Melanconium fuligineum and the Bitter Rot Disease of Grape

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ABSTRACT

Melanconium fuligineum infections result in bitter rot of maturing berries and small necrotic flecks on succulent plant parts of muscadine grape (*Vitis rotundifolia*).

Mycelial growth and spore germination occurred at temp from 8 to 36 C and at pH values as low as 2.3. Spores germinated poorly in water unless yeast extract or casein hydrolysate was added. Mycelial growth occurred in media containing DL-malic and D-tartaric acids at concn as high as 2.4%.

Isolates differed somewhat in rates of growth in liquid media and in symptoms and sporulation on infected berries. No perfect stage of *M. fuligineum* was found.

Inoculations in the greenhouse with spores in distilled water resulted in heavy flecking of immature plant parts and very light flecking of mature plant parts. However, flecking and necrosis of mature leaves resulted from inoculations with spores in yeast extract (0.4%). Penetration of immature leaves by germinating spores appeared to be direct by means of an appressorium. Necrotic areas developed on immature or mature leaves wounded and inoculated with mycelial discs or spores. In laboratory and field inoculations, a wound was necessary for the infection of nearly ripe or ripened bunch and muscadine berries using spores or mycelial discs. The rate of bitter rot development in wound-inoculated berries at temp from 12 to 36 C was most rapid at 28 C. No consistent differences in bitter rot development were noted among isolates or between varieties. *M. fuligineum* rotted fruits of grape, apple, cherry, peach, strawberry, blueberry, and banana. It produced the enzymes polygalacturonase and *trans*-eliminase in vitro.

Early season flecking of immature berries was implicated in the subsequent bitter rot development of mature berries.

All fungicides except Benlate were more effective in inhibiting spore germination than in suppressing mycelial growth. Maneb, Dithane M-45, and ferbam were the more effective fungicides in inhibiting mycelial growth and spore germination. Phytopathology 60:1203-1211.

Grape bitter rot caused by *Melanconium fuligineum* (Scrib. & Viala) Cav. is reported to occur on *Vitis munsoniana* Simpson, *V. rotundifolia* Michx., *V. labrusca* L., and *V. vinifera* L. (4, 5, 6, 8, 12). In North Carolina, *M. fuligineum* has been estimated to cause losses of 10 to 30% of the ripening muscadine berries (*V. rotundifolia*) in recent years (3). To date, no detailed work has been reported on the epidemiology of the disease on muscadine grape with emphasis as to time and nature of infection. Studies on factors affecting sporulation, the thiamine requirements, and carbon utilization by *M. fuligineum* have been reported (7, 16, 17). This paper gives results of research on (i) the physiological and pathogenic aspects of *M. fuligineum*, (ii) the development of *M. fuligineum* and the bitter rot disease under field conditions, and (iii) the development of effective control measures.

MATERIALS AND METHODS.—*Isolates of M. fuligineum*.—Single-spored isolates of *M. fuligineum* designated as 15, 36, 115, 119, 123, and 142 were obtained during 1966, 1967, and 1968 from infected muscadine and bunch berries, leaves, and tendrils in five locations of North Carolina. Subcultures of these isolates are designated as 36-1, 36-2, 115-1, etc.

Media.—The dextrose-potato extract medium (DPE) consisted of 10 g dextrose, 2.5 g Difco dehydrated potato extract, and distilled water to make 1 liter. The composition of the maltose-ammonium tartrate medium (MAT) was the same as that reported by Timnick et al. (16). Sucrose (SAT) was substituted for maltose where indicated. The pH of each medium was adjusted to 5.0 with 1 N HCl.

The glucose-sodium polypectate (GSP) and pectin media used in the pectolytic enzyme study were prepared as described by Sherwood (13) except for the vitamins and microelements. These modifications consisted of adding thiamine hydrochloride, biotin, inositol, Fe^{+3}, Zn^{+2}, Mn^{+2}, and Ca^{+2} at the rates given below. The pH of the pectin and GSP media after autoclaving was 3.3 and 5.1, respectively.

The *Rhizoctonia* medium (RM) used in the cellulolytic enzyme study consisted of 2.5 g cellulose (regenerated cellulose), 1.4 g casamino acid, 0.6 g KH_2PO_4, 0.4 g K_2HPO_4, 0.9 g $MgSO_4 \cdot 7H_2O$, 0.1 mg thiamine HCl, 5.0 μg biotin, 5.0 mg inositol, 0.1 mg Fe^{+3}, 0.2 mg Zn^{+2}, 0.05 mg Mn^{+2}, 10.0 mg Ca^{+2}, 18.0 g agar, and distilled water to make 1 liter of medium. The pH was adjusted to 5.5 before autoclaving.

Difco agar (A) was included at the rate of 20 g/liter of medium where indicated. The liquefied media (30 ml) were dispensed by an automatic pipette into 250-ml Erlenmeyer flasks, plugged with nonabsorbent cotton, and autoclaved at 121 for 15 min.

Measurements and procedures.—Growth of the fungus on agar media is expressed as colony diam. Growth in liquid media is given as dry wt (mg) of mycelia after drying at 80 C for 24 hr.

