Early Botrytis Rot of Grapes: Time of Infection and Latency of *Botrytis cinerea* Pers. in *Vitis vinifera* L.

W. D. McClellan and William B. Hewitt

Farm Advisor and Plant Pathologist, respectively, University of California Agricultural Extension Service, Tulare County, Visalia 93277; and Department of Plant Pathology, University of California, Davis: 9240 So. Riverbend Ave., Parlier, California 93648.

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ABSTRACT

Early Botrytis rot of grapes is a new development in an old disease caused by *Botrytis cinerea*. A brown rot of grapes starts in midseason and may continue to develop until harvest in the absence of rain. The disease may affect only a few grapes or most all the grapes in a cluster. Infection takes place during bloom. The fungus invades the stigma and style and then becomes latent in the necrotic stigma and style tissue at the stylar end of the grape. At véraison or later the fungus resumes growth and rots the grape. The presence of pollen on the grape stigmatic surface increases the germination and germ tube growth of conidia of the pathogen. Experiments with conidia exposed to grape extracts representing different stages of maturity indicated that in extracts of immature grapes, the conidia germinated and grew very poorly. These factors may be involved in the infection process and the latency phenomenon.

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Additional key words: infection at bloom time, latency.

Botrytis cinerea Pers. is a destructive pathogen of grapes and other small fruits throughout the world (2, 4, 7, 10, 11). In grapes, common Botrytis rot has been associated with infections of mature grapes by *B. cinerea* following late season rains or prolonged periods of high relative humidity. Nelson (9, 10) has reported that 12-24 hr of free moisture at 12 C are required for infection to take place on the surface of mature Tokay grapes. In strawberries and raspberries, Powelson (12) and Jarvis (4, 5) have demonstrated that *B. cinerea* will infect senescent floral parts early in the growing season, and remain latent until later in the growing season or even after harvest. The pathogen then renews growth and rots the fruits. A similar phenomenon has been reported by Strobel & Hewitt (13) for summer bunch rot of grapes caused by *Diplodia natalensis*. In contrast to the situation in strawberries where infection takes place in floral parts at the proximal end of the berry, *D. natalensis* infects and remains latent in the stigmatic end of the grape. Upon maturation of the grape berry, the fungus resumes growth and causes rot.

W. B. Hewitt, and Farm Advisors P. La Vine and J. Kissler, respectively, Stanislaus and San Joaquin counties (*unpublished*), observed in California that rot of grapes by *B. cinerea* appeared in some vineyards early in the growing season in the absence of rain. The time of development of the disease was observed to vary with the variety and location of the vineyard. Usually this form of Botrytis rot in grapes developed near the period of véraison; whereas, the common Botrytis rot is associated with mature grapes subjected to late season rains. We choose to name this form of Botrytis rot in grapes, "early Botrytis rot" (8).

This paper provides evidence in support of the hypothesis that early Botrytis rot is caused by *B. cinerea*, and that infection of the grape flower occurs at bloom-time through the stigma at the stylar end of the flower. The fungus remains latent in the stylar end of the grape until later in the season, at which time it renews growth and rots the grape. Factors involved in the infection and latency processes are also presented.

MATERIALS AND METHODS.—*Isolation, growth, and preparation of B. cinerea for in vitro studies.*—The isolates used were from infected grapes of the cultivars 'Grenache' and 'Grey Riesling'. They were grown from single spores and maintained on a modified V-8 juice medium (8). Conidia were collected from 7- to 14-day-old cultures by vacuum into sterile Pasteur pipettes and suspended in 0.001% Tween 20 (polyoxyethylene sorbitan monolaurate). They were then filtered through glass wool, washed three times with sterile distilled water, and resuspended in water with a final concentration of 2,000-3,000 conidia/ml.

Germination and germ tube elongation studies were handled following the method of Kosuge & Hewitt (6) except that small dilution plates (Falcon Plastics, Culver City, Calif.) with 10 wells, each with a capacity of approximately 250 μl were used. One hundred μl of the conidial suspension and 100 μl of the test suspension were incubated at room temperature. Time studies were recorded by taking photomicrographs with an Ultraphot II (Carl Zeiss Co.).

