain

3.5×10^4 spores per ml by the use of a hemacytometer (Levy Ultra Plane, C. A. Hausser and Son, Philadephia, PA.). Using a DeVilbiss #15 hand-held atomizer, the spore suspension was atomized onto the upper surface of the first and second terminal leaves of one-year old rooted 'Concord' grape cuttings in the greenhouse, until run-off occurred. The leaves were then allowed to dry and were tagged with colored ribbons to distinguish them at later times. The leaves were kept dry for 0, 3, 6, 12, 24, 48, 72, and 402 hours. At the end of each designated dry period, one inoculated leaf was removed from the cuttings and brought into the laboratory where three one-cm-square leaf pieces were cut from the area adjacent to the mid-vein. The ascospores were then germinated on the leaf pieces in free water at 30°C for 24 hours and the leaf pieces were cleared as previously described. Staining was accomplished by immersing the leaf pieces in lactophenol - cotton blue in a glass Petri plate for thirty minutes and removing the excess stain by washing the leaf pieces in three changes of lactophenol, approximately two hours each. The leaf pieces were then mounted in lactophenol and the cover slips were sealed with clear fingernail polish. Each leaf piece was examined for the total number of ascospores present and the number of germinated ascospores. Per cent germination was obtained by counting all

ascospores present on a leaf and observing for the presence of germ tubes. The final per cent germination was obtained by averaging the three replications.

To determine the effect of alternate dry and wet periods upon ascospore germination, two inoculated plants were kept dry for twelve hours following inoculation, then placed in a mist chamber for six and twelve hours respectively. At the end of each wetting period, one plant was removed and allowed to remain dry for another twelve hours prior to germination of the ascospores. Germination, clearing, staining, and examination were then carried out as before.

Effect of Temperature on Infection by Ascospores

An ascospore suspension (5×10^4 spores per ml) was sprayed to run-off stage onto the leaves of two- or three-year old 'Concord' rooted grape cuttings. The leaves were resprayed with glass distilled water and the plants were covered with transparent polyethylene bags to maintain free water on the leaves during the incubation period.

The ascospore suspension, obtained as previously described, was centrifuged at 11.5×10^3 revolutions per minute in a Lourdes Model AA-C clinical centrifuge (Venitron Medical Products, Inc., Carlstadt, NJ. 07072) to concentrate the suspension. The upper half of the

supernatant was discarded (examination showed no ascospores present in this fraction) and the lower half was resuspended using a vortex mixer (Cole-Parmer Super-Mixer, Cole-Parmer, Chicago, IL. 60626). The concentration was determined using a hemacytometer and adjusted to 5.0 x 10^4 spores per ml. Sixty-six rooted 'Concord' grapes (three per time-temperature regime) were then inoculated using a DeVilbiss #15 hand-held atomizer powered by compressed air, covered with polyethylene bags, and placed in growth chambers (Sherer-Gillett, Marshall, MI.) at 10°C, 15.6°C, 21.1°C, 26.6°C, and 32.3°C for 6, 12, 18, and 24 hours. Each growth chamber was equipped with a cold water power humidifier to maintain a high humidity. At the end of each incubation period, the designated plants were removed from the chambers, the polyethylene bags were removed, and the plants were allowed to dry. In all cases the leaves were dry within twenty to thirty minutes. At the end of the 24 hour incubation period, all plants were placed at 24°C in chambers without humidifiers, and were checked periodically for the development of lesions. After the first lesions were observed, counts were made periodically for two weeks. Final results were tabulated as lesions per leaf and were the average of three replications.

Host Susceptibility in Relation to Vine Phenology

To determine at which stage of growth the vines are most susceptible to infection by the fungus, artificial inoculations were carried out periodically in a black rot free vineyard at East Lansing with both ascospores and conidia.

The ascospores were collected and a spore suspension was made as previously described. The concentration was adjusted to 5.0×10^4 spores per ml. The suspension was spray inoculated with a DeVilbiss #15 atomizer onto shoots of 'Concord' grapes in the field or on one-year old rooted 'Concord' grape cuttings in the greenhouse, until run-off occurred. The potted greenhouse plants were then placed in a mist chamber for 48 hours, whereas the shoots in the field were resprayed with glass distilled water and covered with transparent polyethylene bags for 24 hours to insure the presence of free water on the leaves during the incubation period. Three potted plants in the greenhouse or three shoots on vines in the field were inoculated at each time. Three plants or shoots, inoculated on each date with glass distilled water and wet-bagged, served as controls. The inoculated shoots were then tagged with colored ribbons and metal tags with the inoculation date and stage of vine development so they could be distinguished at later times. At the end of each incubation period, the bags were removed

and the leaves were allowed to dry. The number of leaves per shoot was noted at this time. The stage of development at each inoculation date was determined by the average lengths of the inoculated shoots, by per cent bloom of the clusters on inoculated shoots, or by the diameter of developing berries on the shoots. Lesions were counted periodically for one month following inoculation. Lesion ratings were discontinued after one month, this being the average length of time before lesions produced from secondary infections could develop. The three replications were averaged to give the lesions per leaf per inoculation date. The percentage of infected berries per shoot was determined.

Naturally infected leaves bearing lesions with pycnidia were gathered in the field and brought to the laboratory where the lesions were excised using a razor blade. These lesions were placed in a glass Petri plate with glass distilled water. The pycnidia were then rubbed with a sterile dissecting needle and the resulting conidial suspension was filtered through several layers of cheesecloth and collected in a glass beaker. The concentration was determined using a hemacytometer and adjusted to 3.0×10^4 spores per ml. The artificial conidial inoculations were then carried out in a black rot free 'Concord' grape vineyard at East Lansing as previously described.

Ascospore Dispersal Pattern in the Field and Rate of Spread of Disease

To determine the pattern of spread of the disease in the field due to ascospores and to determine the apparent infection rate early in the season due to ascospore inoculum, an infection center was established in a black rot free vineyard at East Lansing and the early season development of the disease was observed.

A cluster of four rotted berries from the previous season's infection was collected in the early spring and taken to the vineyard where it was tied to the top wire of the trellis above a vine which had been cut back. A block of eight rows of vines, twenty vines long had been set apart for this study. The rows were eight feet apart with the vines in a row each eight feet apart. At three biweekly intervals, the vineyard was examined for the number of lesions per leaf, the number of leaves infected per vine, and the position of the infected vine sites in relation to the infection focus.

The infection rate, "QR," was determined (31), with the disease being considered as a "simple interest disease" for the period between the first two ratings due to the fact that the early season development of the disease without secondary infection was being considered. For the period between the second and third ratings, the apparent infection rate, "r" (31), was determined with

the disease being considered as a "compound interest disease" due to the fact that both primary and secondary inoculum could have played an active role in the increase of the disease. The proportion of diseased to healthy tissue in both cases was viewed as the proportion of infected to noninfected vine sites, with only a single lesion per vine constituting an infected vine site. In addition, due to the overgrowth of missing vine sites by the adjacent vines, lesions found on tissue in these sites were considered to have been caused by infection of these sites, even though the vines were missing.

Periods of Field Infection as Determined by the Use of Trap Plants

To determine the weather conditions necessary for natural infection to occur in the field, rooted 'Concord' grape cuttings were placed under infected vines in the field for periods of one week, and were then brought back to East Lansing where they were kept in isolation and allowed to develop symptoms.

Five rooted cuttings of 'Concord' grapes were potted in large (#10) cans and placed in a 'Concord' vineyard at the J. Bilger farm in Paw Paw, Michigan during 1974, and in a 'Niagara' vineyard at the R. Ketelhut farm in Scottdale, Michigan during 1975. These plants were replaced weekly and returned to a cold frame outside the greenhouse in East Lansing where symptoms were allowed

to develop. Rooted 'Concord' grape cuttings kept in the cold frame for the year served as controls.

Lesion counts were made periodically for one month from the date the plants were returned from the field. These were expressed as lesions per leaf per trap plant and were the average of five replications.

Weather parameters were also measured.

Spore Dispersal Studies (Ascospores and Conidia)

To determine the weather conditions necessary for spore dispersal in the field, ascospores were trapped from the air using a Burkard seven-day recording, volumetric spore trap (Burkard Scientific Sales Ltd., Rickmansworth, Hertfordshire, England) and conidia were trapped in rainwater run-off using the funnel and jug method (10). The weather parameters were measured using a seven day recording rain gauge (Weather Measure, Inc., Sacramento, California), leaf wetness meter (M. Dewit, Hengelo, Holland), and a sheltered hygrothermograph (Bendix Corp., Baltimore, Maryland).

Ascospores were trapped from the air using the Burkard volumetric spore trap placed in a 'Concord' vineyard at the J. Bilger farm in Paw Paw, Michigan during 1974 season and in a 'Niagara' vineyard at the R. Ketelhut farm in Scottdale, Michigan during the 1975 season. The trap was run continuously from May 9 to October 1, 1974,

and from May 14 to September 16, 1975. The trap was adjusted to draw approximately ten liters of air per minute in both cases.

The spore trap and other recording charts were changed weekly. Ascospores were counted by first cutting the tapes into 48 mm strips (these representing 24 hour periods), staining the strips by floating them face down in 0.5% aqueous safranin for ten to fifteen seconds followed by rinsing in two changes of glass distilled water to remove excess stain, and mounting them on glass slides. Spore counts were made on an hourly basis using the 20X objective of a Wild M20 microscope and 10X wide-field oculars.

Conidia produced in the pycnidia of leaf lesions were trapped in rainwater run-off from infected leaves using the funnel and jug method from June 14 to October 3, 1974, and from June 11 to September 10, 1975. Six one-gallon plastic milk jugs were connected by tygon tubing to plastic funnels which were placed beneath leaves showing lesions with pycnidia or beneath newly rotted berries. Evaporation from the jugs was prevented by inserting tygon tubing through a hole in the cap of the jugs which was then sealed with epoxy resin. The jugs were changed weekly and the water collected was examined for conidia.

Conidial concentrations were determined by centrifuging a given volume of rainwater from each jug for

twenty minutes at 11.5 x 10^3 revolutions per minute. The supernatant was discarded and the remaining material was resuspended in ten mls of glass distilled water using a Vortex mixer. A hemacytometer was then used to determine the conidial concentrations and the average was taken of the counts from the six jugs.

Conidia were also trapped from newly infected berries showing pycnidia from July 30 to September 16, 1975, and from rotted berries which had overwintered and were serving as the primary inoculum source from April 23 to June 25, 1976.

Spray Timing Studies

To determine more precisely when sprays should be applied for maximum disease control, thirteen different spray schedules were tested in 1975. Twenty-five vines at the end of each of the first five rows of a 'Niagara' vineyard at the R. Ketelhut farm in Scottdale, Michigan were used for this study in 1975. Thirteen treatments of Ferbam 76W (76% ferric dimethyldithiocarbamate) at three pounds per acre and the untreated control treatment were assigned to seventy vines in a randomized complete block design (15). Five single vine replications per treatment were used. Every third vine was left untreated to serve as inoculum sources later in the season. Sprays were applied with a hand gun using a power sprayer. Approximately 0.95 liters of spray per vine was applied, which

corresponds to 643.5 liters of spray per acre. The spray schedule is shown in Table 1. Five sprays were applied from the time the shoots were 1.3 to 3.8 cm long (May 17) through petal fall (June 18) according to vine development, with three additional sprays following at intervals of two, four and four weeks. Disease was evaluated on September 10-11. The percentages of rotted berries in ten clusters randomly selected per vine was determined. The five replications per timing schedule were averaged. Analysis of variance was applied to the data and Duncan's Multiple Range Test was used to separate treatment means (15).

Seven rows of a 'Niagara' vineyard at the G. Pohyba farm in Scottdale, Michigan were used for this study in 1976. Twenty treatments and the untreated control treatment were assigned to 147 vines in a randomized complete block design (15). Seven single vine replications per treatment were used. Fungicides used were a combination of Phaltan 50W [50% N-(trichloromethylthio) phthalimide] at four pounds per acre and Benlate 50W [50% methyl 1-(butylcarbamyl)-2-benzimidazide carbamate] at one pound per acre for the first spray (May 7) and a combination of Captan 50W [50% cis-N-((trichloromethyl)thio)-4-cyclohexene-1,2-dicarbozimide] at four pounds per acre and Benlate at one pound per acre, thereafter. The spray schedule is shown in Table 2. Five sprays were applied

from the time the shoots were 3.8 cm long (May 7) through petal fall (June 22) according to vine development, with two additional sprays following at intervals of two and three weeks. Disease was evaluated on August 13. The percentage of rotted berries in two hundred berries randomly selected per vine was determined. The seven replications per timing schedule were averaged. All other procedures followed those previously described.

