Ripe Rot of Muscadine Grape Caused by *Colletotrichum gloeosporioides* And Its Control

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ABSTRACT

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Colletotrichum gloeosporioides, the cause of ripe rot of muscadine grape (*Vitis rotundifolia*), was isolated from mummies, pedicels, and fruit spurs of grape during the winter months of 1979, 1980, and 1982. Infected mummies and pedicels produced abundant conidia during rainy periods in the spring and served as sources of primary inoculum. Conidia of *C. gloeosporioides* were trapped regularly in rainwater runoff from grapevines from March through October 1981 and 1982. Most conidia were caught in the early spring of 1981, when many overwintered mummies remained on the vines, and in the fall of both years, when ripe, rotting fruit were present. Inoculations of immature fruit on the vine, as well as isolations from naturally inoculated green fruit, indicated that infections by *C. gloeosporioides* occur at all stages of fruit development. These infections are probably latent, because symptoms do not appear until ripening. A fungicide bioassay showed that captafol, followed by captan, maneb, and benomyl, had the most activity against *C. gloeosporioides*. Etaconazole and triforine were neither fungistatic nor fungicidal at 20 and 500 μg/ml, respectively. In field tests, captafol, folpet, captan, and maneb suppressed the level of ripe rot when applied every 2 wk from bloom until near harvest.

Ripe rot, caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc., was first reported in the United States on grapes in 1891 (8). Only recently has the disease become a major problem on muscadine grape (*Vitis rotundifolia* Michx.) in North Carolina, with yield losses estimated at 10% (R. D. Milholland, *unpublished*) (4). The primary symptom of this disease is rotting of ripe fruit in the vineyard at harvest. Affected grapes initially develop circular brown spots of decay on their skins that subsequently enlarge to include the entire berry. Rotting fruit are characteristically covered with salmon-colored masses of conidia (Fig. 1A). Symptoms have not been observed on vegetative portions of grapes in North Carolina, although *C. gloeosporioides* is reported to cause leaf spots and cankers on grapes in the Philippines (7).

Control of ripe rot is hampered by the limited epidemiological information available on the disease as well as by the late and rapid development of fruit rot symptoms. The purpose of this research was to determine the mode of overwintering and periods of spore release for *C. gloeosporioides* on muscadine grape, to determine the times of fruit infection, and to develop effective control measures.

MATERIALS AND METHODS

Field experiments were conducted at the Horticultural Crops Research Station, Castle Hayne, NC, and at the Sandhills Research Station, Jackson Springs, NC. Grapevines at Castle Hayne were trained on a Geneva double-curtain trellis and were 9 yr old in 1980. Grapevines at Jackson Springs were grown on a one-wire trellis and were 7–8 yr old in 1980.

Overwintering studies. One hundred healthy-appearing fruit spurs (fruiting wood) were collected at random from grapevines of the cultivar Carlos at Castle Hayne in March 1982. The fruit spurs were surface-sterilized for 3 min in 0.525% sodium hypochlorite, rinsed, and plated on potato-dextrose agar (PDA) acidified with 50% lactic acid. During February 1979 and 1980, grape pedicels and attached mummies were collected from the cultivars Carlos and Magnolia. Mummies also were collected at random from the ground beneath the vines. Each year, 400 mummies from the vines, 400 mummies from the ground, and 200 pedicels with mummies removed were placed on moistened paper towels in plastic boxes and incubated at room temperature. The material was examined 5–6 days later with a dissecting microscope for production of conidial masses of *C. gloeosporioides*.

Periods of spore release. Spore traps were constructed at Castle Hayne to study the release of inoculum of *C. gloeosporioides* from mummies and pedicels, which were suspected to be major sources. Additional spore traps also were placed beneath grapevines to monitor the seasonal production of *C. gloeosporioides* conidia. Spore traps consisted of 1-L plastic bottles supporting 10-cm-diameter saran cloth-lined funnels that collected rainwater runoff. Ten milliliters of a 5% $CuSO_4$ solution was added to each bottle to prevent spore germination. The rainwater was collected weekly and *C. gloeosporioides* conidia were counted with a hemacytometer.