Spore germination was studied by placing a drop of the spore suspension (1×10^5 spores/ml) onto a cover glass contained in a petri plate with the top lined with filter paper. A 0.4% sterile aqueous solution of Difco yeast extract (pH 6.4) was used with the spore suspension as a germination stimulant unless indicated otherwise. Spore germination was recorded

as positive when the germ tube length exceeded the spore width. At least 100 spores were counted/replicate.

To follow berry development during the season, the soluble solids, pH values, and sizes of berries were recorded. The soluble solids content of the berries was measured with a Lafayette Hand Sugar refractometer. Using dilutions of a 20% sucrose solution, the refractometer scale was calibrated at 25 C. Readings were obtained by halving each berry and pressing the juice on the refractometer plate. Readings were recorded and averaged for 10 berries. Using a pH tape, the hydrogen ion concentration of contents of individual berries was recorded at the same time as the soluble solids.

Berry size was expressed as diam (mm). Leaf measurements were taken at the widest point across the leaf perpendicular to the midrib and of the length of the midrib.

The rot development of a berry was followed by rating the per cent surface area rotted in relation to the size of the berry. Flecking of leaves, berries, and stem tissue was recorded as trace, light, moderate, or heavy, according to the extent of over-all flecking of the plant part in relation to its size.

Inoculum preparation.—Inoculum consisted of a 5-mm disc of mycelium from the edge of a 2- to 4-day agar culture, or a spore suspension (1×10^6/ml) collected from 10-day-old cultures grown on MAT or SAT agar medium. The spore concentration was determined with a Spencer haemocytometer.

Greenhouse and laboratory inoculations.—Studies in the greenhouse and laboratory were made on muscadine grape cultivars Thomas, Topsail, NC57-56, Magnolia, Roanoke, and Scuppernong, and bunch grape cultivars Niagara and Portland. Inoculations were made on the following plant parts: 1) immature (8-15 mm × 10-30 mm) and mature (30-50 mm × 50-70 mm) leaves, either detached or intact on 2- to 3-year-old rooted cuttings; 2) berries of size C (13-18 mm) and ripeness C_1 (green), C_2 (nearly ripe), C_3 (ripe); 3) segments of stem tissue cut from current season's shoot growth; 4) leaf petioles of immature and mature leaves; and 5) unopened flower clusters.

The inoculum consisted of either a spore suspension in distilled water or yeast extract (0.2% or 0.4%), mycelial fragments scraped from culture, or mycelial discs taken from PDA or water agar cultures. Isolates 119, 142, 15, and 36 were used. Inoculation involved applying spores with an atomizer to the ventral and dorsal surface of the leaves, either wounded or not berries, and injecting the berries or pedicels with spores with a syringe and 25-gauge needle. Mycelial discs were placed with the mycelial surface against the ventral or dorsal surface of the leaves, either wounded or not wounded. Also, mycelial discs or mycelial fragments were placed on the wounded or nonwounded surfaces of the berries (wound made by a needle puncture), pedicels, or stem segments which were either steamed or nonsteamed.

Plants were covered with polyethylene to reduce runoff and incubated in a mist chamber (mist of water for 15 min each hr). Berries and stem segments were placed on a wire screen in the mist chamber or in a moist chamber (plastic box lined with moistened cheesecloth). Incubation was either at greenhouse temp (20-30 C) or in incubators at 12, 22, 28, or 36 C.

Field inoculation studies.—To study the infection and development of *M. fuligineum* on grape cultivars under field conditions, experiments were conducted in 1967 and 1968 on the University Farm at Method, North Carolina, and the Central Crops Research Station at Clayton using Niagara, Portland, Scuppernong, Willard, NC57-56, and Topsail.

Inoculations were made by (i) injecting a spore suspension into the pedicel 3-5 mm from the berry and into the berry pulp 2-5 mm; (ii) spraying a spore suspension on nonwounded or wounded berries (spores applied either before or after wounding); (iii) applying mycelial discs to pedicels which were either nonwounded or wounded (removal of the epidermis); or (iv) placing a bitter-rotted berry into a cluster of healthy berries and spraying with water until point of runoff.

Inoculations of the muscadine cultivars were made early in the growing season (bloom) and repeated several times until harvest. Inoculations of the Niagara and Portland grapes were not started until 4 weeks before harvest. Individual clusters of berries or one-half arm of a vine containing clusters of berries were selected for each inoculation technique and time of inoculation. Three to five replications were used for each inoculation technique.

To maintain moist conditions in some of the experiments, plastic bags containing wetted cotton were placed for 3 to 5 days over the inoculated and non-inoculated berry clusters. A paper bag was then fastened over the plastic bag and maintained even after removal of the plastic.