Two-year Old Berries As An Inoculum Source

To determine whether two-year old mummified berries could serve as inoculum sources, berries infected during the previous season were allowed to overwinter for a second time and were examined for the presence of ascospores.

Mummified berries which were infected in 1973, and served as the primary inoculum source in 1974, were collected from the J. Bilger farm at Paw Paw, Michigan in late September of 1974, and were brought back to East Lansing where they were placed outside in a predetermined location. During early June of 1975, these berries were again collected and observed for ascospore discharge.

RESULTS

Ascospore Germination

Germination of ascospores in water was obtained in as little as 6 hours at all temperatures tested, with per cent germination being greatest the higher the temperature (Figure 1). Over 90% germination occurred within 24 hours at all temperatures. Germination was greatest at 30°C and least at 10°C.

Ascospore Longevity

Ascospore germination decreased as the period of ascospore incubation on dry leaves was increased prior to rewetting of the leaves (Figure 2). Maximum germination (97%) was obtained with no dry period following inoculation of the leaves. There was a marked decrease in germination from six to twenty-four hours of dryness prior to rewetting the leaves (90.7% to 18.4%). No germination was obtained when the leaves were dry for forty-eight hours or longer prior to rewetting. The increase in germination from three to six hours of dryness was probably due to the natural variability between treatments.

Ascospores incubated on leaves that were dry for twelve hours, wet for six hours, and dry for an additional twelve hours showed only 6.7% germination, and those on

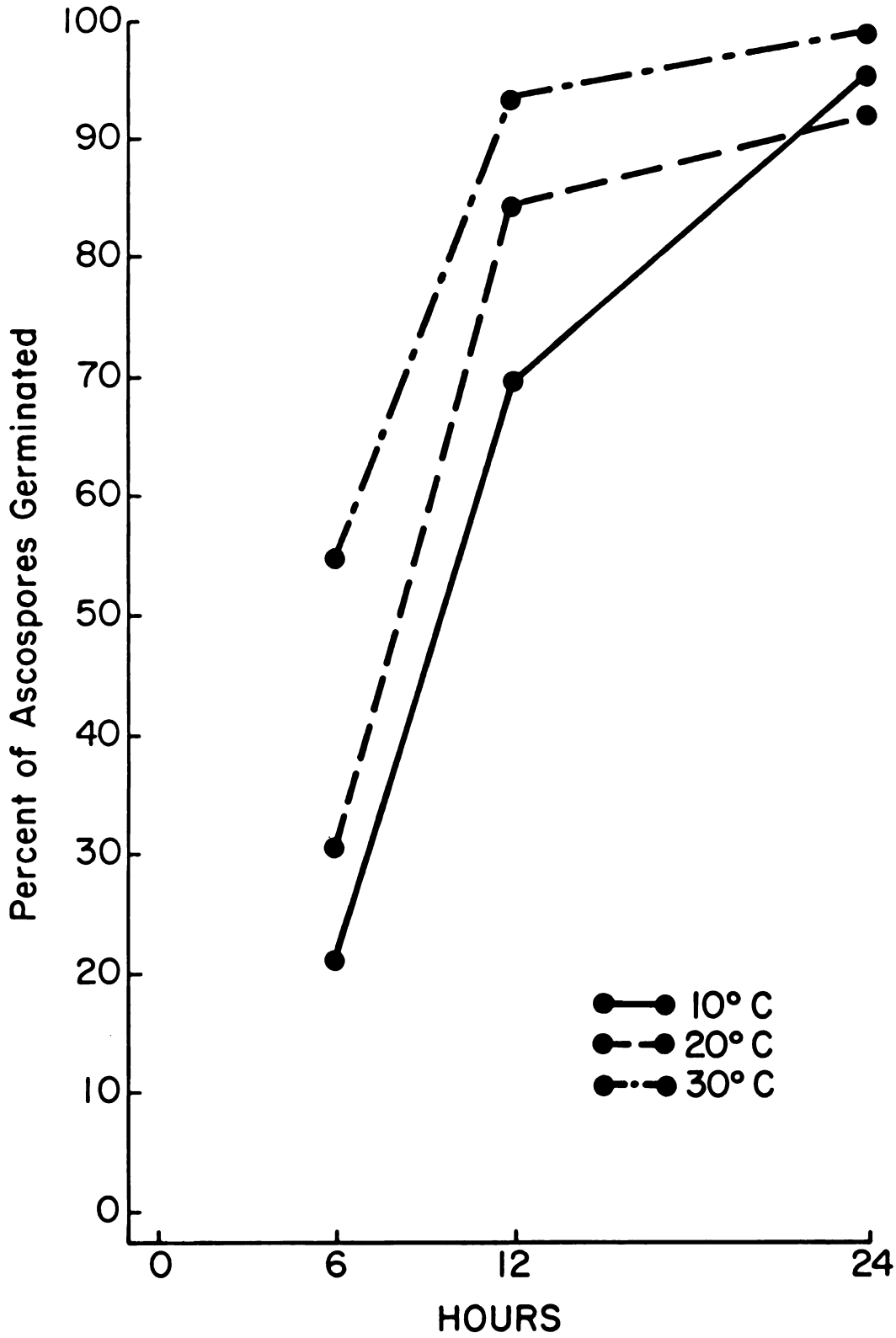


Fig. 1. Germination of ascospores of G. bidwellii in free water on 'Concord' grape leaves.

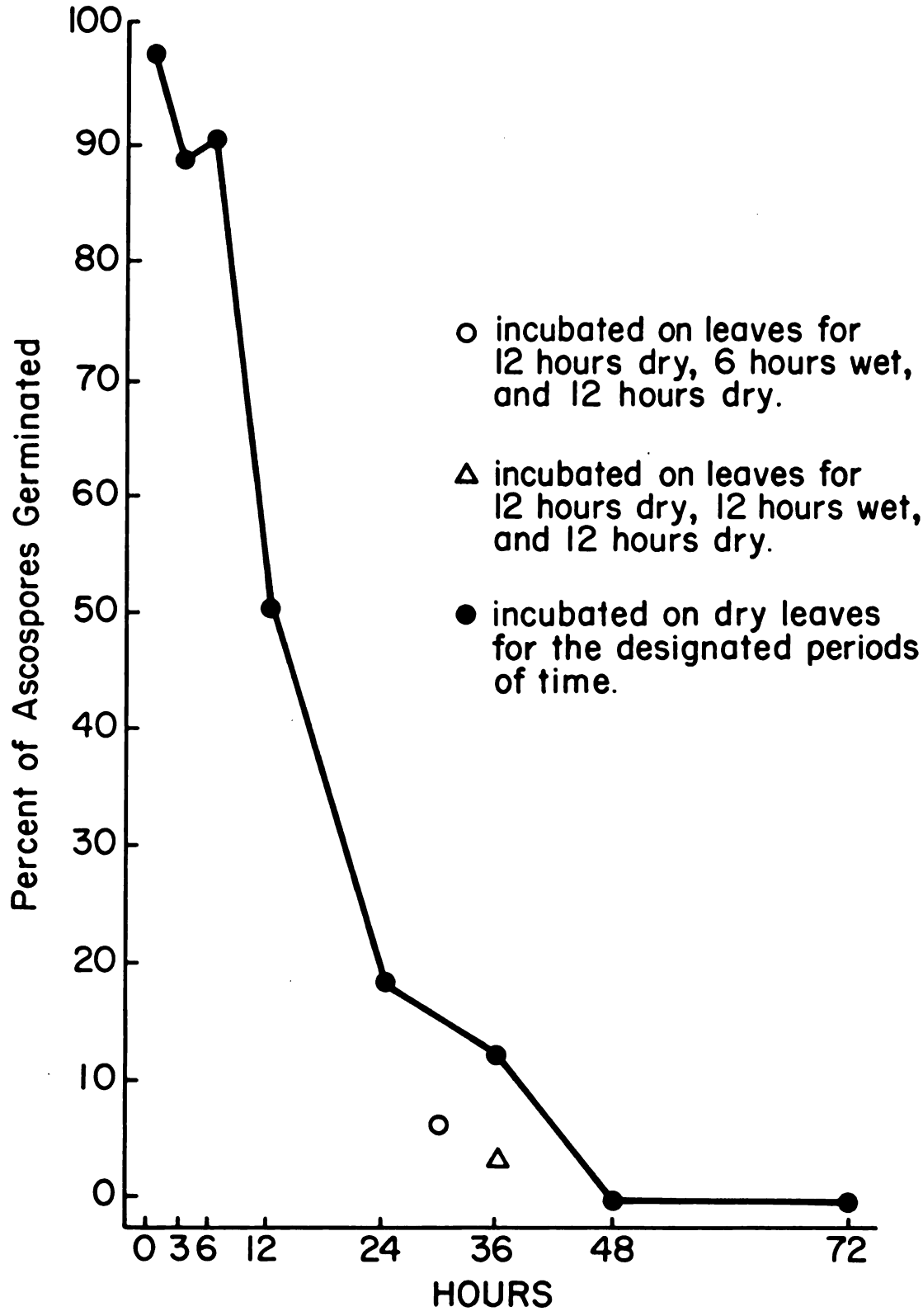


Fig. 2. Germination of ascospores of G. bidwellii in free water on 'Concord' grape leaves following various periods of incubation on dry leaves.

leaves that were dry for twelve hours, wet for twelve hours, and dry for another twelve hours showed only 3.3% germination.

Effect of Temperature on Infection by Ascospores

Infection due to ascospores was most rapid and reached the highest level (9.8 and 16 lesions per leaf after 12 and 24 hours, respectively), at 27°C (Figure 3). It was slower but reached the same level after 24 hours at 16°C. At 10°C and 21°C the infection rate was slower and reached only 8 lesions per leaf in 24 hours. No infection occurred at 32°C.

Host Susceptibility in Relation to Vine Phenology

Artificial inoculations were made with ascospores in 1974 and 1975. The first three inoculations of 1974 were carried out on rooted 'Concord' grape cuttings in the greenhouse. All subsequent inoculations were carried out in the vineyard at East Lansing. Infection of leaf tissue in 1974 was greatest when the shoots were approximately 15.2 to 20.3 cm long (about two to three weeks prior to bloom) resulting in 16 lesions per leaf (Figure 4). Leaf infection continued through bloom with a second peak of twelve lesions per leaf resulting from inoculations at the 80% petal fall stage of development. Leaf infection decreased markedly from this point with no new

