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Brown Spot of Grapes Caused by *Cladosporium cladosporioides* and *Cladosporium herbarum*

Seiji OUCHI, Motomu HATAMOTO*, Hachiro OKU, Tomonori SHIRAISHI, Tatsuo YOKOYAMA**, Michihiro TATEISHI***, and Shintaro FUJII*

(*Laboratory of Plant Pathology*)

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Introduction

Mascut of Alexandria is one of the representative cultivars of grapevine cultivated in greenhouses in the southern part of Okayama prefecture. For the past three or four years, farmers producing grapes of this cultivar experienced dejection due to a disease that had been called 'black navel'. The disease, in most cases, does not cause any significant defect in the essential quality of the grape berries, but spoils the external appearance of grapes to decrease their commercial value. Ikata[2] reported an epidemic outbreak of this disease in 1940 and attributed it to the growth of *Cladosporium* sp. on the epidermis injured by thrips.

In view of its economical importantce and significant outbreak in the recent years, we decided to identify the pathogen, to clarify the cause-effect relationship between the pathogen and thrips, and to devise an efficient control measure of the disease. This paper concerns primarily with identification of the fungus isolated from the diseased berries and characterization of the isolates as pathogen.

Materials and Methods

Grapes : Diseased grapes of different ages were collected from several greenhouses in Tsudaka area of Okayama. Typical symptoms were recorded and diseased berries were subjected to anatomical observation of infection profile and to the isolation of pathogen.

Isolation of pathogen : Fungi in the diseased tissues were isolated on potato sucrose agar by a routine procedure.

Inoculation test : Apparently fresh and healthy berries were, with or without needle-prick, inoculated at the stigma trace with conidia of fungi which had been most frequently isolated. Inoculated berries were placed in a desicator of 100 % relative humidity and incubated at 28 C for certain period of time. Number of berries with apparent symptom was scored with an appropriate interval. Berries at different stages of development were also tested for their susceptibility against the disease. Sugar content of these berries was determined by a Brix refractometer.

Anatomical observation : Thin section of the diseased tissues were prepared by slicing with a razor blade, stained with 2 % lactophenol cotton blue and destained by rinsing with tap water. Sections with or without staining were observed under a microscope.

* Okayama Agricultural Experiment Station, Sanyo-cho, Okayama.
** Institute for Fermentation, Juso-nishinomachi, Higashi Yodogawa, Osaka.
*** Extension Service, Shimane Agricultural Experiment Station, Izumo, Shimane.

Results

Symptoms

The most characteristic symptom of this disease appears at the stigma trace of berries. The primary symptom becomes apparent when berries developed to the size of 8—10 mm in diameter, and is characterized by a small spot at the stigma trace shaded from dark brown to black. The original trivial name 'black navel' seems to be derived from this characteristic symptom. These spots enlarged as the berries developed and became a distinct brown spots with different shades. The size of lesion ranged from 1 to 4 mm with an average at 1.5 mm in diameter. The most remarkable development of lesions occurred at the stage when sugar contents of berries progressively increased. Figures 1 and 3 A represents berries with different sizes and types of lesions.

Microscopic observation

Some of barries exhibited a black moldy sign on the stigma trace, as could be easily discerned with the naked eye, especially when the berries were kept under humid condition. Close examination of the epidermal tissues of these diseased berries revealed that these signs were due to abundant hyphal growth and sporulation of fungus at the stigma trace. Both colour of hyphae, conidiophores, and conidia, and mode of conidiation suggested that the pathogen would belong to *Cladosporium*. Hyphae of greyish to olivaceous shades grew radially from the stigma trace on the epidermal surface. Some of hypodermal cells under these hyphae responded with necrosis. The characteristic browning radiating in all directions from the solid lesion of greyish shade as frequently observed of heavily infected berries, were thought to be due to either direct or indirect action of the fungus. Some of these hyphae growing radially also accompanied with a few conidia. When lesions were larger and accompanied a distinct necrosis at the stigma trace, brownings were also extended to the axial vascular strands of the berries. In many cases, epidermal tissues closely located to the receptacle, especially those directly in contact with pedicel, became brown and were found to harbor the pathogen. In some berries of which this browning of epidermal tissues was progressive, not only axial vascular strands but also peripheral vascular strands exhibited an extensive browning. It is only these cases that the disease significantly affect the quality of the berries to the extent that one should consider the disease important. Otherwise the disease does not affect the quality of berries. Under certain condition, however, this browning became visible on entire surface of the berries as is illustrated in Figure 3 B.