Effect of fungicides on mycelial growth and spore germination.—The relative toxicity of various fungicides that might be used to control bitter rot was studied by determining their effectiveness in inhibiting spore germination and mycelial growth by *M. fuligineum.*

The following fungicides were tested in the mycelial growth and spore germination studies: Benlate [1-(butylcarbamoyl)-2-benzimidazole carbamic acid, methyl ester]; captan[N-(trichloromethyl) thio-4-cyclohexene-1, 2-dicarboximide]; Botran (2,6-dichloro-4-nitroaniline); copper (copper sulfate pentahydrate); dodine (n-dodecylguanidine acetate); Dithianon [5,10-dihydro-5, 10-dioxonaphtho (2,3-b)-p-dithiin-2,3-dicarbonitrile]; Difolatan [N-(1-1,2,2-tetrachloroethyl) sulfenyl-cis-4-cyclohexene-1,2-dicarboximide]; Dikar (74% Dithane M-45 and 6% Karathane); Dithane M-45 (coordination product of zinc ion and maneb); ferbam (ferric dimethyldithiocarbamate); folpet [N-trichloromethylthiophthalimide); Karathane WD [mixture of 2-(1-methylheptyl-4, 6-dinitrophenol and 2-(1-methylheptyl)-4,6-dinitrophenyl crotonate]; maneb (manganous ethylenebis dithiocarbamate); Polyram[ammoniate of (ethylenebis dithiocarbamate)] zinc, ethylenebis (dithiocarbamic acid) bimolecular, and trimolecular cyclic anhydrosulfides and disulfides]; and zineb (zinc ethylenebis dithiocarbamate).

For the mycelial growth study, the concn of active ingredient used were 10, 30, 90, 270, 810, and 2430 ppm. The highest concn was prepared at the $\times 2$ level, and serial dilutions were made to obtain the lower concn at the $\times 2$ level. The fungicidal mixtures were mixed with an equal volume of melted PDA at 45 C. The PDA ($\times 2$ level) consisted of 10 g dextrose, 2.5 g potato extract, and 40 g of agar/liter. Five plates were used for each concentration of fungicide. Inoculations were made within 24 hr with 5-mm mycelial plugs taken from the margin of 7-day-old cultures of isolate 119-1 growing on PDA ($\times 1$ level). Incubation was at 30 C in a moist chamber.

In the spore germination study, the concn of active ingredient used were 10, 30, 90, and 270 ppm. The highest concn was prepared at the $\times 2$ level, and serial dilutions were made in test tubes. To each fungicide preparation was mixed a $\times 2$ concn of spores ($2 \times 10^5/$ ml) in a 0.4% yeast extract prepared from a 10-day-old culture of isolate 119-1 on a SAT medium. Three spore-fungicide drops (replicates) were used for each concn. Incubation was in a moist chamber at 30 C. The percentages of nongerminated spores were recorded after 17-22 hr. At least 100 spores were counted for each replicate.

RESULTS.—*Physiological studies.*—The optimum temp for growth was studied using four isolates inoculated in triplicate in plates of PDA and flasks of liquid DPE medium. Twelve temp from 2 to 40 C were used for PDA, and 5 temp from 20 to 32 C for the liquid DPE medium. Colony growth data after 7 days showed that the optimum temp ranged from 28 to 30 C. The fungus did not grow or survive at 40 C.

The relative growth rates of isolates in a liquid MAT medium were compared after different periods of incubation at 30 C. Mycelial wt were recorded after 5, 9, 11, and 14 days in the MAT medium (Fig. 15). Isolates 36-1 and 123 grew the slowest. Isolates 115-1 and 142 reached maximum wt after 9 days; isolates 15 and 36-2 reached maximum wt after 11 days.

The optimal temp range for spore germination was studied using temp at 4-C intervals from 8 through 24 C, and at 2-C intervals from 28 to 36 C. Within 20 hr, no germination occurred at 8 and 12 C, whereas 22 and 62% germination occurred at 16 and 20 C, respectively. Complete or nearly complete germination occurred at temp of 24 to 36 C. No differences in the percentage of germination or germ tube length were found at 28, 30, and 32 C. The mean germ tube lengths after 20 hr for each temp were 11, 56, 98, 140, 63, and 56 μ for each respective temp of 16, 20, 24, 28-32, 34, and 36 C.

The effect of heat treatment on the viability of spores was studied using two isolates of *M. fuligineum* growing on MAT. Spore suspensions (75,000/ml) in yeast extract (0.4%) were incubated in a water bath for 10 min at temp of 30, 35, 40, 45, 50, 55, and 60 C. Germination was recorded after 20 hr at 30 C. Spores treated at 60, 55, and 50 C failed to germinate, whereas those treated at temp of 45, 40, 35, and 30 C germinated 100%. Viability of spores incubated for 7 hr at

45 C decreased rapidly. At 40 C, spore viability decreased gradually to 76% after 26 hr.

To determine the effect of hydrogen ion concentrations on mycelial growth, pH values were adjusted to include those found in muscadine berries. Four isolates were inoculated into a modified liquid DPE medium (dextrose 5.0 g and potato extract 1.25 g/liter) buffered with 0.05 M D-tartaric acid and 0.05 M NaH$_2$PO$_4$. The pH values of the medium were adjusted at 0.3 increments from pH 2.0 to 4.1. The buffer system chosen was not completely satisfactory, since the tartrate was utilized to some extent and inhibitory at high pH values. Other buffering compounds, such as acetate, citrate, and malic acid were also unsatisfactory because of toxic effects or easy utilization.

After 8 days at 30 C, mycelial growth occurred at pH values as low as 2.3, and was max at pH 3.2 and 3.5 (Fig. 14). The growth rate for all isolates decreased as pH increased from 3.5 to 4.1. The pH values after 8 days and the initial pH values were nearly the same. The final pH of the unbuffered check was 4.2.