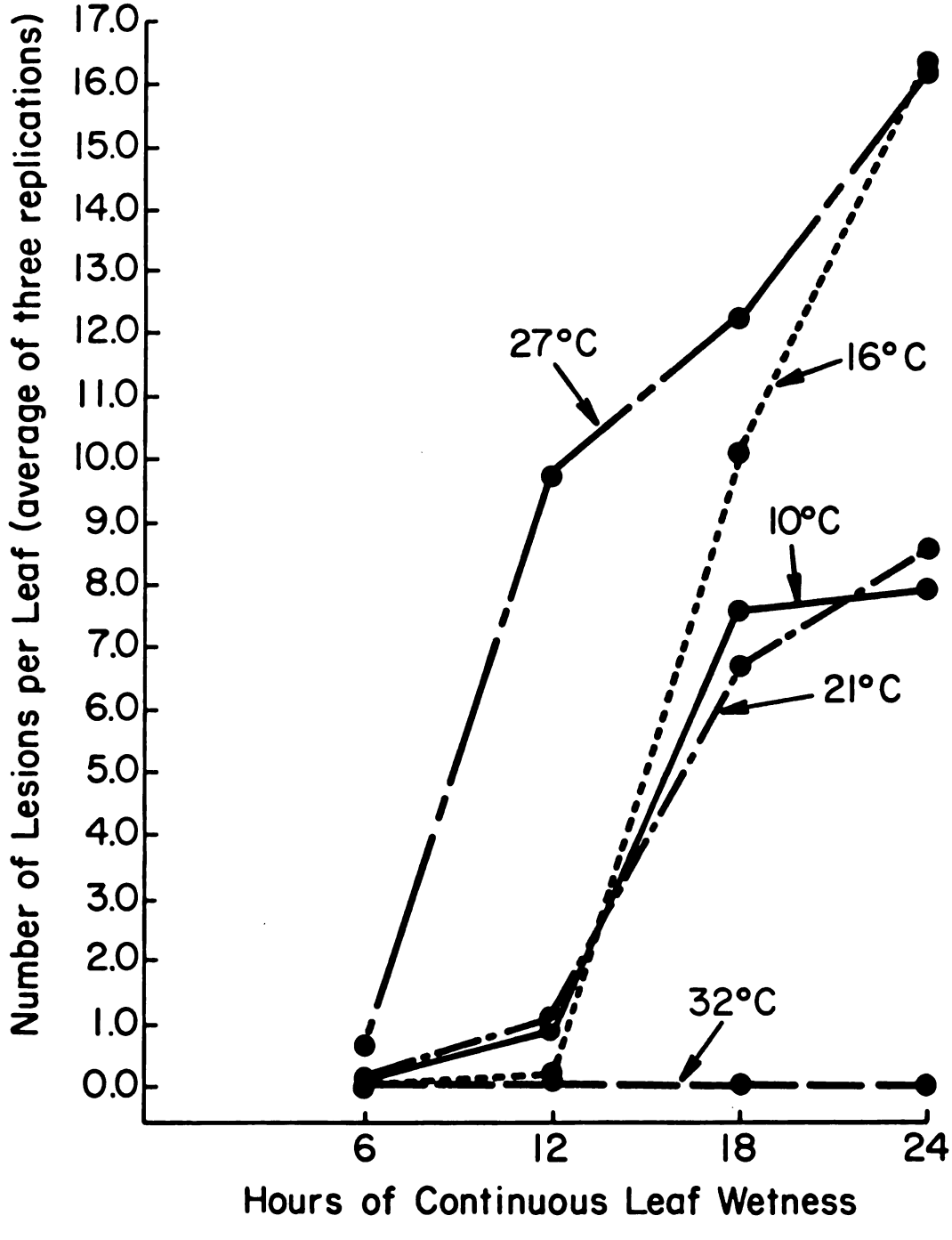


Fig. 3. Effect of temperature on infection of 'Concord' grapes following inoculation of ascospores of G. bidwellii (5×10^4 spores/ml) and various periods of incubation in the presence of free water.

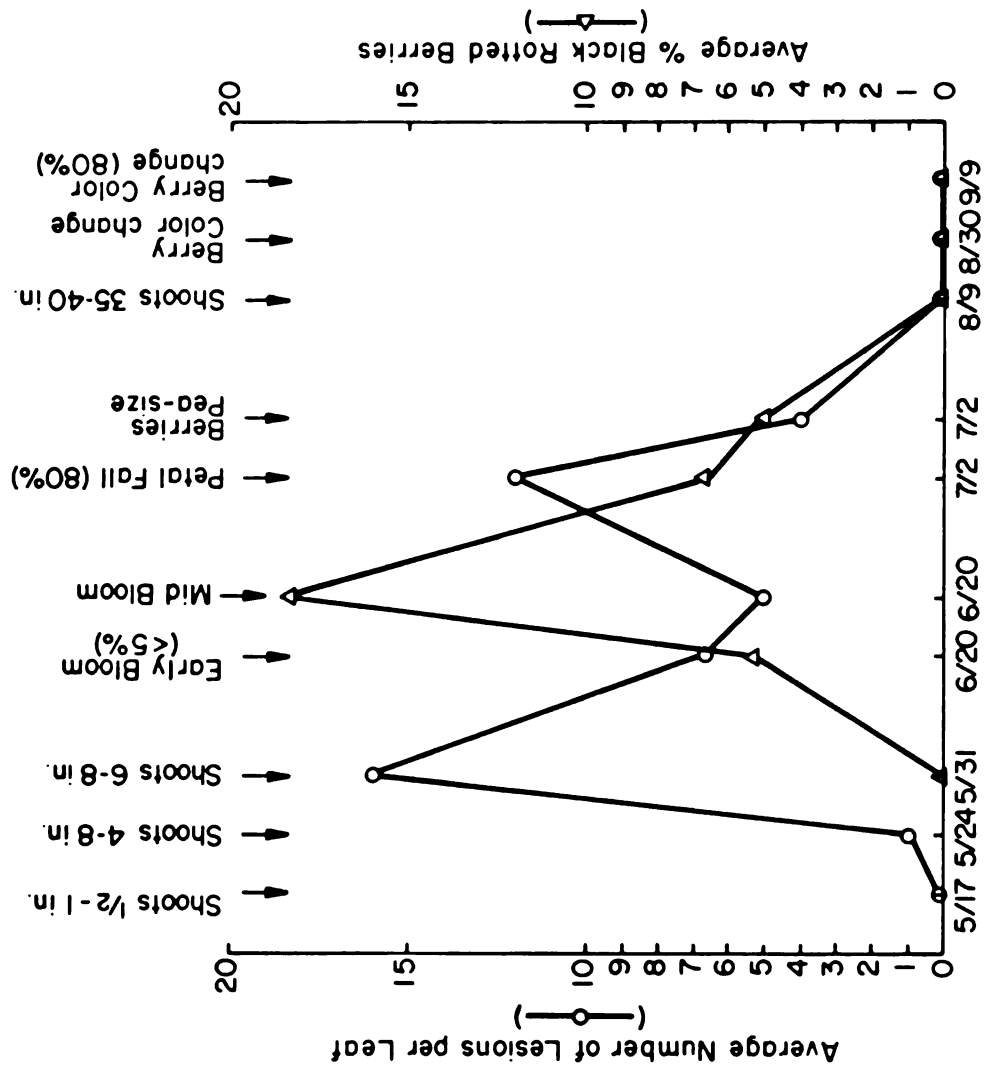


Fig. 4. Infection of 'Concord' grapes by ascospores of G. bidwellii resulting from artificial inoculations (5×10^4 spores/ml) made in the field on various dates during 1974.

infections resulting from inoculations made during August or September. Infection of leaf tissue in 1975 was greatest when the shoots were approximately 33 cm long (about two to three weeks prior to bloom) resulting in 19 lesions per leaf (Figure 5). Leaf infection continued through bloom, as before, with a second peak of 16 lesions per leaf resulting from the mid-bloom inoculations. As in 1974, infection of leaves then decreased markedly with no new infections resulting from inoculations made during August or September. Infection of berries began with the early bloom inoculation in both 1974 and 1975 (5.3% and 16% of the berries infected, respectively) and reached a peak of 18.5% and 29% of the berries infected resulting from the mid-bloom inoculations, respectively. Berry infection decreased markedly from this point with no new berry infection resulting from inoculations of August and September.

Leaf infection resulting from inoculations made with conidia in 1975 reached a peak from the mid-bloom stage of development through the time the berries reached pea-size (approximately 1.0 cm in diameter) with 13 and 12.5 lesions per leaf resulting, respectively (Figure 6). Infection of leaf tissue after this time declined, with inoculations made in September resulting in no infections. Berry infection followed this same pattern, with the mid-bloom inoculation resulting in 21% infection of berries

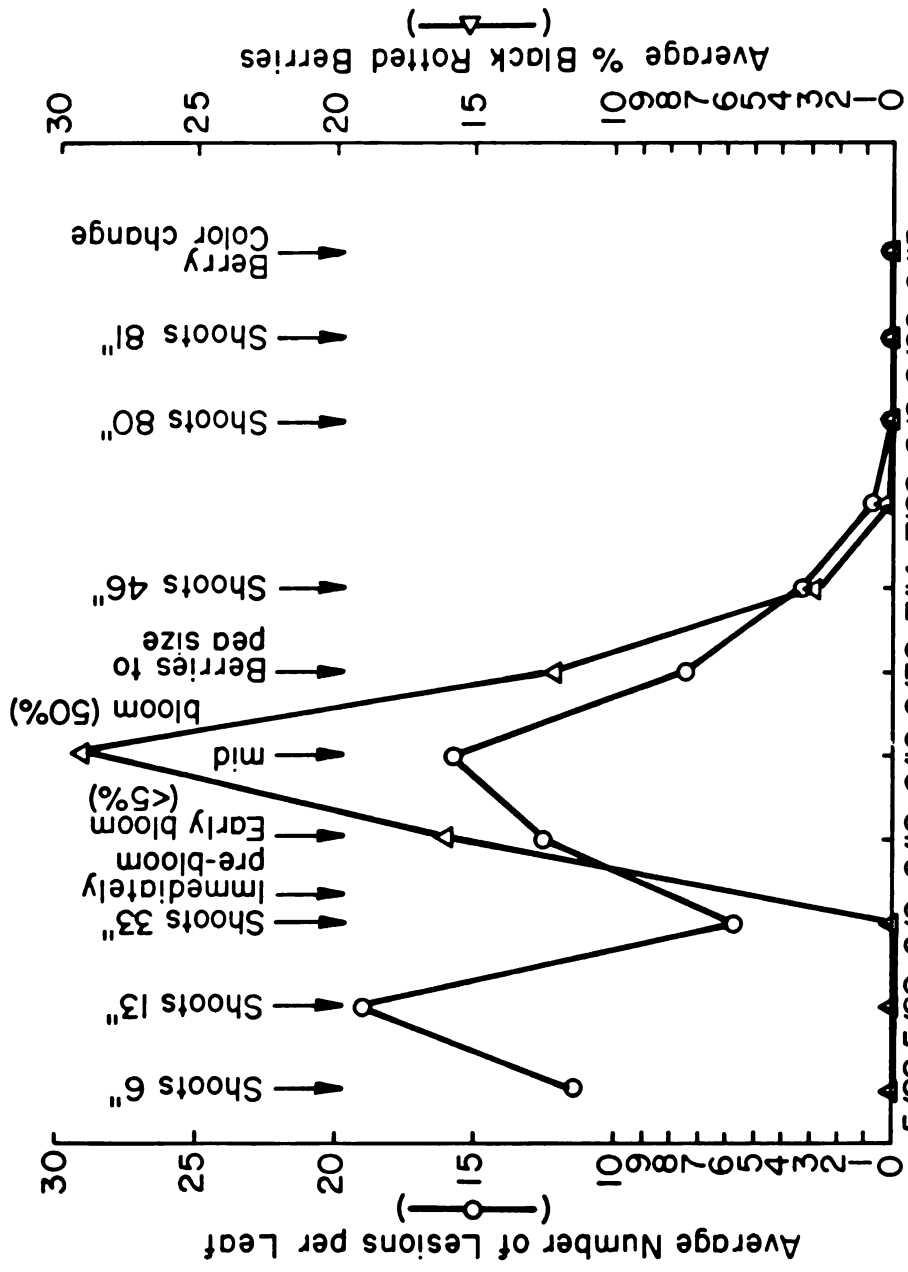


Fig. 5. Infection of 'Concord' grapes by ascospores of G. bidwellii resulting from artificial inoculations (5×10^4 spores/ml) made in the field on various dates during 1975.