Four spore traps, each with funnels containing 50 mummies from Carlos, were set up on 19 February 1980. Three additional spore traps, each with funnels containing 25 detached pedicels from Carlos, were set up on 1 April 1980. The mummies and pedicels placed in the spore traps were collected during the winter of 1980 and had been stored at 2 C. The original mummies and pedicels remained in the funnels throughout the season. The spore-trap bottles were partially buried in the ground adjacent to the vineyard. Additional spore traps with empty funnels were set up nearby as controls to detect any conidia that may have come from other sources.

On 17 March 1981, four spore traps with empty funnels were mounted several centimeters beneath the arms of Carlos grapevines to monitor spore production in live plantings. Spore trapping was repeated at the same locations the following year beginning on 9 March 1982.

Data on numbers of conidia trapped from the mummies, pedicels, and grapevines were recorded through October or until the material ceased producing detectable levels of spores.

Periods of infection. Periods of fruit infection were determined by isolating *C. gloeosporioides* from naturally infected fruit and by inoculating fruit at different stages of development. Five hundred Carlos fruit were collected randomly from unsprayed vines at

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Castle Hayne at 2-wk intervals from 16 June until 8 September 1981. Fruit were surface-sterilized in 0.525% sodium hypochlorite for 2.5 min, rinsed in sterile distilled water, incubated in sterile moist chambers at 25 C, and observed periodically for sporulation of *C. gloeosporioides*. The procedure was repeated with 250 fruits per sampling date in 1982 from 15 June to 7 September, but after surface-sterilization, the grapes were halved and plated on water agar plus 100 μg/ml vancomycin hydrochloride. Infected grape halves showed abundant sporulation by *C. gloeosporioides* after 3–4 wk.

In 1980, Carlos grapes at Castle Hayne were sprayed with a suspension of 10^6 conidia per milliliter of *C. gloeosporioides* isolate GC-7, from a Carlos mummy, at the small and large green fruit stages, when the fruit were beginning to turn bronze, and when the fruit were bronze and nearly ripe. The fungus was grown on PDA for 6 days at 25 C, then plates were flooded with sterile distilled water, scraped, and the conidial suspension filtered through two layers of cheesecloth. A spray of distilled water served as a control. The inoculated clusters were then wrapped in two layers of wet cheesecloth and sealed in plastic bags. The cheesecloth and plastic bags were removed and replaced with paper bags 3 days after inoculation. The experiment was designed as a split plot, with each plot consisting of two arms. Four clusters in each plot were inoculated with *C. gloeosporioides* at one of the four inoculation times and four were sprayed with sterile water. Plots for different times of inoculation were replicated four times in a Latin square design. Inoculated grape clusters were harvested on 1 October, when 85% of the grapes were ripe. Healthy grapes and those sporulating with *C. gloeosporioides* were then sorted and counted.