The effect of pH on spore germination was determined by using pH values from 2.0 to 7.5 at 0.3 and 0.5 units. Three replicates were used for each pH value. After 20 hr, only 9% of the spores germinated at pH 2.0. The percentage of germination increased as the pH was increased from pH 2.3 to 2.9. One hundred % germination occurred at pH 3.2 to 7.5, inclusive. The germ tube lengths increased from pH 2.0 to 2.9 and showed no differences at pH 2.9 to 6.5. At pH 7.0 to 7.5, they were markedly shorter. No changes in pH values were detected during this experiment.

Spore germination stimulants.—The effects of different nutritional compounds or plant extracts on spore germination were studied using spores which were rinsed after each of three centrifugations for 20 min at 10,000 g. Sterile stock solutions of Difco yeast extract, salt and vitamin-free casein hydrolysate, thiamine hydrochloride, dextrose, glycine, L-asparagine, and ammonium chloride were serially diluted and mixed with the spore suspension to give the final concentration of nutrient and spores.

Spores incubated in water at 30 C for 20 hr did not germinate. The addition of asparagine (0.4%), dextrose (1.0%), glycine (0.4%), NH$_4$Cl (0.4%), or thiamine (0.04%) to water singly and in combination was relatively ineffective in stimulating spore germination. Less than 5% germination occurred after 20 hr. Casein hydrolysate (0.4%) or yeast extract (0.4%) in aqueous solution stimulated at least 90% of the spores to germinate within 20 hr.

The concn of the stimulant affected spore germination (Fig. 12). After 8 hr, spore germination increased as the percentage of yeast extract or casein hydrolysate was increased to 0.8%. After 18-hr incubation, germination was 100% in all concn of yeast extract. With casein hydrolysate, germination ranged from 50% at the lowest to 99% at the highest concentration. In addition, after 18 hr the length of the germ tubes were shorter as the concn of yeast extract increased, while with casein hydrolysate, the lengths of the germ tubes were the same at concn of 0.2% to 0.8%. The lengths

of germ tubes were greater in the yeast extract than in the casein hydrolysate at each respective concentration. Spore germination in extracts from mature and immature leaves after 22 hr at 30 C was 96%.

The effect of different concn of D-tartaric and DL-malic acids, the two predominant acids of muscadine berries, on mycelial growth was studied using two isolates of *M. fuligineum*. Because the total concn of these two acids in ripening muscadine berries ranges from 0.7% to 1.5% (D. E. Carroll, *personal communication*), serial dilutions were made with DPE (pH 3.3) to give the final concn of 1.6, 0.8, 0.4, 0.2, and 0.1% of each acid. Also, dilutions were arranged so that a final concn of 0.4% tartaric acid was combined with each concn of malic acid, and 0.8% malic acid was combined with each concn of tartaric acid. The checks included the DPE medium at pH 3.3 without added malic or tartaric acids. After 8 days at 30 C, growth was greater at all acid concn than in the DPE medium without the added acids. Growth of each isolate increased as the concn of acids, singly and combined, were increased, except at the 0.8 and 1.6% concn where some growth inhibition was noted. Each isolate showed the greatest inhibition of growth in the malic + 0.4% tartaric acid medium at both 0.8 and 1.6% levels of malic acid. The initial pH value of 3.3 changed very little during the experiment.

Production of pectolytic and cellulolytic enzymes in vitro.—An assay for pectolytic enzymes was conducted with 4-, 7-, and 12-day-old culture filtrates of *M. fuligineum* grown in liquefied GSP and pectin media. The media were inoculated with mycelial discs from PDA cultures. The culture filtrates were assayed using the Brookfield rotating spindle viscometer (13, 14) and the thiobarbituric acid test described by Sherwood (13). Pectin methylesterase was assayed in 14-day-old culture filtrates from the pectin medium according to the method of Smith (15). Commercial pectinase was included in the assay.

The results of these studies are given in detail by Ridings (11). In summary, *M. fuligineum* was found to produce polygalacturonase in 4- and 7-day-old cultures and *trans*-eliminase in 12-day-old cultures from the GSP and pectin media. No pectin methylesterase was found in 14-day-old cultures grown in the pectin medium.

The production of cellulolytic enzymes by *M. fuligineum* was studied using the method of Rautela & Cowling (10). *Rhizoctonia solani* was included in this study as a standard for comparison. Each of five test tubes of the *Rhizoctonia* medium was inoculated with a 5-mm mycelial disc from PDA and incubated at room temp. After 4 days, *R. solani* produced a 3.8 mm depth of clearing that reached 18.0 mm after 30 days. No depth of clearing was produced by *M. fuligineum* after 30 days.

Greenhouse and laboratory inoculations.—The fungus grew and sporulated heavily within 4 days on steamed stems inoculated with mycelial discs (PDA or water agar). A slight necrosis developed on inoculated green stems. No necrosis developed on nonsteamed leaf

petioles inoculated with mycelial discs (PDA) after 10 days.

Inoculation of the ventral or dorsal surface of attached or detached leaves with mycelial discs (PDA or H_2O agar) resulted in a spreading necrosis only where a wound was present prior to inoculation. The area of necrosis was larger on the immature leaves (Fig. 1, 2).