Field inoculation experiments.—Artificial inoculation experiments were made on the variety Grey Riesling in the University of California, Department of Viticulture vineyards, Davis. Twenty clusters at each of six stages of flower and berry development were sprayed with a spore suspension of *B. cinerea* ($7\text{-}8 \times 10^4$ conidia/ml of a 0.001% Tween 20 solution). Following inoculation the clusters were covered with brown paper bags. These were removed some 6-9 weeks later around midseason when symptoms first developed. The clusters were then evaluated for the presence of early Botrytis rot. A sterile 0.001% Tween 20 solution was applied to clusters at each stage as a control.

Assay of natural infections of floral parts.—Natural infection of floral parts by *B. cinerea* and other fungi was followed by random sampling of flowers and berries. The samples were collected bimonthly throughout the growing season from two vineyards in the Napa Valley, California. The flowers and berries were surface-sterilized in 10% sodium hypochlorite for 2 min and plated on 1% water agar. Tissue platings were incubated at room temperature and then examined for fungal growth after 48 hr.

Collection of floral exudates.—Grape flowers in the prebloom stage were dissected into the following parts: (i) stamen, (ii) stigma and style, (iii) calyptra, and (iv) ovary and pedicel. Figure 1 shows three stages in the development of the grape flower and the floral parts referred to in this paper. At prebloom, the ovary is completely enclosed in a modified calyx or

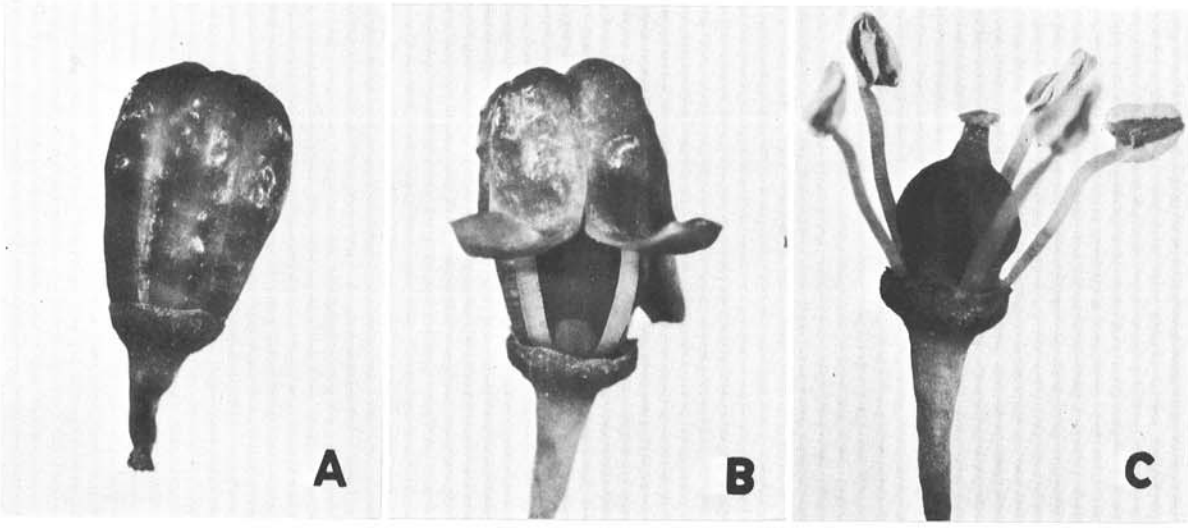

Fig. 1. Stages of grape flower development. **A)** Prebloom stage; **B)** calyptra dehiscing, marking the start of bloom; and **C)** full bloom with the calyptra detached and the stamens, stigma, and ovary fully exposed.

calyptra. The calyptra dehisces at the pedicel to begin bloom, leaving the floral parts exposed. "Shatter" is the stage of flower development which marks the onset of the loss of stamens, ovary enlargement, and drop of some of the ovaries.

Two hundred stamens and 40 of each of the other floral parts were collected. Each floral part was suspended in 2-4 ml of deionized distilled water for 10-12 hr at 5 C. These washings were then collected by filtration and stored in the freezer.