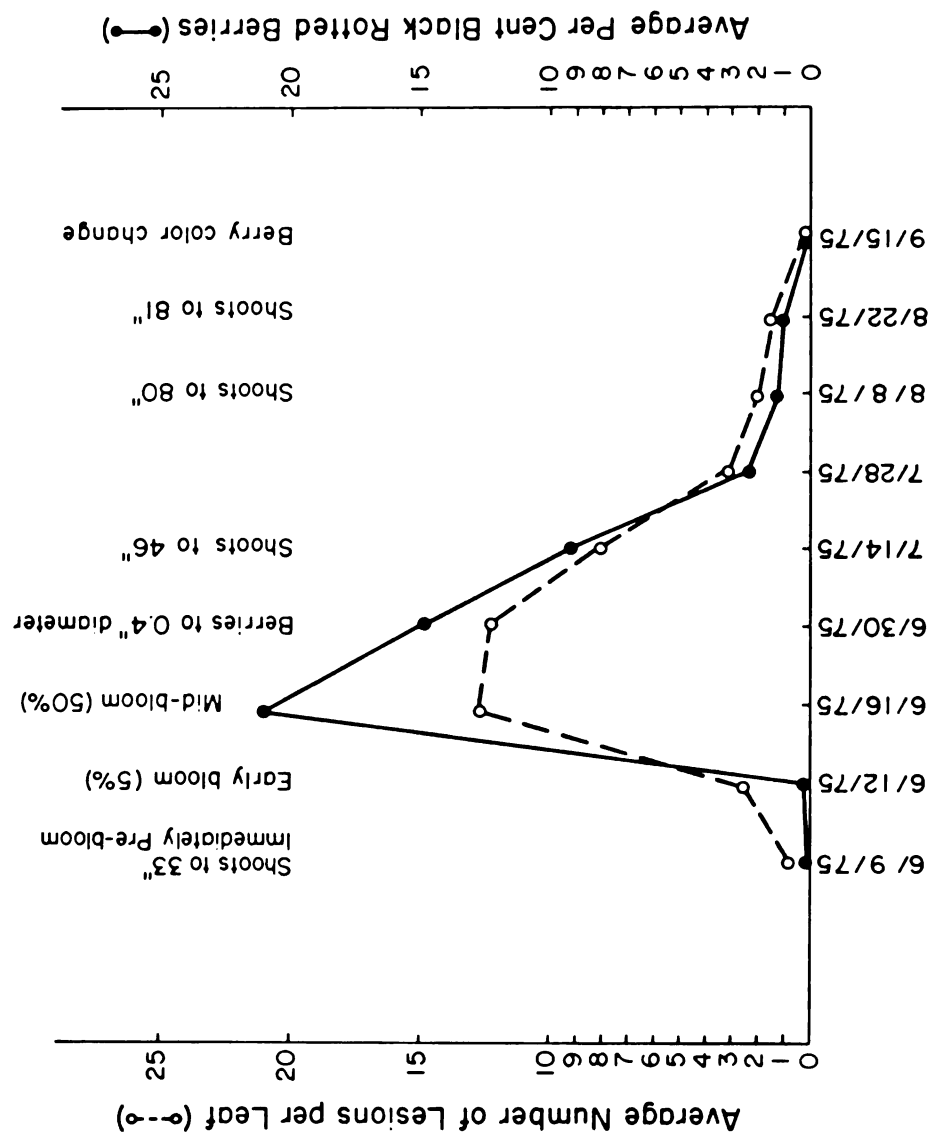


Fig.6. Infection of 'Concord' grapes by conidia of G. bidwellii resulting from artificial inoculations (5×10^4 conidia/ml) made in the field on various dates during 1975.

and the inoculation made when berries were 1.0 cm in diameter resulting in 15% berry infection. The mid-July inoculation resulted in 9% infection. No berry infection resulted from inoculations made in September.

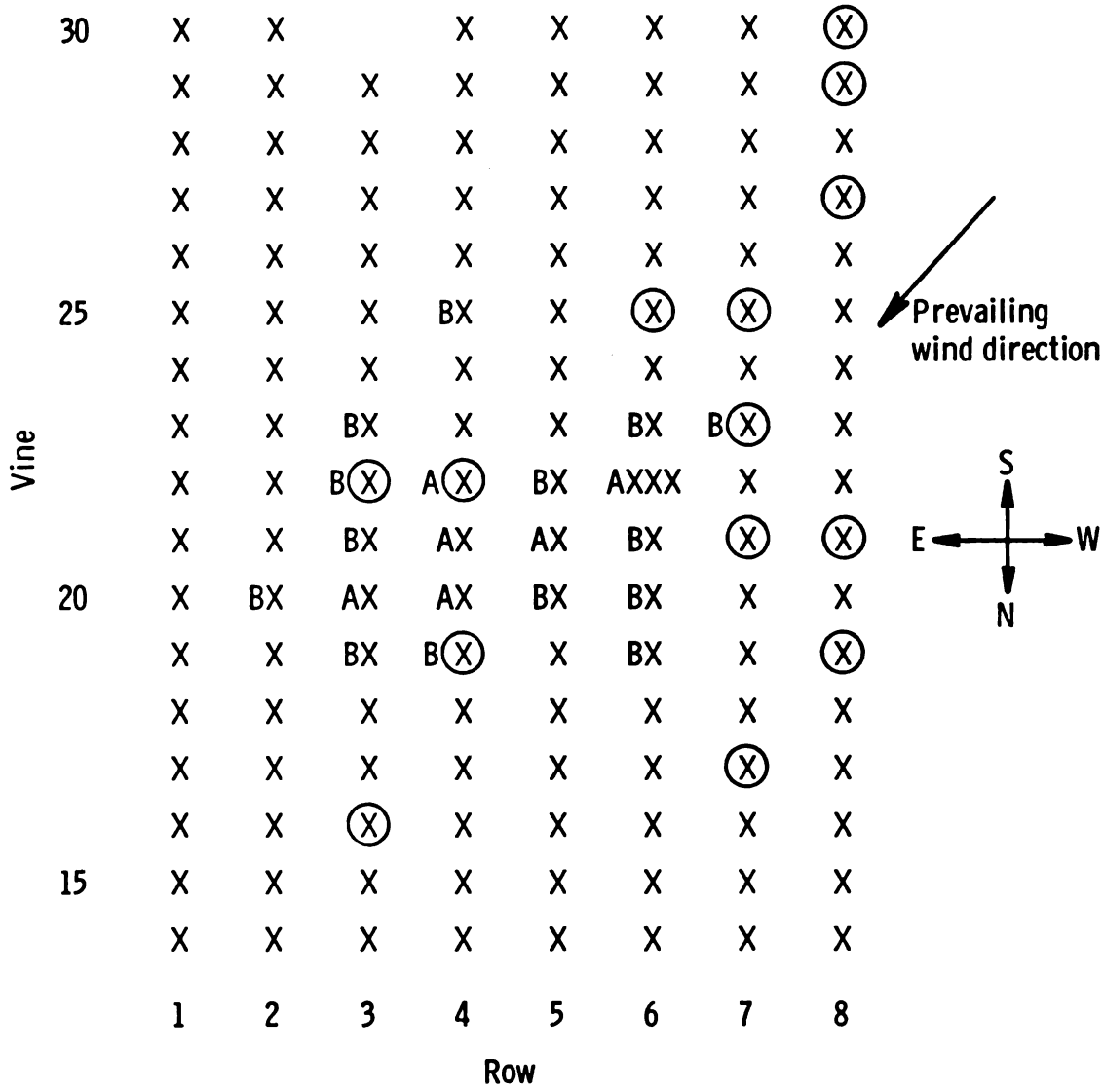
Ascospore Dispersal Pattern in the Field and Rate of Spread of Disease

Figure 7 is a plot map of the vineyard at East Lansing in which the early season disease dispersal was studied. The first lesions were observed on June 12, when the vines were at early bloom (ca. 5%), with six vine sites showing infections (28 leaves showing 99 lesions). The final evaluations, done on June 25, when the vines were immediately past bloom, showed twenty vine sites with infections (191 leaves showing 1812 lesions).

Initial infections could be attributed only to ascospores and the pattern of dispersal was in the direction of the prevailing winds, as would be expected for air-borne inoculum. The simple interest rate, "QR," was calculated in relation to these infections, for the period from June 1 to June 12. The formula used to calculate this "QR" taken from van der Plank was:

$$QR = \frac{1}{t_2 - t_1} \left(\log_e \frac{1}{1 - X_2} - \log_e \frac{1}{1 - X_1} \right)$$

where X_1 and X_2 are the proportion of disease at times t_1 and t_2 respectively (31). The "QR" value was calculated



X = 'Concord' grape vine site
XXX = infection focus
Ⓧ = missing vine site (due to the overgrowth of these sites by adjacent vines, they were still considered infectable).

AX = infected sites, 6/12/75
BX = infected sites, 6/25/75

Figure 7. Early season pattern of disease dispersal due to inoculum released from an infection center established in a black rot free vineyard.

at 0.004 per unit per day for this primary infection period, prior to bloom.

Infections found on June 25 could have been caused by both ascospores and conidia. With this in mind, van der Plank's formula for a compound interest disease was used to calculate the apparent infection rate, "r," for the period from June 12 to June 25, the period through bloom. The formula used to calculate this was:

$$r = \frac{1}{t_2 - t_1} \left(\log_e \frac{X_2}{1 - X_2} - \log_e \frac{X_1}{1 - X_1} \right)$$

where X_1 and X_2 are the proportions of disease at times t_1 and t_2 respectively (31). The "r" value was calculated at 0.101 per unit per day for this infection period during bloom.

Periods of Field Infection as Determined by the Use of Trap Plants

In viewing the relationship between the number of lesions developed per leaf on trap plants, the amount of rainfall, and the number of hours of leaf wetness during the week the plants were in the field, it can be seen that no lesions developed on plants in the field during a week of very little or no rainfall (Figure 9) (Question marks (?) on these figures indicate missing data due to equipment malfunction). However, it can be seen that the amount of rainfall was not directly proportional to the

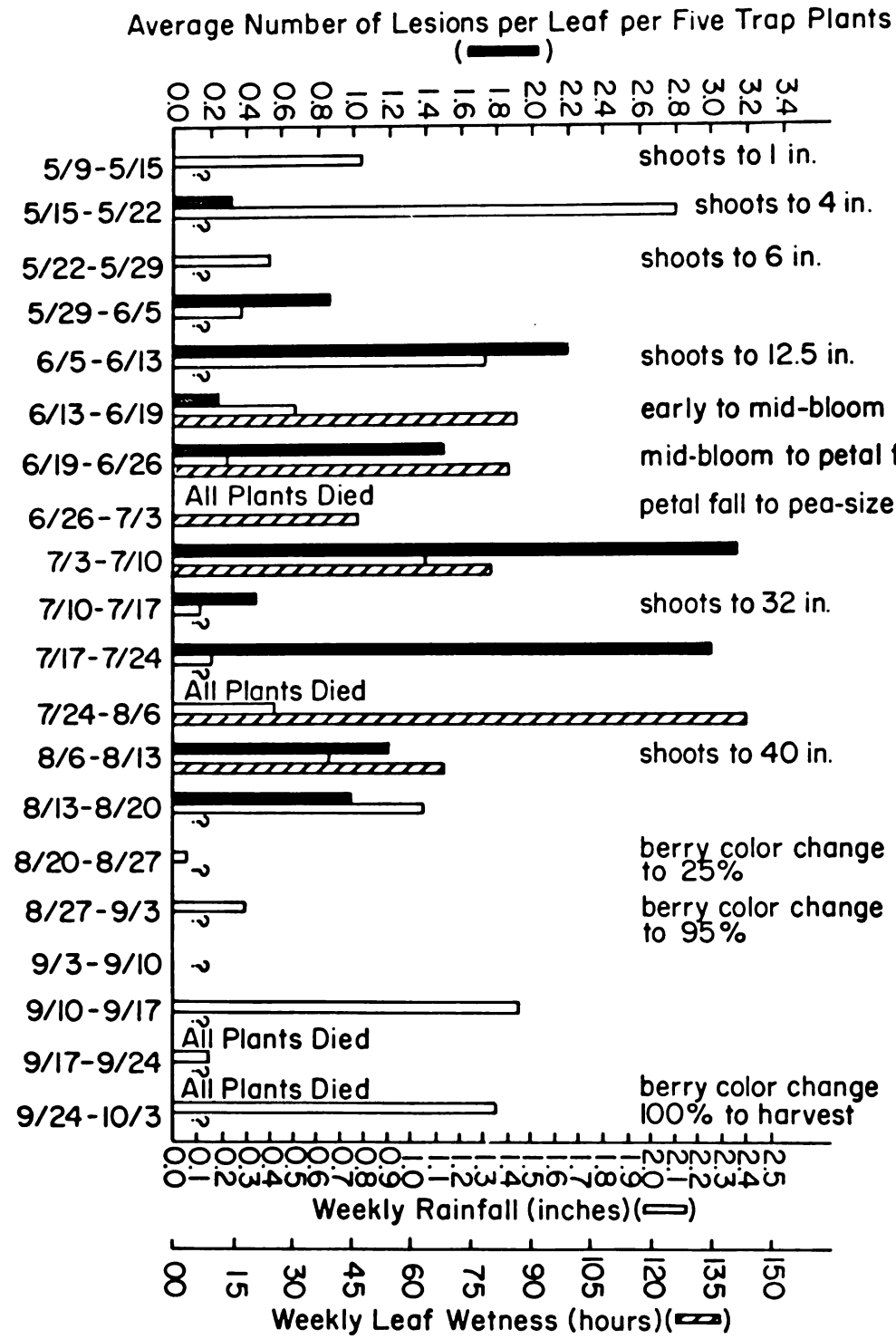


Fig. 8. Infection of 'Concord' trap plants placed under infected vines of a 'Concord' vineyard during 1974.