Inoculation of the ventral or dorsal surface of attached leaves with a spore suspension in water resulted in heavy infection (flecking) of the immature leaves and no infection to light flecking of the mature leaves. The flecking reaction appeared within 17 hr, and was rated after 48 hr. A concn of 1×10^4 spores/ml produced light flecking of immature leaves; 1×10^6 spores/ml produced heavy flecking. Flecks on plants subjected to repeated mist did not increase in size. The fungus failed to sporulate in the flecks, but remained alive. *M. fuligineum* was isolated from the flecks for at least 44 days after inoculation.

The use of 1×10^6 spores/ml in 0.4% yeast extract resulted in heavy flecking and necrosis of immature and mature leaves as well as unopened flower clusters. Only slight necrosis was noted on flower clusters inoculated with spores in water.

Penetration by germinating spores of *M. fuligineum* was studied on inoculated leaf discs cleared with chloral hydrate. The technique of Boedijn (1) using Trypan blue in lactophenol was then employed to stain the germ tubes of the spores. Leaf penetration by germinating spores was followed closely on the dorsal surface of immature leaves. At 0 hr, no spores were detected, possibly because they had been washed off the leaves in the process of staining. Germinating spores were detected after 10 hr with the germ tubes showing elongation after 14, 23, and 35 hr. Flecking of the leaves and leaf penetration were detected at 72 hr. The flecks had enlarged considerably by 135 hr (Fig. 8, 9). Penetration of leaves appeared to be direct by means of an appressorium. At the point of penetration, a browning reaction occurred in the cell walls (Fig. 7). No penetration through stomata was observed. Inoculations of either the dorsal or ventral leaf surface showed that flecking occurred at equal intensity on both surfaces.

Inoculation of ripening berries with mycelial discs taken from PDA or H_2O agar resulted in bitter rot development consistently where a wound was present. A brown rot area was noted after 19 days on unwounded berry surfaces. Further incubation to 29 days resulted in typical bitter rot, and *M. fuligineum* was isolated.

The inoculation of berries with spores in water resulted in bitter rot only where a wound was present. The use of a spore germination stimulant (yeast extract, berry extract) did not result in infection of muscadine berries, but did allow infection of berries of *V. vinifera* 'Tokay' within 6 days. No bitter rot developed in clusters of muscadine berries inoculated with a diseased berry.

Inoculation of wounded or nonwounded pedicels with a spore suspension in water resulted in bitter rot on berries after 20 days. Slightly more bitter rot developed on berries from wounded pedicels. Bitter rot

of berries developed within 7 days when wounded pedicels were inoculated with mycelial discs of PDA.

A study of the rate of bitter rot by five isolates on inoculated Magnolia and Topsail berries at temp of 12, 22, 28, and 36 C showed that 28 C was optimal after 7 days (Fig. 13). No consistent differences were noted in bitter rot development among isolates or between varieties. At 36 C, bitter rot development was almost nil except for isolate 15, which caused slight rot. Differences were noted in type of sporulation resulting from the bitter rot development of Scuppernong berries by isolates 115, 123, 119, and 36 (Fig. 6). The type of sporulation exhibited by isolate 115 is quite typical of most isolates.

The ability of *M. fuligineum* to decay various fruits and vegetables was determined by inoculating wounded fruits or vegetables with mycelial discs or spores and incubating at 30 C in a moist chamber. Soluble solids and pH values were recorded from small segments of the specimen tissue where the inoculum was placed.

Within 7 days, isolates 119-1 and 142 had initiated rotting of the mature fruits of Golden Delicious apple, grape, cherry, Earlibelle strawberry, peach, and banana. Rotting of immature blueberries and Stayman apples occurred only after 3 to 4 weeks' incubation. Inoculated tomato fruits or potato tubers showed no rot after 6 weeks.

Field inoculations.—Immature berries, pedicels, tendrils, and leaves were inoculated with a spore suspension in distilled water or aqueous yeast extract (0.2%). Infection (flecking) developed in 2 to 3 days (Fig. 10, 11). However, bitter rot development could not be related to the above inoculations applied either early or late in the growing season. Bitter rot developed (consistently) within several days on inoculated, wounded immature muscadine berries which had a diam 10 mm or greater, a pH of 2.7, and a soluble solids content of 5.0.

Mycelial strands of *M. fuligineum* were detected on naturally infected Niagara berries and found to increase in number as the berries approached maturity (Fig. 3, 4). Insect wounds were found on approximately 30% of the bitter-rotted Niagara berries.

The soluble solids content of the muscadine berries remained at 5% in the berries of C_3 size until 21 August, when the soluble solids in the bunch berries increased rapidly to approximately 15% by 21 September. The soluble solids in the bunch berries increased from 5 to 17% from 5 July to 10 August. In the same periods, the pH values of the berries (bunch and muscadine) increased from pH 2.5 to pH 3.3.

Attempts to find a sexual stage of reproduction by examining inoculated and naturally infected leaves, stems, berries, and cultures grown on agar media failed.