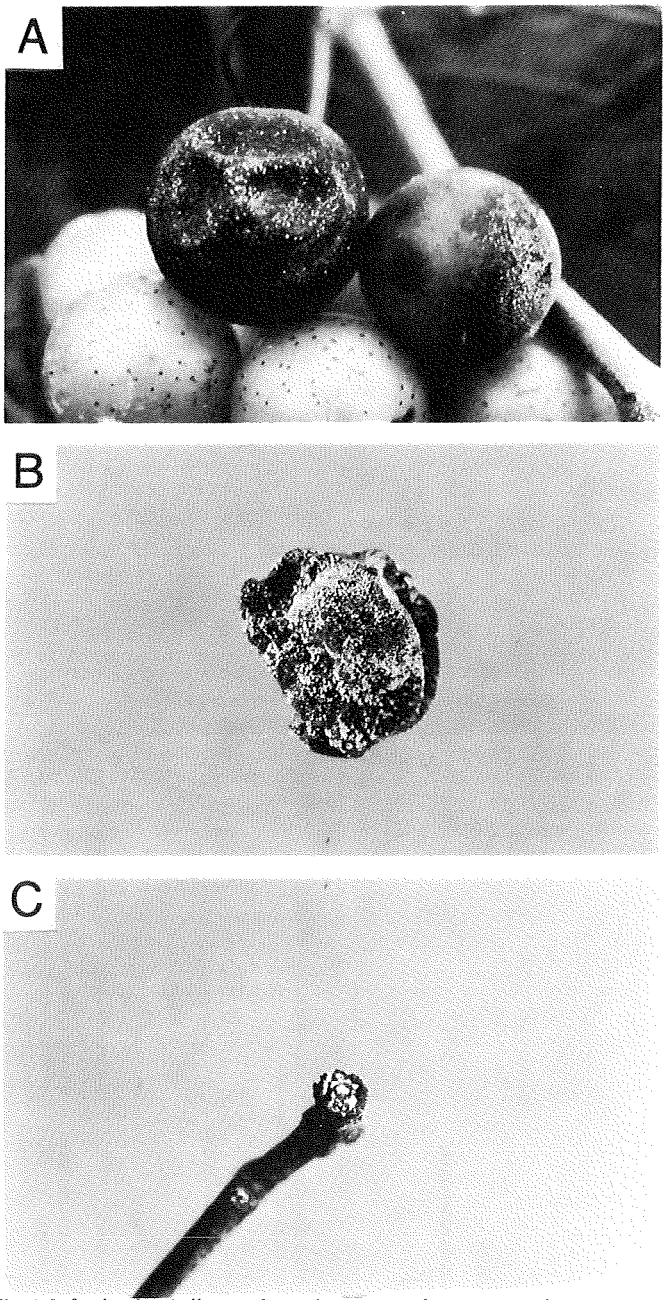

Fig. 1. Infection by *Colletotrichum gloeosporioides* on muscadine grape. **A,** Ripe rot symptoms on mature fruit of the cultivar Carlos. Decayed areas are covered by masses of *C. gloeosporioides* conidia. **B,** Overwintered mummy from Carlos grapevine with masses of light-colored *C. gloeosporioides* conidia exuding from its surface. **C,** Overwintered pedicel from Carlos grapevine with masses of *C. gloeosporioides* conidia produced at the site of fruit attachment. Sporulation was observed on the mummy and pedicel after 5–6 days of incubation in a moist atmosphere at room temperature.

The experiment was repeated at Castle Hayne in 1981, with twice as many clusters inoculated in each plot. Grapes were harvested on 1 October, when 97% of the fruit were ripe.

A similar experiment also was conducted on Carlos grapes at Jackson Springs in 1980. Grapes were inoculated at the same four stages of fruit development as described previously. In this experiment, each plot consisted of two vines, with each plot containing four clusters inoculated with *C. gloeosporioides* and four uninoculated clusters. Treatments for the four stages of development were replicated four times in a randomized complete-block design. Grapes were harvested on 26 September, when 98% of the fruit were ripe.

Control studies. A procedure developed by Neely and Himelick (6) was used to evaluate fungicidal and fungistatic properties of six fungicides in laboratory tests. The fungicides chosen for testing were benomyl (Benlate 50W), captan (Captan 50W), etaconazole (Vangard 10W), captafol (Difolatan 4F), maneb (Dithane M-22 special 80W), and triforine (Funginex EC, 18.6 g/L). Penicillin assay disks no. 740-E (Schleicher and Schuell, Inc., Keene, NH 03431) were saturated with various concentrations of the chemicals. Cellophane disks 6.5 mm in diameter were placed on top of the filter-paper disk and seeded with spore suspensions (2×10^5 conidia per milliliter) of *C. gloeosporioides* isolate GC-7. The suspensions had been prepared from 6- to 8-day-old cultures grown on PDA. Fungicide concentrations of 500, 100, 20, 5, 1, and 0 μg a.i./ml were used in the fungicidal portion of the test, and concentrations of 20, 5, 1, 0.2, 0.04, and 0 μg a.i./ml were used in the fungistatic portion. A concentration was considered fungicidal if *C. gloeosporioides* failed to grow when transferred to PDA after being in contact with the chemical for 3 hr. Concentrations were considered fungistatic if 99% or more of the spores failed to germinate during a 24-hr exposure to the chemical.

Field trials also were conducted at Castle Hayne to evaluate the efficacy of several fungicides for control of ripe rot. Preliminary results from the spore-trapping studies in 1980 revealed that large numbers of spores were being released weekly from infected mummies in March and April. Therefore, fungicides were applied immediately after bloom, when small green fruit developed; applications were repeated at 2-wk intervals until 1 mo before harvest.