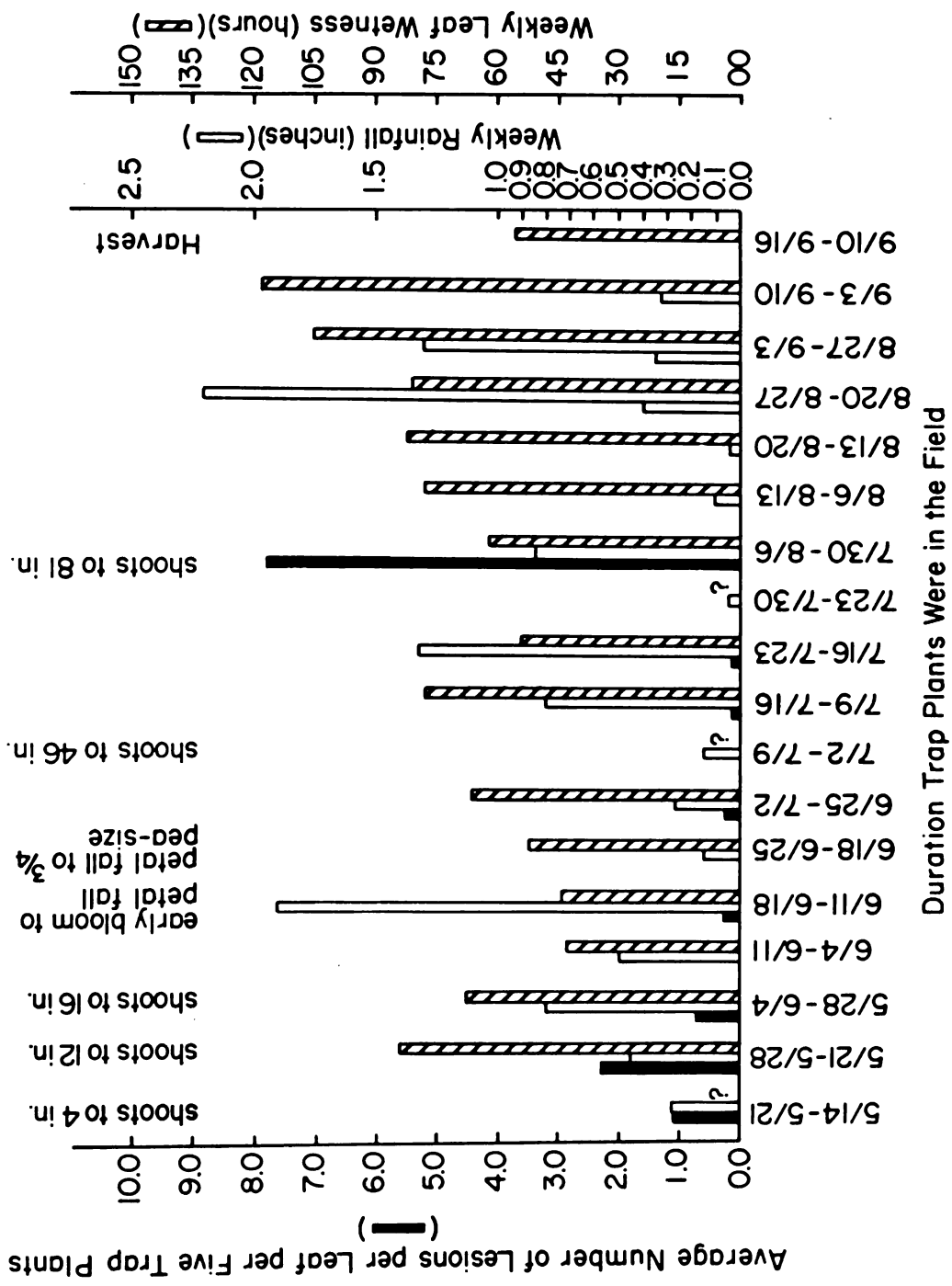


Fig. 9. Infection of 'Concord' trap plants placed under infected vines of a 'Niagara' vineyard during 1975.

number of lesions produced. Weekly amounts of leaf wetness also failed to show a direct relationship to the number of lesions produced.

To determine what environmental factors could be correlated to infection in the field, the Pearson Correlation Coefficients were determined from the data of 1975 (Figure 9), for the following eight variables: the weekly average temperature, the weekly high temperature, the weekly low temperature, the weekly hours of 100% RH, the weekly hours of leaf wetness, the longest interval of continuous leaf wetness per week, the weekly amount of rainfall, and the weekly duration of rainfall. These computations were made using the SPSS - Statistical Package for the Social Sciences (Vogelback Computing Center, Northwestern University, Version 6) run on the Model 6500 computer (Control Data Corporation) at Michigan State University. The highest correlations were obtained with the weekly high temperature ($r = 0.6113$, $P = 0.004$) and the weekly duration of rainfall ($r = 0.3960$, $P = 0.052$). All other variables considered were not significant at less than the 0.150 level of significance.

Spore Dispersal Studies (Ascospores and Conidia)

As viewed on a daily basis, the ascospore trapping data in relation to rainfall for 1974 and 1975 show that ascospores were trapped only on days on which there was

rainfall (Figures 10, 11, and 12). As little as 0.08 and 0.10 cm of rain on May 19 and May 31 in 1974 triggered the catch of 4 and 28 ascospores, respectively (Figure 10). As little as 0.03 cm of rain on May 29, July 12, and August 10 triggered the release of 5, 4, and 3 ascospores, respectively, in 1975 (Figure 12). However, there appears to be no direct relationship between the amount of rainfall and the number of ascospores trapped. For example, more ascospores were trapped on June 11 than June 9, even though there was four times as much rain on June 9 (Figure 10). Ascospores were first trapped on May 14, 1974 and on May 20, 1975. These dates correspond to the time ascospores were first found to be released from mummified berries collected in the field, and the first week infection occurred on trap plants in the field. Peak ascospore catches in 1974 occurred on June 7 and June 10 (84 and 82 ascospores, respectively). This corresponds to the time the shoots were from 22.9 to 31.8 cm long, about one week prior to bloom. Peak ascospore catches in 1975 occurred on May 30 and June 15 (63 and 65 ascospores, respectively). This corresponds to the time when the shoots were from 30.5 cm long to mid-bloom. The general trend is for the ascospores to begin being released about the middle of May, reaching a peak prior to and into bloom, and finally decreasing to low levels after the middle of July.

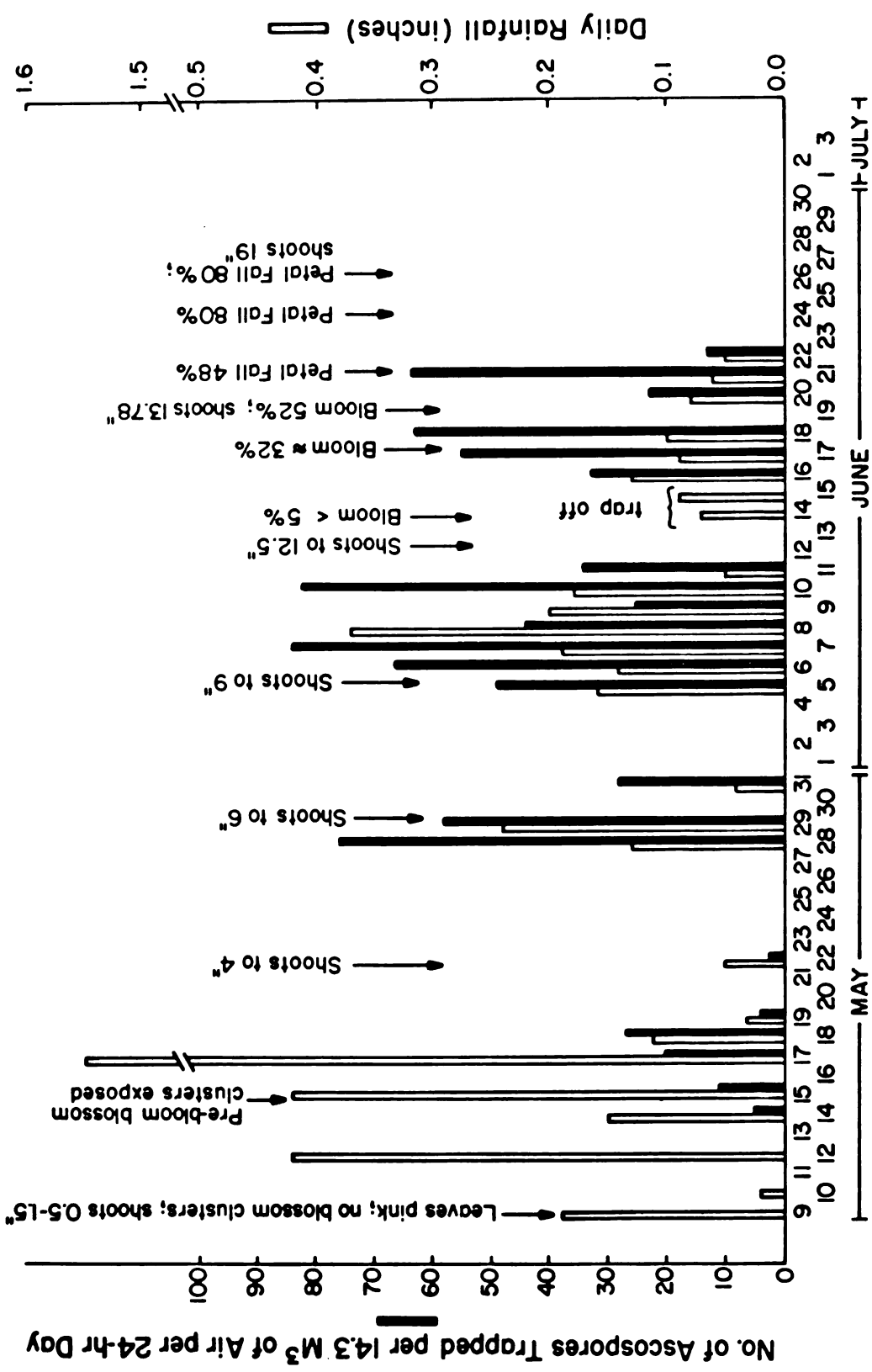


Fig. 10. Daily ascospore catches through petal fall during 1974.