Isolation and identification of the fungi

Although several fungi belonging to different genera were isolated from the diseased tissues, the most frequently isolated were two fungi that belonged to *Cladosporium*, as had been expected from microscopic observation of the diseased specimen. These two isolates were subjected to identification. Both isolates were similar in colony type and conidial structure. Chains of holoblastic brownish conidia were formed on the apex of unbranched, dark brown conidiophore. These morphological characteristics indicate that both the isolates belong to the genus *Cladosporium* Link ex Fr. Consequently these isolates were referred to the type cultures of *Cladosporium* deposited in the culture collection of the Institute for Fermentation, Osaka. Morphological comparison and literary citation[1] indicated that the isolate 1 was *Cladosporium cladosporioides* (Fresenius) de Vries. This species is considered the most common *Cladosporium* on dead organic materials. However, pathogenicity of the present isolate on grapes and slightly reduced number of conidiophores of the present isolate suggest that there is

considerable variation among this species. The isolate 2 was found to be identical with *Cladosporium herbarum* (Pers.) Link ex Fr. which is also prevalent on dead organic matters, very often occurring side by side or together with *C. cladosporioides* on the same substrate. However, *C. herbarum* could be differentiated from *C. cladosporioides* on the basis of several criteria such as larger size of conidia, greater number of 2-or more-celled conidia, inflation of conidiophore at the portion where conidial chains arise, prolonged conidiophores, and much rougher conidial wall.

Inoculation test

a) Pathogenicity of the isolates. The two isolates identified as above were tested for their pathogenicity against berries. Healthy berries at the stage of harvest were inoulated with these isolates by placing a drop of respective conidial suspension at the portion of stigma trace. Ripe berries were used for this purpose because younger ones were suspected to be infected without apparent symptom. The result is shown in

Table 1. Infectivity of *Cladosporium cladosporioides* and *C. herbarum* against mature grape berries*

Isolates	Percent Berries infected		
	Uninoculated Control	Inoculated with iniury	Inoculated without injury
C. cladosporioides	0	100	100
C. herbarum	0	100	80

* Berries at the stage of harvest were used. Final scoring was made 14 days after inoculation.

Figure 2 and Table 1. Apparently *C. cladosporioides* and *C. herbarum* were both pathogenic to grape berries as evidenced by the characteristic symptom, even though lesions were slightly darker in colour comparing with those found in field. The berries which received only the needle-prick (line B in both rows in Figure 2) also exhibited black lesion at the injured portion, probably being due to oxidized polyphenols. *C. herbarum* were found to be less aggressive than *C. cladosporioides* as was exemplified by the production of much smaller lesion. Infection frequency was also lower with *C. herbarum.*

b) Susceptibility of berries as affected by the maturity. In view of rapid development of lesions at the maturing stage, berries at the different stages of growth were tested for their susceptibility. The result is shown in Tables 2 and 3. It is apparent that berries at the younger stage were resistant to the disease and they became

Table 2. Infectivity of *Cladosporium cladosporides* against grape berries at differnet stages of development

Age of Berries	Sugar Content*	Percent Berries Infected**	
		Uninoculated	Inoculated without injury
130	18.8	0	69
100	15.8	0	45
85	15.6	0	79
70	11.0	0	0

* Determined by Brix refractometer.