RESULTS.—*Field inoculations.*—Figure 2 shows results of field inoculation studies in two growing seasons, 1970 and 1971, in the grape variety Grey Riesling. Results show that maximum rot developed when inoculations were made during the bloom period. At the time symptoms developed, the treated clusters were evaluated for rot; less than 1% of the clusters in the open vineyard on grapevines not in the experiment had early Botrytis rot. It would appear from the differences between the percentage of rot in the control bagged clusters and in clusters in the open vineyard, not in the experiment, that the presence of the paper bag over clusters favored the development of the disease.

Field inoculations were also made with different conidial concentrations applied at bloom time with an atomizer to clusters of the variety 'Thompson Seedless'. Twenty-four hr after inoculation, random samples of flowers were collected, surface-sterilized, and plated on 1% water agar. After 48 hr of incubation at room temperature, the number of infected flowers was tabulated. When a conidial suspension of approximately three conidia/flower was applied, 7% of the flowers sampled became infected. With concentrations of 30 to 30,000 conidia/flower, 50-63% of the flowers sampled were infected by *B. cinerea*. More than 75% of the infected flowers sampled had fungal growth originating from the stigmatic end. Also when dry spores were dusted on open flowers in clusters, and flowers were randomly collected and cultured as before, 67% of the flowers in these clusters yielded *B. cinerea* in culture.

Location of latent mycelium in grapes.—Evidence for infection by *B. cinerea* through the stigma and style at the stylar end of the grape flower was also obtained by culturing whole young grapes after inoculation and surface sterilization. After bloom, the stylar end of the grape undergoes morphological changes. In the prebloom and early bloom stages, the stigma and style are very turgid and remain in this condition usually for a short period of time after the calyptra has dehisced. The stigma and style progresses from a turgid, filamentous structure to a dried or decayed necrotic tissue which remains attached to the berries of some varieties through maturity and harvest.

Figure 3 shows the presence of conidiophores and conidia of *B. cinerea* on young grapes which have been surface-sterilized and incubated for 3 days on water agar. The fungus growth originates either from the stigmatic portion of the flower or from the adhering necrotic stamens which did not fall during shatter. Stamens which remained adhering to

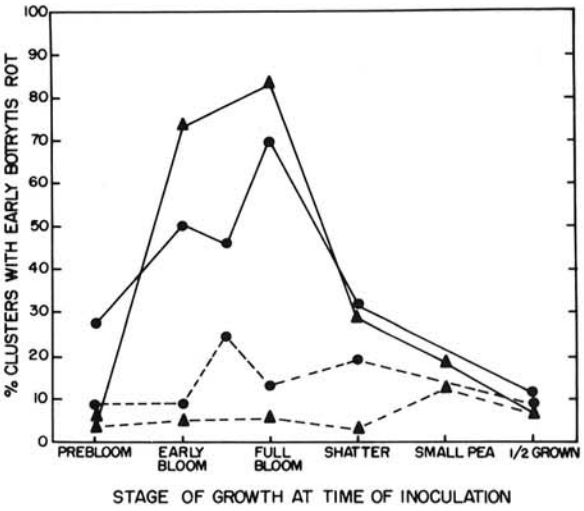

Fig. 2. Percentage of grape clusters that developed early Botrytis rot after inoculation at each of six stages of maturity, experiments during two seasons. ●——● 1970; ▲——▲ 1971. Solid lines–inoculated with *Botrytis cinerea*; broken lines–noninoculated controls.

maturing grapes were only rarely seen in the field.

Sampling of natural vineyard populations for infected grape flowers and fruits.—In two commercial vineyards, of the cultivars 'Pinot St. George' and 'Sauvignon vert', grape flowers and later grapes were sampled at intervals throughout the growing season. All flowers and grapes were symptomless at the time of sampling. Figure 4 shows the percentage of flowers and grapes infected with *B. cinerea*. Table 1 gives the genera of the fungi observed and the location of the fungal growth from the grape. In the variety Pinot St. George, more than 90% of the flowers sampled were infected with *B. cinerea* in the bloom period, and all these had Botrytis growth originating from the stylar end of the berry. Only 50% of the flowers of Sauvignon vert were infected at bloom time; however,