In 1980, grapes were sprayed with treatments consisting of captafol, captafol at two rates plus captan, captan, and maneb. The sprays were applied with a low-volume air-blast sprayer at 187 L/ha. Treatments were replicated three times in two plant plots and arranged in a randomized complete-block design. All grapes also were sprayed with benomyl at 0.6 kg a.i./ha once every 2 wk throughout the same period to control powdery mildew.

The timing and number of applications in the 1981 tests were similar to those in 1980 and were based on the results of the 1980 studies of spore release and periods of infection. Grapes were treated with three rates of captafol, folpet (Phaltan 50W), and maneb. The experimental design and methods of fungicide application were the same as in the 1980 test. Sulfur at 4.48 kg/ha was used to control powdery mildew. Ripe grapes were harvested over a 3-wk period and data on the number of fruit with ripe rot were recorded.

RESULTS

Overwintering studies. *C. gloeosporioides* was isolated from 21% of the Carlos fruit spurs. The fungus sporulated on 88 and 73% of mummies collected from vines in 1979 and 1980, respectively, whereas it sporulated on 33 and 36% of the mummies collected from the ground during the same years (Fig. 1B). Conidia of *C. gloeosporioides* were produced on 46 and 45% of the pedicels in 1979 and 1980, respectively (Fig. 1C). Sporulation on pedicels usually occurred at the point of berry attachment, often in a ring over the open ends of vascular bundles.

Periods of spore release. Detached Carlos mummies and pedicels released the greatest numbers of *C. gloeosporioides* conidia in the spring, during the first weeks of collection (Fig. 2). Conidia continued to be produced from the mummies throughout the remainder of the season but at gradually decreasing levels. Numbers of conidia produced from pedicels also decreased during the summer months and conidia were not detected after 5 August. Conidia of *C. gloeosporioides* were never found in the control spore traps except during the weeks ending 30 September and 7 and 28 October, when an average of 9.23×10^5 conidia per trap per week were found.

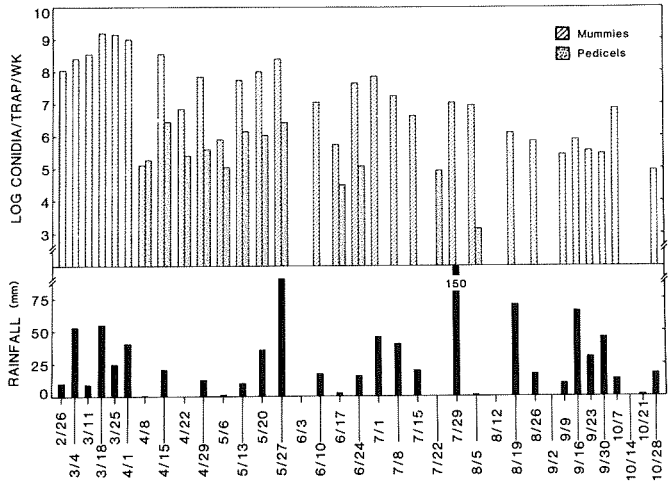

Fig. 2. Weekly totals of rainfall and levels of *Colletotrichum gloeosporioides* conidia released from detached mummies and pedicels of Carlos grapes at the Horticultural Crops Research Station, Castle Hayne, NC, in 1980. Each date marks the end of one week's collection. Data on the number of conidia produced from mummies are an average obtained from four spore traps holding 50 mummies each. Data on the number of conidia produced from pedicels are an average obtained from three spore traps holding 25 pedicels each and were collected only during the period 8 April through 26 August.

Conidia of *C. gloeosporioides* were trapped in rainwater runoff from grapevines during most weeks when rainfall occurred. In 1981, two peak periods of spore production were observed (Fig. 3A). The first peak occurred from 17 March to 12 May, when many infected mummies were still attached to the vines. The second peak occurred from 5 August to 29 October, when infected ripe fruit were present. The greatest number of conidia were trapped during the week ending 27 October. In 1982, there were fewer mummies present in the vicinity of the spore traps. Consequently, only one peak period of spore production occurred, during 1 September to 12 October, as the fruit ripened (Fig. 3B). Greatest spore production was during the week ending 7 September.