Effect of fungicides on spore germination and mycelial growth.—The effects of four concentrations of several fungicidal chemicals on spore germination after 21 hr are given in Table 1. Further testing showed that captan, dinocap, dodine, folpet, and Polyram were 100% inhibitory at 5 ppm. Dithianon, Difolatan, Dikar, maneb, and Dithane M-45 completely inhibited germination at 2.5 ppm. The fungicides dinocap, folpet, and

TABLE 1. The effect of different concn of fungicides on spore germination by *Melanconium fuligineum* after 21 hr

| Fungicide | % Nongerminated spores at stated concn (ppm active ingredient) | | | |
	10^a	30	90	270
Botran	28	41	59	97
Copper	7	20	100	100
Zineb	56	92	97	100
Benlate	76	95	96	100
None (check)	3			

[a] At 10 ppm, the following fungicides, captan, Difolatan, Dikar, dinocap, Dithane M-45, dithianon, dodine, ferbam, folpet, maneb, and Polyram, resulted in 96-100% inhibition.

Polyram completely inhibited germination only at 30 ppm; copper ($CuSO_4$) was effective at 90 ppm; and zineb and Benlate were effective at 270 ppm. Botran inhibited germination by 97.3% at the highest concentration tested (Table 1).

The pH values of most of the fungicide-yeast extract mixtures were 6.4 to 6.7. The pH of copper sulfate was 5.9 and 4.2 at 10 and 270 ppm, respectively. Benlate gave readings of pH 6.5 and 7.5 at 10 and 270 ppm, respectively.

The results of the effect of six concn of each fungicide on mycelial growth after 6 days at 30 C are given in Table 2. The fungicides Polyram, zineb, folpet, and captan were not effective in inhibiting mycelial growth. Benlate was outstanding in that no growth occurred with this fungicide even at the lowest concentration tested.

The pH values were quite variable at the high concn, but tended to be the same at 270 ppm and lower except for the copper sulfate (Cu) and Benlate. All fungicides at 10 ppm gave the same pH values. No buffer was employed to control the pH.

At the fungicide concn where no growth occurred, the inoculum plug was removed after 6 days and inverted in a PDA plate. No growth occurred after 6 days' incubation.

DISCUSSION.—The ability of *M. fuligineum* to rot berries may result from its production of the enzymes polygalacturonase and *trans*-eliminase which were demonstrated in vitro. Its ability to rot incubated fruits of peach, apple, strawberry, banana, and blueberry indicates that this fungus is not restricted in its pathogenicity to the fruits of *Vitis* sp., even though it has not been reported to affect these fruits.

From inoculations in the laboratory and greenhouse, immature leaves, shoots, or berries became heavily infected (flecked), while little or no flecking occurred on the mature tissues. These differences could have resulted from some stimulant which was only present (or present in larger concn) in the immature tissues. Inoculations of mature leaves using an external spore germination stimulant resulted in heavy infection (flecking and necrosis). A study of spore germination in vitro showed that the addition of yeast extract or casein hydrolysate to water stimulated spores to germinate 95-100% within 20 hr, while the per cent germination in water was zero. Critopoulos (4) has similarly reported only 2% germination in water. The

TABLE 2. The effect of different concentrations of fungicides on mycelial growth of *Melanconium fuligineum* after 6 days[a]

Fungicide	Mean colony diam (mm) at stated concn (ppm active ingredient)					
	10	30	90	270	810	2,430
Polyram	73	64	53	43	31	8
Zineb	79	76	67	52	36	0
Folpet	65	54	38	18	7	3
Captan	74	49	6	4	2	0
Copper	54	29	0	0	0	0
Dithianon	31	19	5	3	3	0
Difolatan	17	9	6	5	4	4
Dikar	32	12	1	0	0	0
Dinocap	10	7	2	0	0	0
Dodine	7	5	2	1	0	0
Ferbam	32	3	0	0	0	0
Dithane M-45	62	0	0	0	0	0
Maneb	58	0	0	0	0	0
Benlate	0	0	0	0	0	0
None (check) = 79						

[a] Least significant difference at 5% = 10.0; at 1% = 13.1. Coefficient of variation = 37%.

demonstration of spore germination stimulants on other plant species has been reported by Brown (2). The sources of a stimulant during berry ripening could be immature plant tissues or injured berries. Field and laboratory inoculations using a spore germination stimulant, however, did not result in bitter rot development to any noticeable extent. The differences in the development of necrosis of wounded mature and immature leaves showed that the latter were physiologically more favorable for the development of *M. fuligineum*.

The results from laboratory and field inoculations showed that bitter rot developed consistently in wounds of berries and pedicels. The pedicel inoculations with spores resulted in less disease than inoculations with a mycelial disc. It was surmised that bitter-rotted berries developing from insect wounds might serve as a source of inoculum for the infection of other berries, but inoculations in the field and laboratory using bitter-rotted berries pinned into healthy berry clusters did not produce infection of adjacent nonwounded berries.

Inoculation experiments on nonwounded muscadine plant parts from bloom to harvest resulted only in flecking of immature tissues. Because the bitter rot infections frequently arise at the points of pedicel attachment in the absence of detectable wounds, the role of early season flecking on the pedicels in subsequent bitter rot development needs further study under controlled environmental conditions. The data of Luttrell & Murphy (9) showed that bitter rot was reduced by half when fungicidal sprays of bordeaux or zineb were

applied soon after bloom 3 times at 2-week intervals. It appeared that protection early in the season was important in reducing bitter rot. The inoculum from overwintered tendrils, pedicels, and mummies has been observed to be heavy in spring and light in late summer. Also, no sporulation has been found in early season infections (flecks).