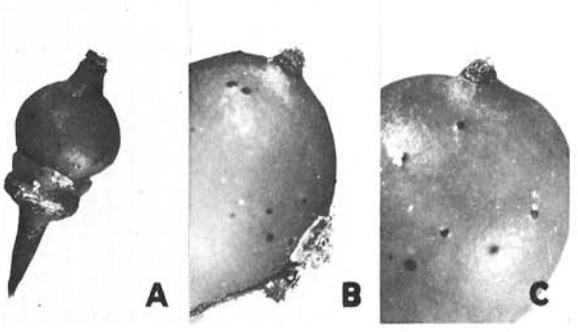

Fig. 3. Growth of *Botrytis cinerea* from naturally infected grape floral parts after incubation for 3 days on 1% water agar. **A)** Growth from stigmatic end of berry 3- to 4-mm diam, **B)** growth originating from both decayed stamens and stigma of berry 6- to 7-mm diam, **C)** growth originating from necrotic stigma of berry 8- to 10-mm diam.

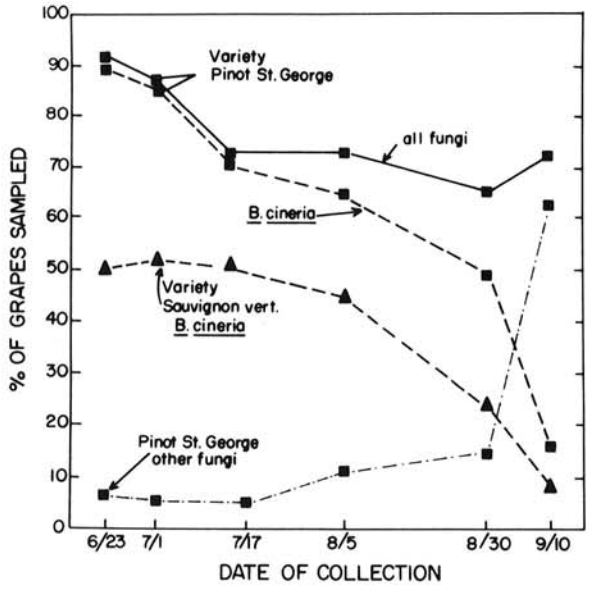

Fig. 4. Percentage of flowers and grapes sampled in two vineyards having fungal growth from their floral parts after surface sterilization and incubation on 1% water agar. ■——■ 'Pinot St. George'; ▲——▲ 'Sauvignon vert'. Solid lines–percentage of all fungi, dashed lines–percentage with *Botrytis cinerea*; dash-and-dot lines–percentage of berries with fungal growth other than *B. cinerea*.

fungal growth from all infected flowers was at the stylar end. Many decayed stamens, most of which remained attached to the outer surface of the berry, were also infected by the fungus. As the grapes matured, infected stamens adhering to the berries were found only infrequently. Less than 1% remained attached to the grape at maturity. The fungus was still viable in the decayed stylar end of the grape at the time symptoms developed in the field. Even though the two grape varieties showed high percentages of infection at bloom, only 2% of the clusters of Pinot St. George and 9% of those of Sauvignon vert developed early Botrytis rot. In the previous year these two vineyards had 60% and 25% clusters with early Botrytis rot, respectively.

Factors influencing the infection and latency of B. cinerea in grapes.–Figure 5 shows germination of conidia and germ tube growth of *B. cinerea* on extracts of the different floral parts. Extracts of the stigma and style, stamen, and calyptra all increased germination of the conidia and germ tube growth over that in the distilled water controls. In the control the conidia germinated only to a limited extent and the germ tube was usually only as long as the conidium itself. When stamen, stigma, and style exudates were added together, the percent that germinated was 40% and the germ tube length was 80 μ after 4 hr of incubation.

Germination and growth of *B. cinerea* conidia in the presence of two media, one natural and the other artificial, representing different stages of fruit maturity indicated that substances found in the grape in the stages between shatter and the period of veraison inhibited germination and growth of *B. cinerea* conidia. The artificial liquid medium (S-1) was made to simulate the sugar and organic acid concentrations that Strobel & Hewitt (13) found in the seven different stages of fruit development in the variety Thompson Seedless from prebloom to full maturity. The percent soluble solids ranged from 3.0% at stage 1 to 20.0% at stage 7 with amounts of glucose and fructose increasing sharply in stages 5 and 6. The pH ranged from 3.8 in stage 1 to 2.3 in stage 4 and then increased to 3.2 in stage 7 (13). In the S-1 media, germination was reduced from a mean of 87% and 80% for stages 1 and 2 to only 39% in stage 4. The germ tubes were distorted and elongation proceeded very slowly during stage 4. At maturity (stage 7) the fungus germinated well (84%) and growth was rapid and comparable to that in the first stages.