** Estimated 5 days after inoculation.

*** Inoculated and uninoculated berries were incubated at 18 C.

Table 3. Infectivity of *Cladosporium cladosporioides* attested with berries at different stages of development*

Age of Berries	Sugar Content**	Percent Berries Infected***	
		Uninoculated Control	Inoculated without Injury
132	20.0	0	0
102	18.2	0	33
87	17.8	0	38
72	11.4	0	0

* Experiment was conducted at 32 C.
** Determined by Brix refractometer.
*** Scored 3 days after inoculation.

Table 4. Disease development on grapes inoculated with *Cladosporium cladosporioides**

Age of Berries	Sugar Content**	Percent Berries Infected***	
		Uninoculated	Inoculated
130	18.8	5	100
100	15.8	5	100
85	15.6	5	95

* Experiment was conducted in a greenhouse.
** Determined by Brix refractometer.
*** Scored 9 days after inoculation.

susceptible as they matured. Similar experiment was conducted with berries in greenhouse. The result is shown in Table 4. In both experiments, some berries that had not been inoculated developed symptom. These must have been infected prior to inoculation.

Discussion

Several species of fungi belonging to *Cladosporium* cause different types of disease in various plants[5,6,7]. *Cladosporium herbarum* is primarily a saprophytic fungus inhabiting on dead organic materials. However, it causes sooty mold, black mold, and false blast of rice plants, sooty mold of barley and rye as well as black mold of wheat[5]. *Cladosporium cladosporioides*, together with *C. herbarum*, is known to cause sooty mold of poplars[7]. *Cladosporium viticolum* Cesati was reported to be the pathogen of leaf scab of grapevine[3]. This is the only *Cladosporium* sp. reported to cause disease of grapevine but apparently needs to be verified for its pathogenicity[6].

IKATA [1] reported in 1940 an epidemic disease of grapes that apparently corresponds to the disease we have described in this paper. However, neither identification nor pathogenicity test of the candidate pathogen had been done. IKATA attributed this disease to a synergistic action between *Cladosporium* sp. and thrips which has recently been identified as *Scirtothrips dorsalis* Hood[4]. The result in this paper, however, provided evidence that the disease could be incited by *C. cladosporioides* and *C. herbarum* without a concomitant presence of thrips. The extensive brown stains observed of some berries were also proved to be associated with these fungi, but should be further studied for thier cause-effect relationship with thrips.

In view of the characteristic brown spot appeared at the mature stage we propose to name the disease brown spot of grapes.

Although parasitic trait of these pathogens was in most cases confined to a limited area of or arround the stigma trace, the disease is significantly important because of its effect on commercial value of grapes, Mascut of Alexandria. Search for efficient control measures of the disease is in progress.

Summary

Cladosporium cladosporioides (Fresenius) de Vries and *Cladosporium herbarum* (Pers.) Link ex Fr. were isolated from grape berries that had been commonly called 'black navel', and were found to cause the disease. In view of the colour of lesions that were observed most frequently at the stage of maturing, the disease was named brown spot of grapes.

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クラドスポリウム属菌によるブドウの褐点病について

大内成志・畑本　求*・奥　八郎・白石友紀・横山竜夫**・立石道博***・藤井新太郎*
(岡山大学農学部植物病学研究室)

岡山県下のマスカットに発生した通称へそ黒病について，病原菌の分離・同定を行った。その結果，病原菌は *Cladosporium herbarum* (Pers.) Link ex Fr. および *Cladosporium cladosporioides* (Fresenius) de Vries であることが明らかになった．成熟果上の病徴に鑑み本病をブドウ褐点病と命名した．