Periods of infection. Isolations from naturally inoculated grapes in 1981 revealed that infections by *C. gloeosporioides* occurred as early as 16 June, the first date collections were made (Fig. 4). The infection level was low from 16 June through 11 August, then increased rapidly from 25 August to 8 September. In 1982, a low level of infection (1.27%) also was detected on the first date of collection (15 June). Levels of infection then remained below 3% from 29 June to 24 August and rose to a final level of 24% on 7 September.

An analysis of variance showed that grapes inoculated with *C. gloeosporioides* had significantly more ripe rot ($P = 0.05$) than grapes in control treatments at harvest (Table 1). Rot observed on control clusters probably resulted from natural inoculations in the field.

Control studies. In the laboratory assay, captafol, captan, and maneb were fungistatic at 0.04, 0.2, and 1 μg a.i./ml, respectively, and fungicidal at 1, 4, and 100 μg a.i./ml, respectively. Benomyl was also fungicidal at 100 μg a.i/ml. Benomyl, etaconazole, and triforine were not fungistatic at 20 μg a.i./ml, the highest concentration tested, and etaconazole and triforine were not

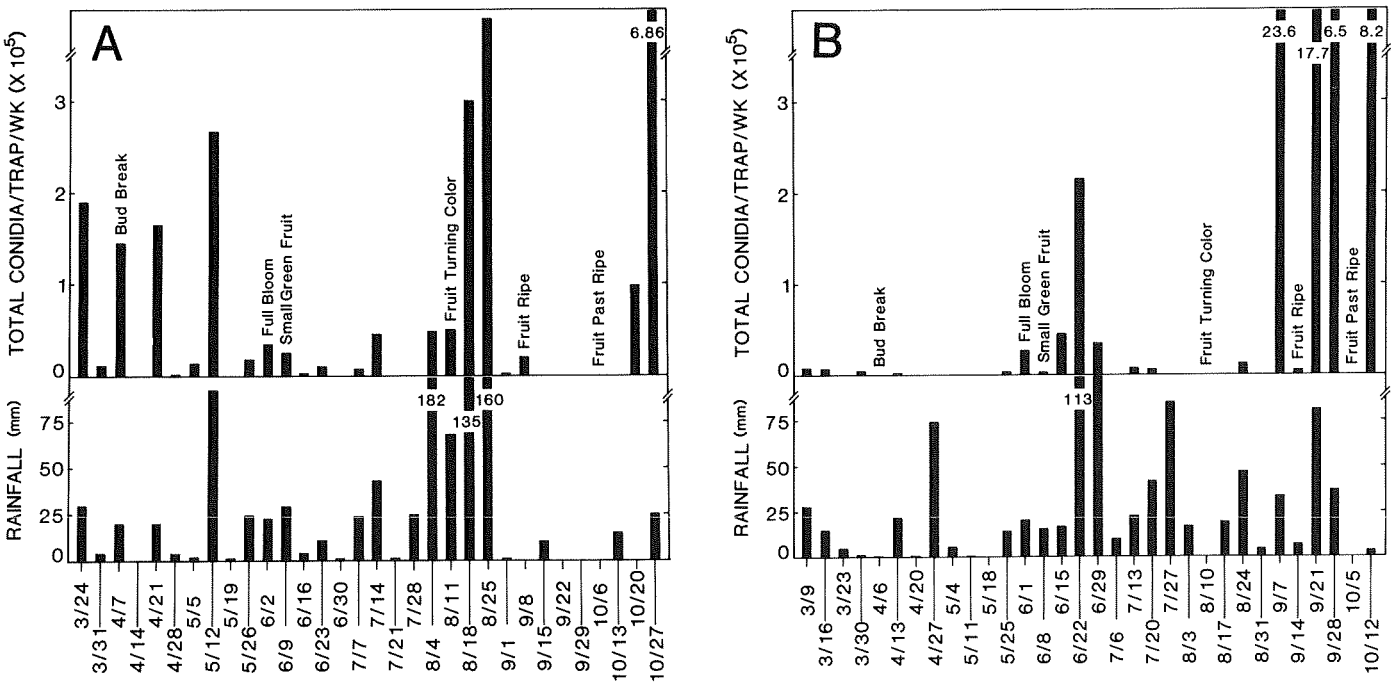

Fig. 3. Weekly totals of rainfall and levels of *Colletotrichum gloeosporioides* conidia trapped in a Carlos vineyard at the Horticultural Crops Research Station, Castle Hayne, NC, in **A,** 1981 and **B,** 1982. Each date marks the end of one week's collection. Data on the number of conidia are an average obtained from four spore traps.

fungicidal at 500 μg a.i/ml.