Critopoulos (4), using berries of *V. vinifera*, found that infection by *M. fuligineum* occurred on nonwounded berries. Results in the present study showed that infection of nonwounded *V. vinifera* 'Tokay' berries did not occur unless a germination stimulant was added to the spore inoculum.

The results of the fungicide treatments on spore germination and mycelial growth indicated that spores were more sensitive to fungicides than mycelia. Only Benlate was more effective against mycelial growth than spore germination. Maneb and Dithane M-45 were both highly effective in spore germination and mycelial growth tests. The maintenance of fungicides in suspension in the mycelial growth test was difficult because they settled rapidly at the highest concentration. The recommendations for the use of a fungicide for bitter rot control can be reliably made only after testing in the field for several years, but the results of this study suggest that maneb and Benlate show promise for testing under field conditions. These fungicides have been found very effective in recent study of their relative effectiveness for the control of bitter rot under field conditions (3).

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Fig. 1-11. 1) Immature grape leaf showing: (a) no necrosis from noninoculated wounded area; (b) necrosis from inoculation with agar mycelial disc of *Melanconium fuligineum* on wounded area; (c) no necrosis from inoculated and unwounded area. 2) Mature grape leaf showing necrosis from inoculation with mycelial disc on wounded area. Positions of inoculations and check are the same as in Fig. 1. 