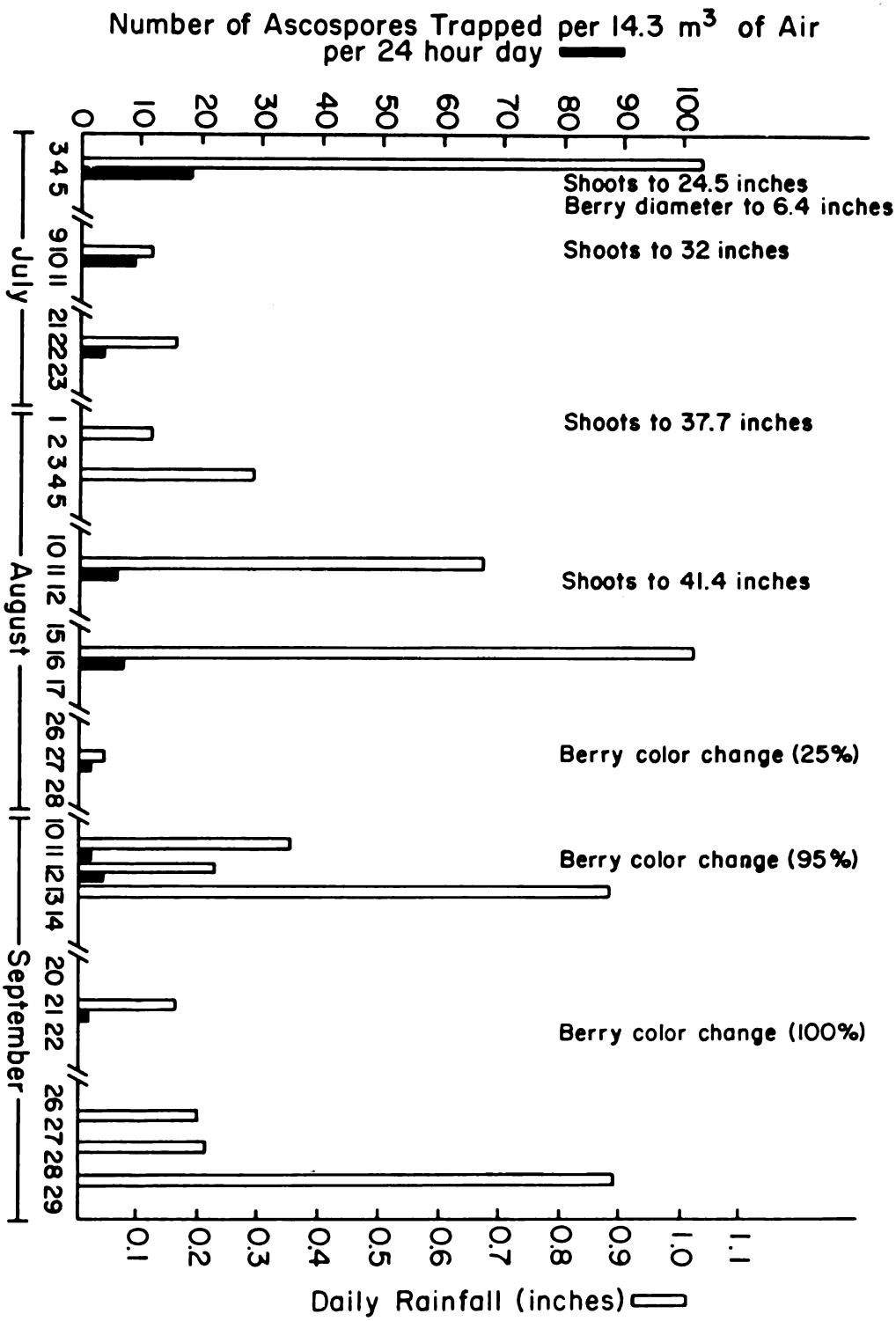


Fig. 11. Daily ascospore catches late in the season during 1974.

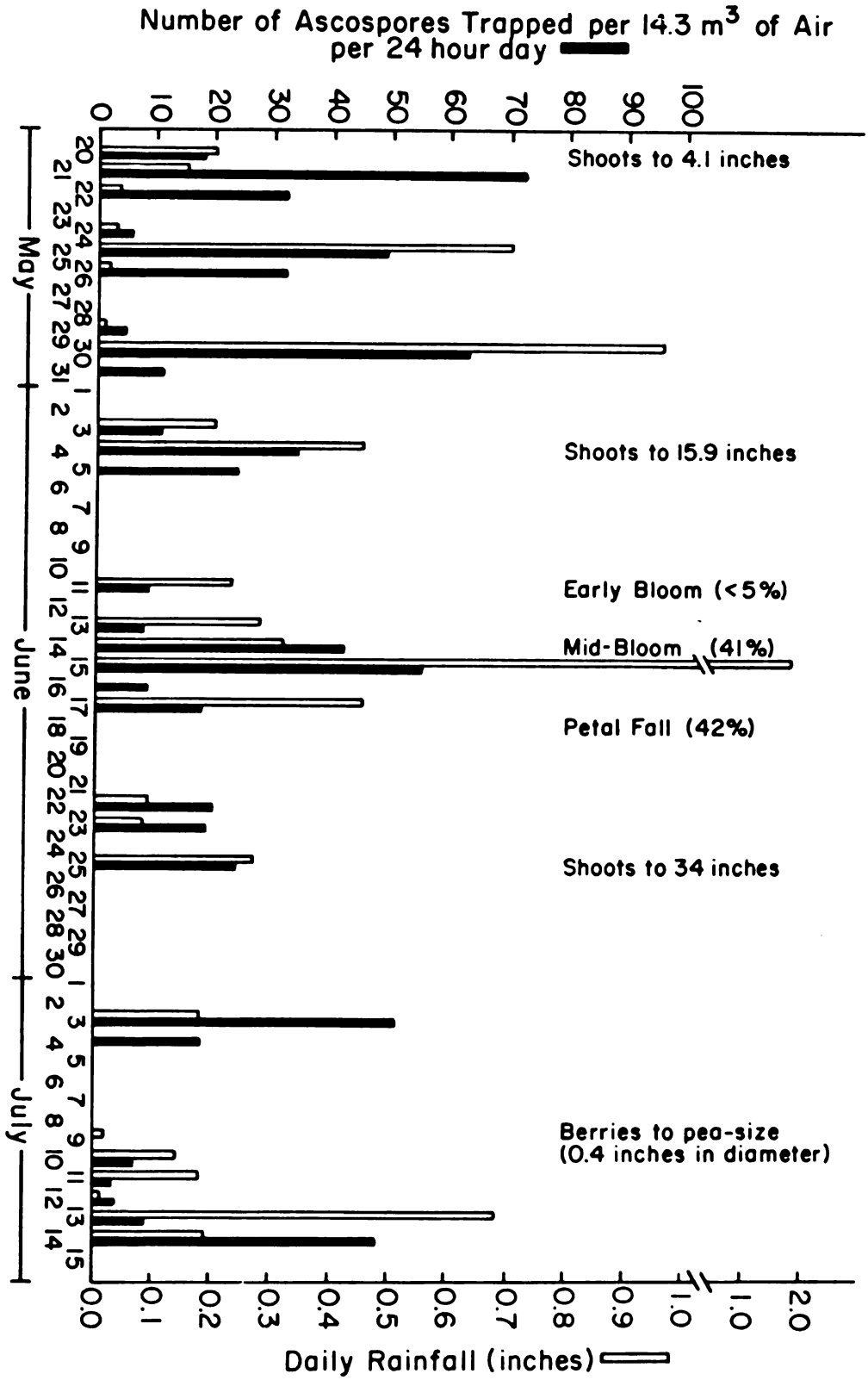


Fig. 12. Daily ascospore catches through early berry development stage during 1975.

However, some ascospores were still being trapped as late as September 21, 1974 and September 5, 1975.

Viewed on an hourly basis, an excellent representation of the manner in which rainfall triggers the liberation of ascospores can be seen (Figure 13). With the onset of rainfall, or shortly thereafter (usually within the hour), ascospores are released into the air. The greatest number of ascospores were trapped during the first and second hour after the cessation of rainfall. They continued to be released in diminishing numbers from this point, in some cases for as long as eight hours.

To determine the climatological factors involved in the release of ascospores, Pearson Correlation Coefficients were calculated for the following variables on a daily basis: the average temperature, the high temperature, the low temperature, the number of hours of 100% RH, the number of hours of leaf wetness, the amount of rain, and the duration of rainfall. The highest correlations were obtained with the duration of rainfall, $r = 0.6738$ ($P = 0.001$); the amount of rainfall, $r = 0.6500$ ($P = 0.001$); the number of hours of 100% RH, $r = 0.2798$ ($P = 0.002$); and the number of hours of leaf wetness, $r = 0.2008$ ($P = 0.020$). The temperature variables were not significant at less than the 0.134 level of significance.

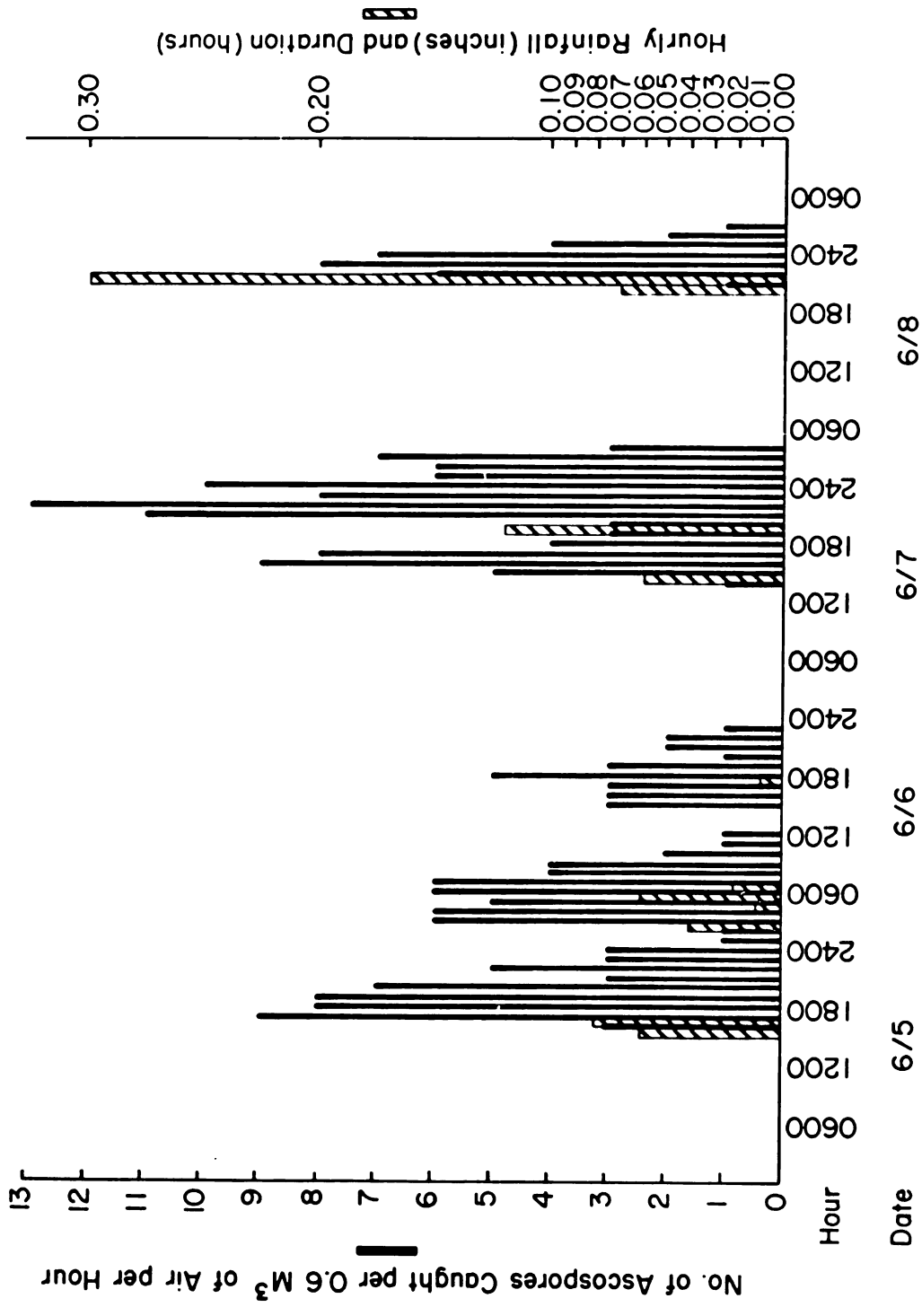


Fig. 13. Effect of rainfall on the hourly trapping of ascospores during 1974.