All fungicides used in the test at Castle Hayne in 1980 suppressed the incidence of ripe rot compared with the unsprayed control plots, which averaged 29.5% over the three harvest dates (Table 2). Although the differences among fungicide treatments were not significant, the average levels of ripe rot in the captan and maneb treatments (11.7 and 13.1%, respectively) tended to be greater than the levels found in treatments where captafol was used (6.7–9.3%). The additional sprays of captan given in some captafol treatments did not reduce the level of ripe rot compared with captafol by itself.

In the 1981 experiment, the effect of fungicides on ripe rot development was most evident on the first harvest date (Table 2). At this time, the two higher rates of captafol and folpet suppressed ripe rot the most. Levels of ripe rot increased in all treatments during the following 2 wk of harvest. Grapes in the unsprayed control treatment averaged 16.2% ripe rot over the harvest period, which was not significantly different from grapes in the maneb-treated plots, which averaged 15.5% ripe rot. The average level of ripe rot in treatments containing either the two higher levels of captafol or folpet was less than 6%.

DISCUSSION

Conidia of *C. gloeosporioides* were produced in abundance from mummies and pedicels of grapes during rainy periods throughout the growing season. Conidia are probably spread to other parts of the vines by splashing, blowing rain. As the season progresses, this primary inoculum source is gradually reduced as the mummies detach and decompose. *C. gloeosporioides* also survives in the fruit spurs, which may be another source of inoculum. The presence of ripe fruit sporulating with *C. gloeosporioides* near harvest greatly increases the number of conidia trapped during rainfall in the vineyard and provides secondary inoculum. Splashing rain and visits by numerous insects observed on the rotting fruit probably serve to spread this inoculum.

Results of field inoculation studies, as well as isolations from naturally inoculated fruit, indicate that fruit are equally susceptible at any time during the growing season. Macroscopic symptoms, however, do not appear until ripening. A histological study of the infection process by *C. gloeosporioides* in muscadine grape indicates that conidial germination, appressoria formation, and penetration of developing berries occurs within 48–72 hr; however, further growth of the pathogen ceases until fruit mature (M. E. Daykin, *unpublished*). Establishment of such latent infections is characteristic of diseases caused by *C. gloeosporioides* on avocado (2), mango (5), orange (1), and tangerine (3). The relative activity of fungicides in the laboratory test appeared to correlate with their activity in the field tests. Treatments with captafol, the fungicide most toxic to *C. gloeosporioides* in the laboratory, consistently had the lowest amounts of ripe rot, whereas maneb, which was somewhat less toxic in the laboratory, did not always control ripe rot. Folpet and captan, which are structurally related to captafol, also suppressed ripe rot development. Currently, maneb is the only fungicide recommended for use against ripe rot on muscadine grapes in North Carolina. These experiments indicate that folpet and captafol may be superior to maneb in controlling ripe rot, although further testing is required.

Our results show that infections by *C. gloeosporioides* are

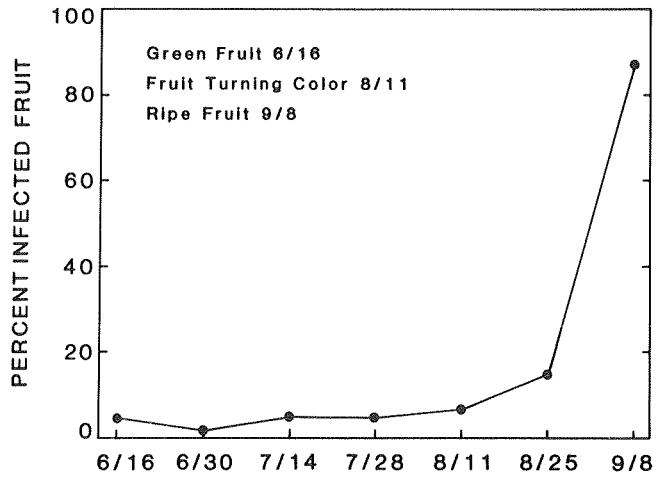

Fig. 4. Percentage of Carlos grapes naturally infected with *Colletotrichum gloeosporioides* in a vineyard at the Horticultural Crops Research Station, Castle Hayne, NC, in 1981. Sporulation by *C. gloeosporioides* on surfaces of surface-sterilized detached fruit was used to indicate infection. Percentages are based on collections of 500 fruits.