TABLE 1. Percentage of total fungi isolated from flowers and grapes collected at random on dates shown from clusters in vineyards of two cultivars, 'Pinot St. George' (A) and 'Sauvignon vert' (B)

Date[b]	Variety	Organism[a]						Location of growth
		Bot	Alt	Pen	Clad	Stem	Other	
6/23	A	97					3	All at stigma; many
	B	96	2				2	decayed stamens
7/1	A	100						All at stigma; many
	B	100						stamens still attached
7/17	A	100						131 stigma; 29 stamen
	B	94				4	2	106 stigma; 6 stamen; 1 stem end
8/5	A	90			4		6	161 stigma; 1 stamen
	B	85	7			1	7	153 stigma; 1 stem end
8/30	A	83		2			15	All stigma
	B	78					22	All stigma
9/10	A	14	5		43	38	38	70 stigma; 2 split berry; 4 stem
	B	10	13	68			9	

[a] Bot = *Botrytis*; Alt = *Alternaria*; Pen = *Penicillium*; Clad = *Cladosporium*; Stem = *Stemphyllium*.
[b] Each collection date represents approximately 200 flowers or grapes sampled from each vineyard.

Figure 6 shows the germination and growth of *B. cinerea* conidia after 6 and 9 hr in extracts of Grey Riesling grapes taken at different stages of development. Flowers and berries were collected from the variety Grey Riesling at different stages of maturity and juice was extracted from them with a hand press. The juice was clarified by centrifugation, sterilized by filtration, and stored in the freezer. Total soluble solids in the extracts were measured with a Bausch & Lomb hand refractometer. Table 2 characterizes the different stages of development and gives the percent germinated and germ tube growth after 6 and 9 hr of incubation at room temperature. For germination after 6 hr there was a decrease from 84% in the bloom extract to 7% in the extract from immature berries. Germination and growth increased again as the berries increased in size and again as the berries became more nearly mature. As can be seen in Fig. 6, the germ tubes of the conidia grown in extracts of immature berries were enlarged and distorted in comparison to growth in extracts from the other stages.

DISCUSSION.—The experimental findings presented here support the hypothesis that *B. cinerea* infects the grape flower at bloom time, and that the path of infection is through the stigma and style and then into the stylar end. It then becomes latent and remains in the necrotic stigma and style tissue until later in the season, at which time it renews growth and rots the berry. The mode of infection and time of development of rot in the vineyards distinguishes early Botrytis rot from common Botrytis rot of grapes associated with mature grapes and late season rains (9, 10).

Infection and latency of *B. cinerea* in the grape is different from that reported in other fruits (3, 12). In strawberry and raspberry (5) the pathogen infects senescent floral parts and remains in a quiescent state until later in the growing season. The infections in these fruits occur at the receptacle end by way of the decayed stamens and calyces. When conditions are favorable, the fungus renews growth and invades the receptacle and the fruit causing Botrytis rot. The stamens in strawberries remain attached to the receptacle throughout the growing season.

Our unpublished observations with strawberry show that there is no abscission zone formed at the base of the stamens and that the stamens become necrotic shortly after the pollen had been released from the anthers. By contrast, in the grape flower the stamens dehisce during the shatter stage just after bloom. As a result, the necrotic stamens, even though they may have been infected by *B. cinerea* (Fig. 2, Table 1), were not major avenues of entry for *B. cinerea* into grapes later in the growing season.

Studies of artificial inoculation and natural infection in vineyards presented here show that *B. cinerea* entered the grape flower through the stigmatic end and that maximum infection took place during bloom. Chu Chou & Preece (1) reported the enhanced germination of conidia of *B. cinerea* in the presence of strawberry pollen. They demonstrated the increased aggressiveness of the fungus in the presence of aqueous pollen diffusates in producing lesions on strawberry petals. In this paper it is demonstrated that aqueous extracts of the pollen and stigma and style stimulated the germination and germ tube growth of *B. cinerea* conidia (Fig. 5). In the grape flower this stimulatory effect may enhance the ability of the pathogen to establish itself in the stylar end of the young grape.