3) Mycelial strands of *M. fuligineum* developing on bunch berry. 4) Sporulation by *M. fuligineum* on infected bunch berry. 5) Spores of *M. fuligineum* from an acervulus (×1,600). 6) Acervuli and mycelial production on muscadine grapes 14 days after inoculation with 4 isolates, 115, 123, 36, 119-1, and check (left to right, respectively). 7) Penetration of immature leaf tissue by germinated spore after 72 hr (×1,500). 8) Flecking of immature leaf 72 hr after inoculation (×160). 9) Flecking of immature leaf 132 hr after inoculation (×160). 10) Natural infection (flecking) of immature muscadine plant parts (leaf, leaf petiole, tendril, stem, unopened flowers). 11) Flecking of immature berries and berry pedicels.
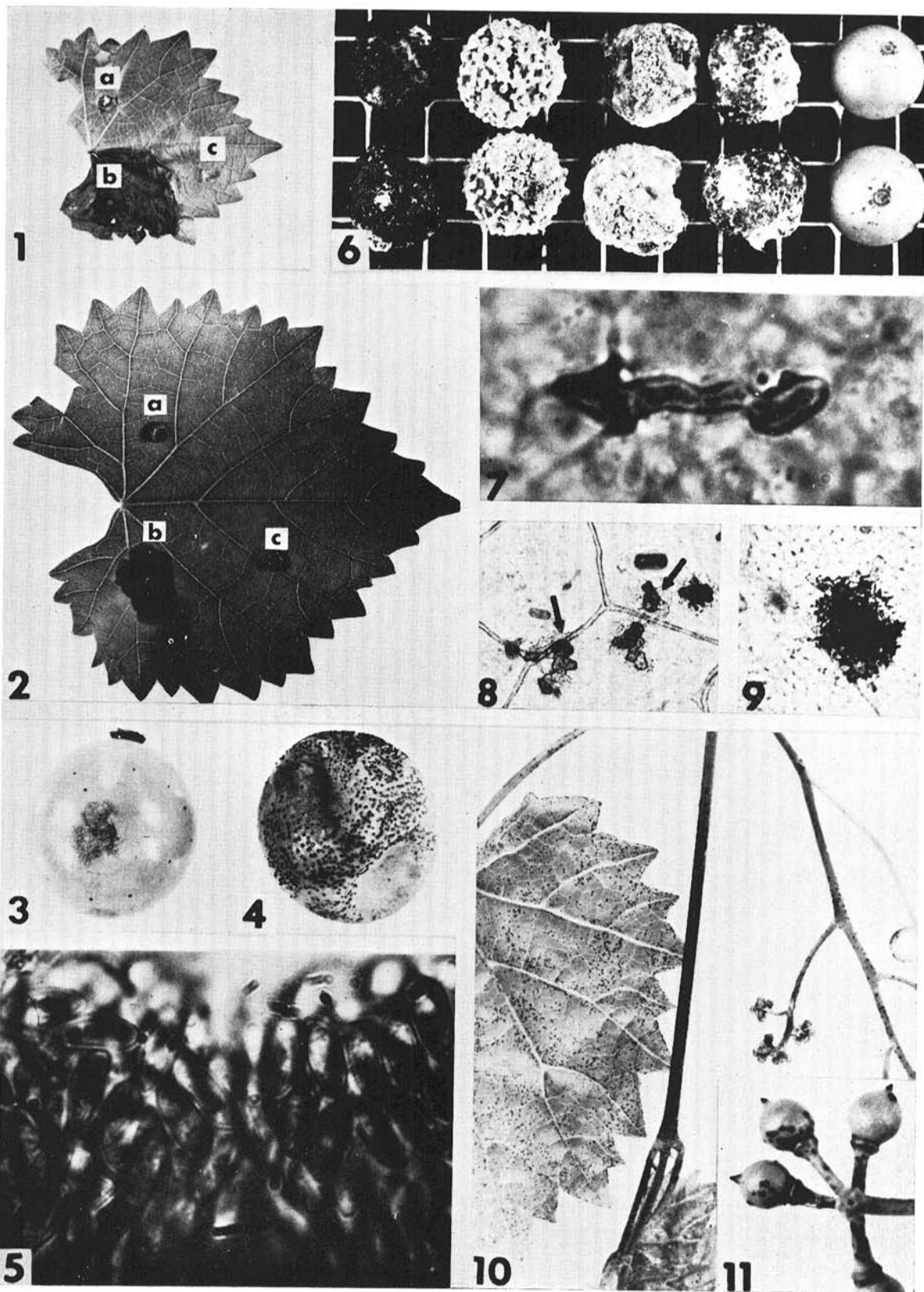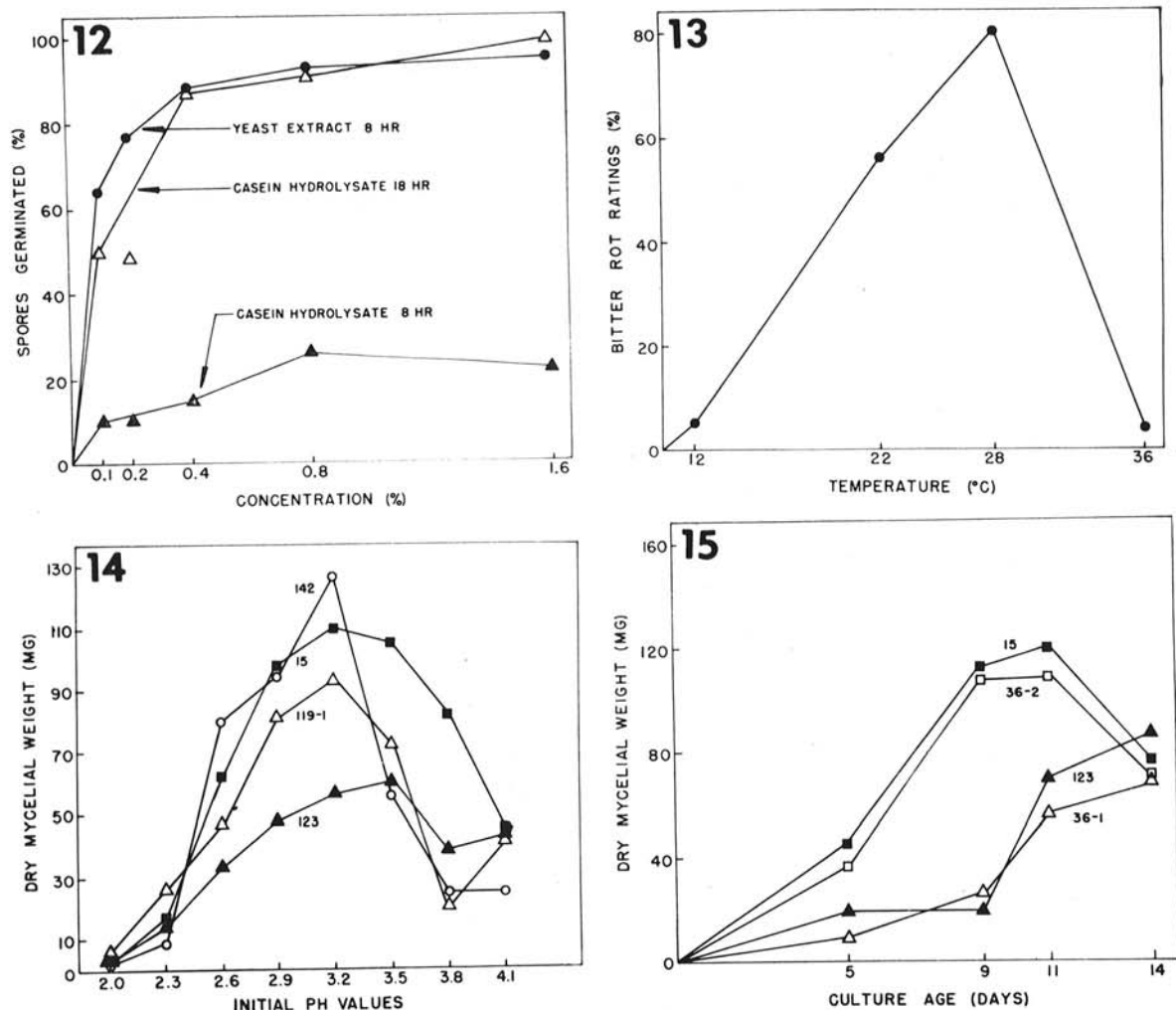
Fig. 12-15. 12) The effect of different concentrations of yeast extract and casein hydrolysate upon germination of spores of *Melanconium fuligineum* after 8 and 18 hr. 13) Bitter rot development on berries of Magnolia grapes 7 days after inoculation with isolate 123 of *M. fuligineum* at 4 temp. 14) The effect of hydrogen ion concn on mycelial growth by four isolates of *M. fuligineum* in a dextrose-potato extract medium after 8 days. All initial pH values were buffered with tartrate-phosphate except for the nonbuffered check at pH 4.9. 15) Mycelial growth by four isolates of *M. fuligineum* in a maltose-ammonium tartrate medium during 14 days.

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