TABLE 1. Effect of time of inoculation of Carlos grapes on infection by *Colletotrichum gloeosporioides* in 1980 and 1981

Location	Growth stage	Date of inoculation	Percent grapes sporulating with *C. gloeosporioides*[a]	
			Inoculated[b]	Uninoculated[c]
Castle Hayne	Small green fruit	17 Jun. 1980	53.4	1.1
		16 Jun. 1981	9.5	3.6
	Large green fruit	15 Jul. 1980	25.3	5.6
		14 Jul. 1981	25.6	1.8
	Fruit turning bronze	12 Aug. 1980	34.0	9.1
		11 Aug. 1981	76.3	13.3
	Fruit bronze	3 Sept. 1980	29.9	0.0
		1 Sept. 1981	58.7	10.9
Jackson Springs	Small green fruit	19 Jun. 1980	61.1	6.5
	Large green fruit	24 Jul. 1980	87.4	25.3
	Fruit turning bronze	14 Aug. 1980	81.5	25.6
	Fruit bronze	5 Sept. 1980	78.6	35.2

[a] Ripe fruit were harvested on 1 October 1980 and 1981 at Castle Hayne and on 26 September 1980 at Jackson Springs. Fruit were evaluated 1–2 days after harvest. Percentages for the 1980 test are based on the means of four replicates with four clusters per replicate. Percentages for the 1981 test are based on the means of four replicates with eight clusters per replicate.
[b] Grape clusters were inoculated with a suspension of 1×10^6 *C. gloeosporioides* conidia per milliliter of water.
[c] Grape clusters were sprayed with sterile distilled water.

TABLE 2. Control of ripe rot at the Horticultural Crops Research Station, Castle Hayne, NC, in 1980 and 1981

Year	Treatment[x]	Rate (kg a.i./ha)	Grapes with ripe rot (%)[y]			
			17 Sept.	24 Sept.	1 Oct.	Avg.
1980	Captafol	2.24	1.1 b[z]	2.7 b	22.1 b	8.5 b
	Captafol + captan	2.24 + 2.24	2.9 b	4.0 b	22.0 b	9.3 b
	Captafol + captan	4.48 + 2.24	0.6 b	2.7 b	19.5 b	6.7 b
	Captan	2.24	4.0 ab	5.5 ab	25.7 b	11.7 b
	Maneb	3.59	2.1 b	9.4 ab	28.8 b	13.1 b
	No spray	···	12.7 a	22.4 a	53.8 a	29.5 a
1981	Captafol	4.48	1.7 d[z]	1.9 b	7.2 b	3.6 b
	Captafol	2.24	1.9 d	2.2 b	3.9 b	2.7 b
	Captafol	1.12	4.9 bc	6.3 ab	12.1 b	7.8 b
	Folpet	2.24	2.9 cd	4.7 ab	9.3 b	5.6 b
	Maneb	3.59	5.9 b	13.5 a	27.2 a	15.5 a
	No spray	···	11.9 a	13.1 a	23.7 a	16.2 a

[x] Grapes in 1980 were sprayed once every 2 wk from 9 June through 18 August. Additional sprays of captan were given to captafol and captan treatments on 27 August and 3 September 1980. Grapes in 1981 were sprayed once every 2 wk from 9 June through 18 August.
[y] Data are the means of three replicates with 500 grapes per replicate.
[z] Means for each year in a column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

initiated any time from bloom until harvest, which coincides with periods of spore release. Therefore, it is important that an effective fungicide be first applied early in the season (at bloom), followed by repeated applications to protect the berries at all stages of development. Use of a highly persistent fungicide, or one with eradicative activity, may help reduce the number of applications needed.

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