Once established, the pathogen remains latent either in the necrotic portion of the style end or, having bridged the layer of cells between the style and the ovary, in the tissue abaxial to the abscission zone.

Although the ability of *B. cinerea* to colonize senescent plant tissue is well documented in the literature, the fungus did not appear to colonize the decayed stigmatic portion of the grape during stages

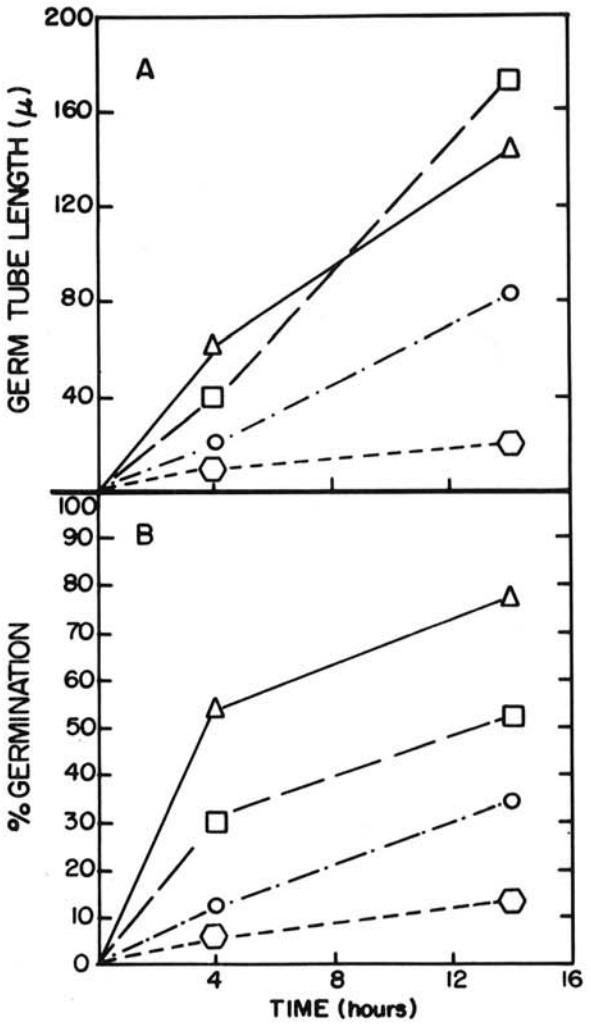

Fig. 5. The effect of floral exudates on germination and germ tube growth of conidia of *Botrytis cinerea*. **A)** Germ tube elongation; **B)** germination. Symbols: △——△ stamens; □——□ calyptras; ○——○ stigma and style; ⬡——⬡ control (water).