* 岡山県農業試験場
** 財団法人醗酵研究所
*** 島根県農業試験場

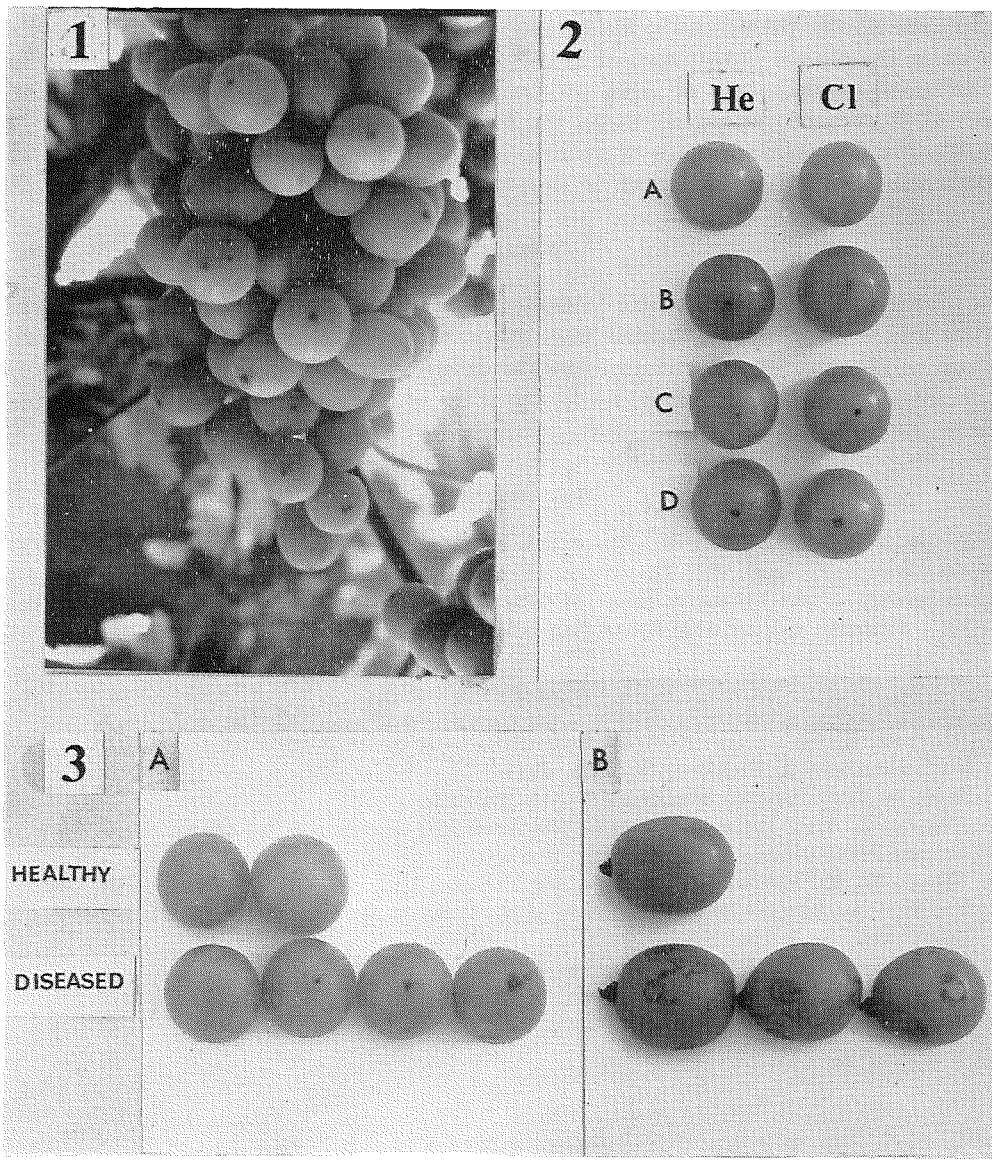

Figure 1. Grapes infected by the brown spot as observed in a greenhouse

Figure 2. Symptoms of brown spot on berries inoculated with conidia of *Cladosporium herbarum* (He row) and *C. cladosporioides* (Cl row). A ; uninoculated control, B ; injured control C; inoculated without injury, D ; inoculated after needle-prick.

Figure 3. A. Berries with different size of lesions.

Figure 3. B. Berries with irregular brownings all over the surface. Notice the brown ring that had been considered as injuries by thrips.