Conidia were first caught in rainwater running from infected leaves during the weeks of June 14 to June 19, 1974 (Figure 14) and June 11 to June 18, 1975 (Figure 15). These dates also represent the weeks immediately following the initial development of leaf lesions in the field. Peak conidial catches in 1974 occurred during the week of July 10 to July 17 (4.9×10^5 conidia per ml of rainwater) when the shoots were 76 to 90 cm long, about one week after the berries had reached pea-size (ca. 1.0 cm in diameter). In 1975, peak conidial catches occurred during the week of July 2 to July 9 (6.1×10^5 conidia per ml of rainwater) when the berries were approximately pea-size. These times correspond to a period of maximum berry susceptibility, as seen in the artificial inoculation data. During 1974, conidia were trapped at relatively high levels late in the season, from August 20 to September 24, averaging 6.8×10^4 conidia per ml of rainwater per week for the four weeks during this period when there was rain. Trap plants in the field during this time developed no lesions (Figure 8), indicating that these conidia were probably of little consequence in causing infection. This fact is also confirmed by the artificial inoculations (Figure 6), which indicated little or no infection taking place at this time. These high levels of conidia late in the season were also observed during 1975, during the three week period from August 20

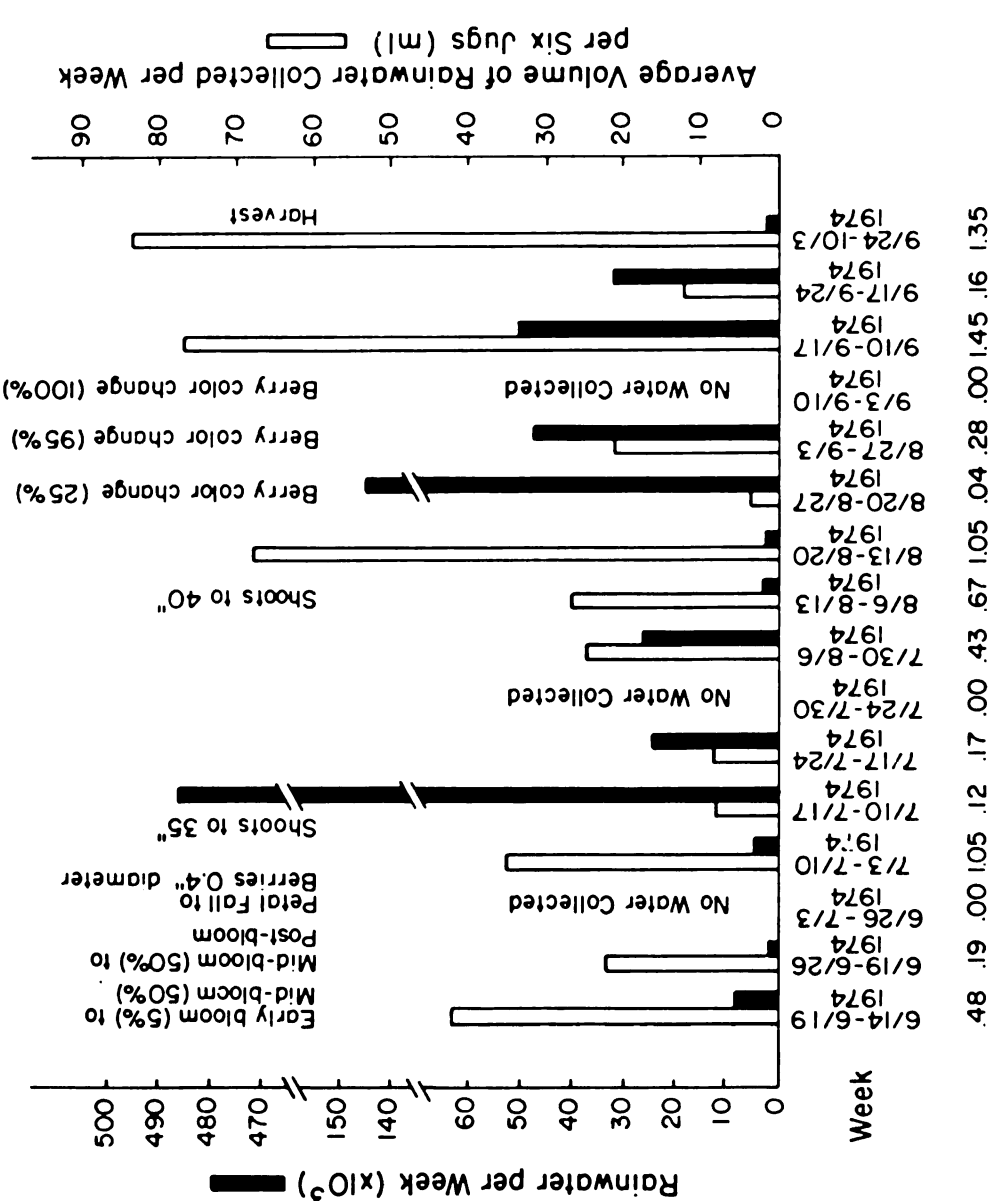


Fig. 14. Weekly conidial catches in rainwater run-off from infected leaves during 1974.

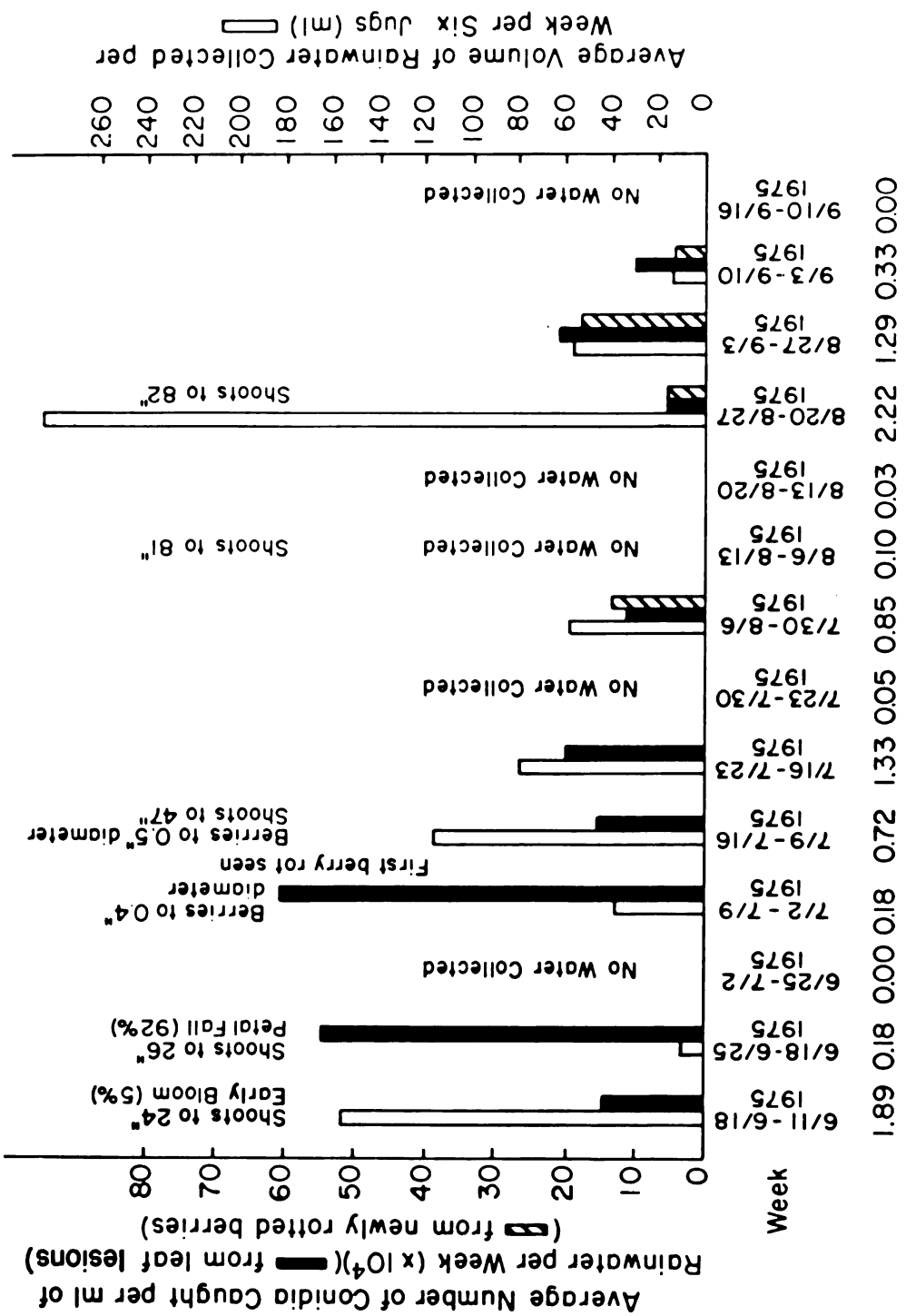


Fig. 15. Weekly conidial catches in rainwater run-off from infected leaves and newly rotted berries during 1975.

to September 10, averaging 1.2×10^5 conidia per ml of rainwater per week.

Conidia were also trapped from newly rotting berries beginning the week of July 30 to August 6 and extending through harvest in 1975 (Figure 15). During the weeks in which rain occurred, levels comparable to those observed for conidia from leaf lesions at this time were obtained, averaging 9.8×10^4 conidia per ml of rainwater per week. However, conidia were undoubtedly being released prior to this time as evidenced by the fact that berries were first observed to be rotting as early as July 9.

Conidia were trapped early in the season from rotted berries which had overwintered and were serving as primary inoculum sources during 1976 (Figure 16). The levels of conidia trapped were relatively low from April 23 to June 18, after which time the level gradually increased to a peak of 4.4×10^5 conidia per ml of rainwater during the week of July 23 to July 30. This corresponds to the period immediately after the berries had reached pea-size (ca. 1.3 cm in diameter).

There appears to be no correlation between the numbers of conidia trapped and the amount or duration of rainfall, the only prerequisite being that rain must occur during the week to provide a means of dispersal.

The general trend in the dispersal of conidia seems to be that relatively low levels are present early

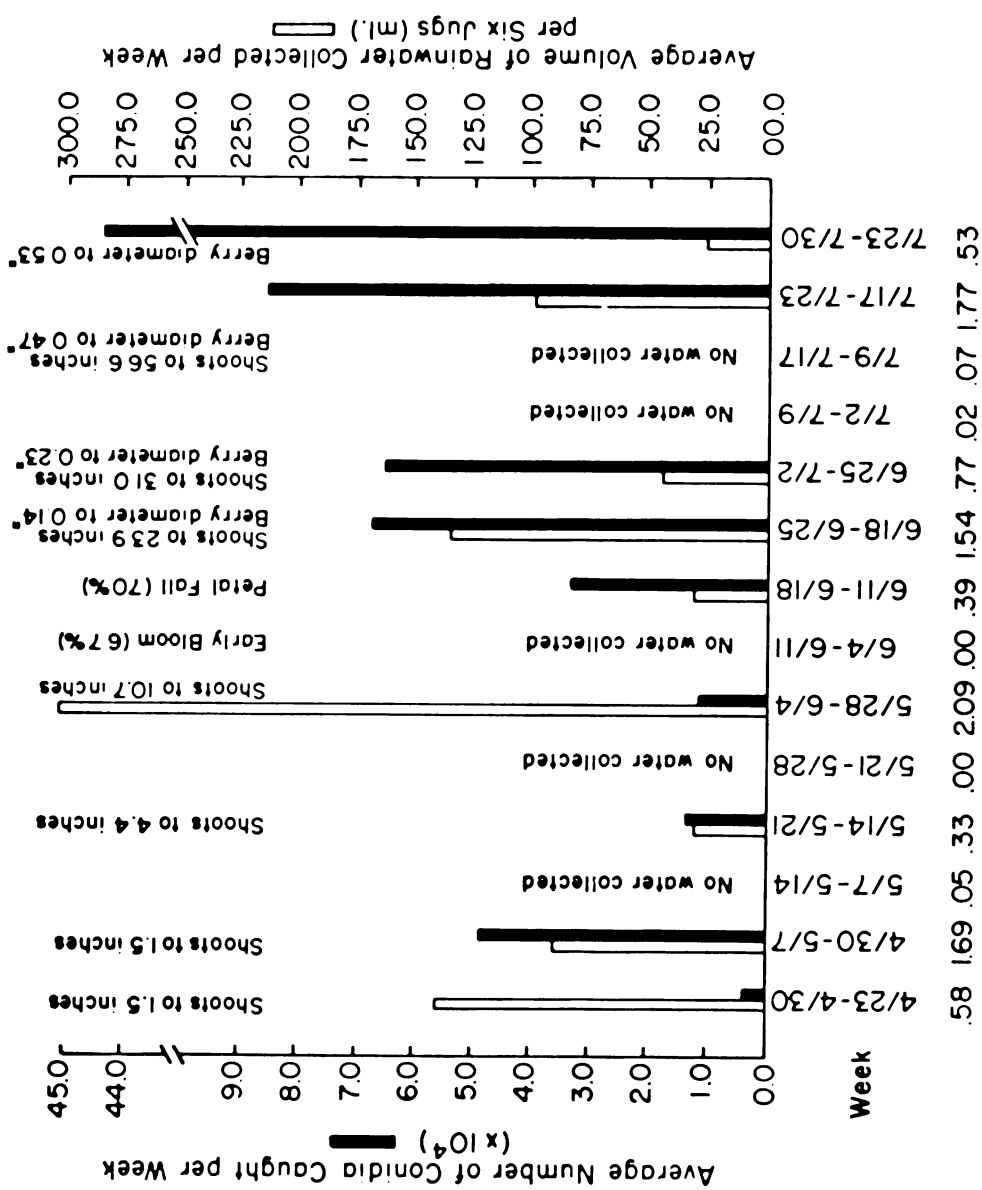


Fig. 16. Weekly conidial catches in rainwater run-off from overwintered rotted berries during 1976.

in the season, reaching a peak about the time the berries reach pea-size, in early July. The levels then decrease, but maintain a rather high level throughout harvest, although late in the season they seem to be of little consequence.