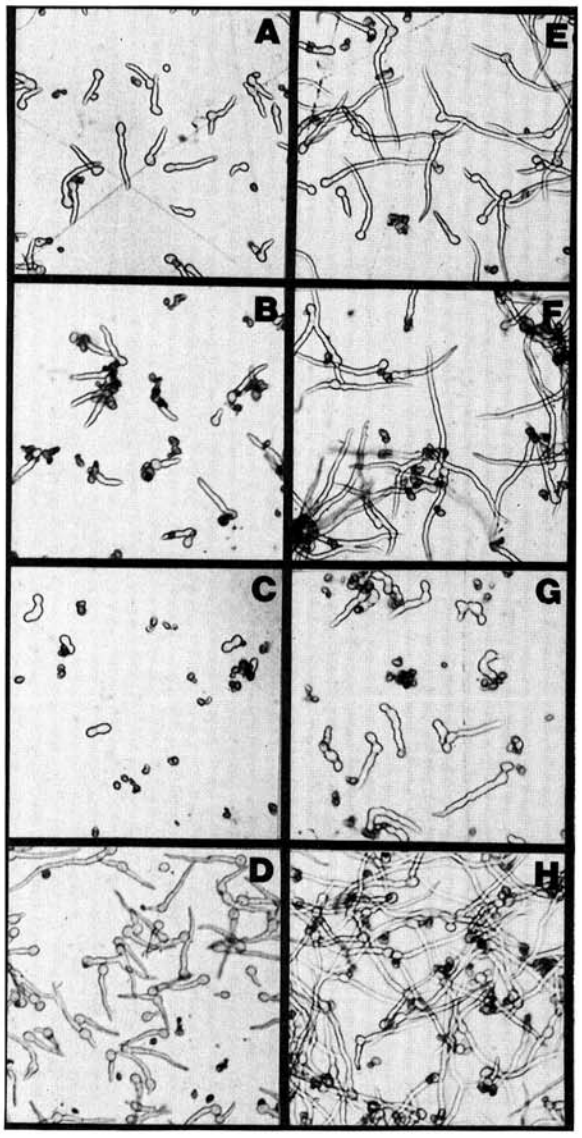

Fig. 6. Growth of germ tubes of *Botrytis cinerea* conidia in extracts of 'Grey Riesling' grapeberries at different stages of maturity. **A-D)** 6 hours of incubation; **E-H)** 9 hours of incubation; (See Table 3)—A and E = Stage A; B and F = Stage B; C and G = Stage C; D and H = Stage D.

of growth past the bloom period. The lack of moisture in this tissue and the inhibiting effect of berry extracts (Fig. 6), may partially explain the fact that the fungus does not establish itself in the necrotic stigma and style tissue after bloom.

Ogawa (11) found that intact necrotic floral tissue was essential for infection of green apricot fruit by *B. cinerea*. He stated that styles which failed to dehisce were avenues of infection of apricots. The absence of necrotic floral parts retarded the aggressiveness of *B. cinerea* in infecting apricot fruit. This observation has also been reported for *B. cinerea* in strawberries (1).

TABLE 2. Germination and germ tube elongation of *Botrytis cinerea* conidia in extracts of 'Grey Riesling' grape blossoms and berries of four stages of maturity

Stage	°B[a]	pH	Percent germination		Germ tube elongation (μm)	
			6 hr	9 hr	6 hr	9 hr
(A) bloom		3.6	84	88	55	180
(B) shatter	4.0	3.4	54	48	45	160
(C) berry 10 mm diam	5.7	2.7	7	25	20	45
(D) berry 12.4 mm diam	16.0	3.1	88	79	100	180
Control (water)		5.5	1	4	20	20-40

[a] °B = °Brix, a measure of total soluble solids in extracts.

In strawberry, which has persistent stamens, Powelson (12) demonstrated that the mycelium in the receptacle end are the result of growth through the necrotic stamens. In the grape flower, the stylar portion is persistent and there is a layer of closely aligned cells at the base of the style, possibly an abscission layer or nonfunctional zone. The fungus apparently has to bridge this layer to infect the grape. Observations of flowers and young grapes in culture in the laboratory indicated that only 5 to 10% of the grapes that show fungal growth from the stylar end after 48 hr will develop a rot of the entire berry after 5-7 days' incubation in a saturated atmosphere (Fig. 4). Examination of these berries provided no evidence that the fungus entered the berry in any way except through the stylar end.

This low percentage of rot of grapes with high percentage of infected styles observed in the laboratory perhaps helps to explain why the two vineyards sampled has such low percentages of early Botrytis rot in the field even though they had a high percentage of the stigma and styles of berries infected by the pathogen. How the pathogen penetrates the barrier between the style and the ovary of only a few grapes and what physiological and environmental conditions are required for penetration of the barrier are not known. The importance of the "abscission zone" layer of cells separating the style from the ovary and the function of the inhibitory effect demonstrated to be present in the extracts of immature grapes are not known. It is also not known just what permits the fungus to overcome the latent state, resume growth, and rot the grape.

The studies do show that the fungus, *B. cinerea*, invades the stylar tissue of the grape at bloom time and at some stage bridges an "abscission zone" layer of the style to the ovary (grape). Later, at véraison, the fungus proceeds to rot the grape. The studies demonstrate that pollen and stigma extracts enhance germination of conidia and stimulate growth of germ tubes. No doubt these factors are important in invasion of the stylar end of the grape and bridging the stylar "abscission zone". The studies further show that the tissue extracts of grape parts early in development have an inhibitory effect on germ tube growth, a phenomenon probably involved in latency of the fungus in the grape.

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