Spray Timing Studies

The results of the spray timing studies for the 1975 season show that the best control was achieved with the program which applied only five sprays, from the time the shoots were from 1.3 to 3.8 cm long (May 17) through petal fall (June 18) (Table 1). This program reduced the incidence of berry rot from 17.47% for the unsprayed control to 1.00%. However, this was not significantly different (by Duncan's Multiple Range Test) from seven other spray schedules. Equally good control was obtained using the five sprays late, from mid-bloom through petal fall (2.47% and 4.69%, respectively). The data indicate that a full program of seven sprays was not necessary for economic control of the disease in 1975.

Preliminary results from the 1976 season show that the best control was achieved with the full spray program which applied seven sprays from the time the shoots were 3.8 cm long through July 30 (Table 2). This program reduced the incidence of berry rot from 30.29% for the unsprayed control to 0.71%. However, this was

Table 1

Control of black rot on "Niagara" grapes with Ferbam applied according to various timing schedules during 1975.

Stage of Development	Treatment Code[x] I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	Date of Spray Application
Shoots ½ to 1½	X														May 17
Shoots 4 to 8"	X	X	X				X								May 24
Early Bloom (< 5%)	X	X	X	X			X	X		X	X				June 11
Mid-bloom (41%)	X	X	X	X	X		X	X	X	X		X			June 14
Immediately Post Bloom	X	X	X	X	X	X		X	X				X		June 18
10-14 Days Later		X	X	X	X	X									July 2
Next Cover Spray			X	X	X	X									July 30
Next Cover Spray			X	X	X	X									August 27
Harvest[y]	1.00 a	5.03 abc	3.50 abc	3.62 abc	2.47 ab	6.13 abc	6.74 bcd	4.69 abc	7.98 cd	11.22 de	18.18 f	15.13 ef	4.93 abc	17.47 f	Sept. 10-11

% Rotted Berries (per 10 clusters per vine)[z]

[x] Each numeral represents a different spray timing schedule. The X's represent when the sprays were applied.

[y] Numbers followed by the same letter are not significantly different at the 0.05 level of Duncan's Multiple Range Test.

[z] Treatments were arranged in a Randomized Complete Block Design with 5 rows, 14 treated vines per row. Percentages are the averages of five replications.

Table 2

Control of black rot on "Niagara" grapes with a combination of Captan and Benlate applied according to various timing schedules during 1976.

Treatment Code [w]

Stage of Development	I	II	III	IV	V	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI [x]	XVII	XVIII	XIX	XX	XXI	XXII	Date of Spray Application
Shoots to 1½"	X														X	X	X			X	X	May 7
Shoots to 8"	X	X	X				X								May 31	X	X	X		X		May 28
Early bloom (6.7%)	X	X	X	X			X	X		X	X					X			X		X	June 11
Mid-bloom (40%)	X	X	X	X	X		X	X	X	X		X			June 19	X		X				June 15-16
Immediately Post Bloom	X	X	X	X	X	X		X	X				X		June 30	X			X		X	June 22
Next Cover Spray		X	X	X	X	X										X	X					July 9
Next Cover Spray			X	X	X	X									July 21	X	X					July 30
Disease [y] Rating	3.21 abcd	2.00 ab	0.79 a	2.50 abc	2.93 abc	11.57 fgh	3.57 abcd	12.86 gh	8.21 defg	5.93 bcde	14.86 hi	9.87 efg	17.79 i	30.29 j	5.21 abcde	0.71 a	7.43 cdef	6.14 bcde	11.00 fgh	5.93 bcde	11.97 fgh	August 13

% Rotted Berries (per 200 berries per vine) [z]

[w] Each numeral represents a different spray timing schedule. The X's represent when the sprays were applied.

[x] This spray timing schedule was determined by weather parameters as measured in the field and analyzed by a computer to determine if spraying was necessary.

[y] Numbers followed by the same letter are not significantly different at the 0.05 significance level of Duncan's Multiple Range Test.

[z] Treatments were arranged in a Randomized Complete Block Design with 7 rows, 21 treated vines per row. Percentages are the averages of seven replications.

not significantly different (by Duncan's Multiple Range Test) from seven of the other spray schedules. Effective control could apparently be achieved with less than the full spray schedule. Equally good control was obtained using five sprays early, from the time the shoots were 3.8 cm long through petal fall; four sprays late, from mid-bloom through July 30; or three sprays timed from when the shoots were 20.3 cm long through mid-bloom (3.21%, 2.93%, and 3.57%, respectively).

Effective control was also obtained with five sprays applied immediately following periods of sufficient rainfall and leaf wetness to constitute infection periods, from the time the shoots were 3.8 cm long through July 30. The necessity of spraying was determined by the use of the Model 6500 computer (Control Data Corporation) into which daily weather parameters were entered for analysis. The variables considered were the amount of rainfall, the duration of rainfall, the length of leaf wetness period associated with periods of rainfall, the stage of development of the vines, and the date of the last spray. If conditions were such that an infection period had occurred, the computer would recommend that a spray be applied. Such sprays were then applied within 24 hours of the end of the leaf wetness period. Since infection would have already occurred, Benlate was included in the spray mixture because of its systemic

action. Disease was reduced from 30.29% for the unsprayed control to 5.21% which was not significantly different (by Duncan's Multiple Range Test) from the best control obtained.

Two-year Old Berries As An Inoculum Source

Two-year old rotted grapes were collected about June 1 and checked for the presence of ascospores by discharging them onto glass slides. Ascospores were present at this time. It was intended that germination studies be carried out with these ascospores to determine their viability. However, no ascospores were observed during early July when this was to be done.

DISCUSSION

Prior to this research, it was known that black rot of grapes caused its greatest devastation during years with frequent rains followed by long periods of fog or high humidity which prevented the vines from drying out (21, 22, 24, 26). The importance of free water in the form of precipitation had been associated with both spore dispersal and infection (2, 24, 26, 28, 35), yet little quantitative evidence had been published to support these observations. Previous spray timing studies indicated that the periods of bloom and early berry development were most crucial in controlling the disease (5, 6, 7, 16, 35). Yet today, a full spray program conducted throughout the season is still employed to combat the disease. As a result of this research, it is now possible to quantitatively define the conditions necessary for dispersal of and infection by the fungus and to seriously consider reduction of the full spray program without adverse effects.

The findings presented here verify earlier observations as to the importance of rainfall in the dispersal of and infection by the fungal spores. Reddick described the process by which rainfall affects ascospore discharge

as follows: "With each succeeding rain during the summer, mummies are moistened, the mature asci absorb water, swell, protrude beyond the perethecial wall, and discharge ascospores into the air" (24). I found that as little as 0.03 cm of rain was capable of triggering ascospore release. Although this relatively small amount of rainfall is not sufficient to thoroughly wet all of the mummies, or even the total surface area of one mummy, those portions of the mummies which are wetted will absorb water and their ascospores will be discharged. The fact that ascospore release is best correlated with the duration of rainfall is not surprising, for the longer rainfall occurs, the more likely the mummies are to be wetted. That the amount of rainfall should correlate so well, despite the observation that there was no apparent relationship between the amount of rainfall and the number of ascospores trapped, is explained in that small amounts of rain will only partially wet the mummified berries, thereby triggering the release of relatively small numbers of ascospores. As the amount of rain increases, the proportion of the mummies becoming wet increases and larger numbers of ascospores are released. Eventually, a point is reached at which the amount of rain causes a thorough wetting of the berries, and additional rainfall no longer triggers the release of additional ascospores. The correlation of periods of 100% RH and of leaf wetness

was also expected. During periods of rainfall and ascospore release, the relative humidity generally reached 100% and the leaves became wet. However, because there were many periods of 100% RH and leaf wetness not associated with rainfall, they were not associated with ascospore release. This accounts for the correlation coefficients for these factors being considerably lower than those for the amount and duration of rainfall.

Once the ascospores have been discharged into the air, they may be picked up by air currents and deposited on vines, ready to germinate and cause infection. Reddick commented on this also: "This is a most effectual means of dissemination, and at the appropriate time, too, since the moisture, as will be seen later, affords opportunity for the germination of the spores" (24). However, he found that ascospore germination proceeded slowly, and he was unable to obtain ascospore germination in less than 36 hours under any conditions. Contrary to this, I found that ascospores germinated in as few as 6 hours on wet leaves at all temperatures tested.

There may also be involved some soluble substance(s) in the leaves which enhances ascospore germination. This was indicated by greater germination of ascospores on glass slides in glass distilled water in which young, immature grape leaves had been soaked for an hour than in plain glass distilled water. Further

studies are needed to confirm this, as well as to study the effect of leaf exudates from mature leaves as compared to immature leaves. This may possibly answer the question of why leaves become resistant to infection as they mature.

The reduced germination of ascospores following incubation on dry leaves for 6 to 48 hours was probably due to the intolerance of ascospores to dry conditions, or perhaps the effect of ultraviolet radiation, rather than to leaf maturation. A 48 hour period is insufficient for such extensive leaf maturation to occur. Moreover, Reddick observed lesions of different ages on the same leaf, which he explained as being due to separate infections caused over a period of three days (24). This same phenomenon was observed with conidia. When incubated for 48 hours or longer on dry leaves, conidia did not cause infection, whereas a fresh conidial suspension inoculated at the times the leaves were rewetted did cause infection (28).

The effect of temperature on the germination rate, but not the final per cent germination of ascospores, is somewhat surprising when viewed in relation to its effects on the infection rate. Germination after 24 hours was essentially the same for all temperatures tested whereas infection after 24 hours was not, indicating that germination alone is not sufficient for infection to occur.

What effect temperature had on the rate of formation of appressoria was not studied, but it seems evident that it did have some effect. The difference observed between the final per cent germination and the final amount of infection may therefore be a consequence of the effect of temperature on the rate of appressoria formation.

Infection in the field was strongly related to the stage of vine development and thereby, host susceptibility, rather than to an excess amount of rainfall or leaf wetness. This was seen when the artificial inoculations in the field were coupled with the data from the trap plants. There were several instances, especially late in the season, where infection periods did indeed occur, with no lesions developing on the trap plants. From the artificial inoculations, it was shown that no infection, or very small amounts, was obtained late in the season, despite there being sufficient inoculum and wetness for infection to occur. This fact was also shown in the spray timing studies which indicated the periods of bloom and early berry development were the critical times for control, this also being the period of maximum vine susceptibility.

Temperature and duration of rainfall correlated best with infection in the field. This coincided with earlier observations made concerning periods of infection. Infection occurred only during periods of rain or shortly

thereafter, with infection most likely to have occurred when the temperatures were high (the average weekly temperature high was 29.2°C during the 1975 season), and when the duration of rainfall was great (the average weekly duration of rainfall was 7.3 hours during the 1975 season). The role that leaf wetness plays should not be overlooked, however. Because there were long periods of leaf wetness not associated with rainfall and no infection resulted, the correlation between leaf wetness and infection was probably not accurate.

In comparing the numbers of ascospores trapped from the air to the numbers of conidia trapped in rainwater run-off from infected vines, the relative importance of the two spore forms in causing the disease may be evaluated. Whereas the levels of ascospores trapped were in the range of hundreds of ascospores or less per week, the levels of conidia caught were in the range of hundreds of thousands of conidia or less per week. The artificial inoculations indicated that the levels of infectiveity of both spore forms were about equal. With this in mind, the conidia may be viewed as being more important, especially once the disease has become established in a vineyard. However, the role of ascospores must not be overlooked, for it is two-fold. It is the ascospores and their dispersal by the wind which allows the disease to gain a foothold in a vineyard, as well as

starting the current disease cycle in the spring. It is also in the ascospores, the sexual stage of the fungus, that genetic changes result and influence the pathogen population. Thus, neither spore type may be overlooked if the disease is to be controlled.

The spray timing studies indicated that the full program of seven sprays currently in use in Michigan for the control of black rot may effectively be reduced to three or four sprays timed to coincide with bloom and the period of early berry development. In addition, the possibility of timing sprays to coincide with periods of infection as well as vine development has been indicated. Further studies and refinement of the computer program used to analyse weather parameters in relation to infection periods and the necessity of spraying are now in order.

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