

Bunch Rot of Grapes Caused by *Aspergillus aculeatus*

W. R. JARVIS and J. A. TRAQUAIR, Agriculture Canada Research Station, Harrow, Ontario N0R 1G0

ABSTRACT

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Aspergillus aculeatus is recorded as a pathogen of grape berries in southwestern Ontario. It is a wound pathogen that enters the berry through fractures caused by partial detachment of fruits at the pedicel in tight bunches and through splits and insect punctures. The disease is associated with high temperatures. Sclerotia were formed in culture but have not been seen in the field or in inoculated grapes.

In the summer of 1983, laboratory examination of rotted white grapes from Essex and Kent counties in southwestern Ontario showed that the principal associated fungus was *Aspergillus aculeatus* Iizuka (11). Although other *Aspergillus* spp. have been reported from grapes (2-5,7,8,10,12-14,17), *A. aculeatus* has apparently only been reported from *Citrus* spp. (1,6), from soil (11), and from humans with upper respiratory infection (9). *A. violaceo-fuscus*, a possible synonymous species (11), has been reported from grape berries (8).

Because *Aspergillus* spp. are usually reported detrimental to wine quality, producing the so-called "pourriture vulgaire" (12), we wished to determine the plant-pathogenic capabilities of *A. aculeatus* in the relatively new vineyards of southwestern Ontario.

MATERIALS AND METHODS

Bunches of grapes were submitted to us for disease diagnosis by scouts monitoring vineyards in Essex and Kent counties in southwestern Ontario. In addition to black rot (*Guignardia bidwellii* (Ell.) Viala & Ravaz) and gray mold (*Botrytis cinerea* Pers.: Fr.), which was less common, an *Aspergillus* sp. with black conidial heads was frequently associated with a bunch rot. The fungus was readily isolated on potato-dextrose, Czapek-Dox, and malt-extract agars by mass spore transfer. Colony morphology was determined with Czapek-Dox agar (11), and microscopic dimensions were measured in lactophenol mounts. Radial growth rate was determined from two diametric measurements on each of three replicate colonies of Czapek-Dox agar.

Inoculations of punctured grape berries were made by dipping whole ripe

bunches in an aqueous suspension of 8.75×10^6 conidia per milliliter. The bunches, each composed of 31-108 berries, replicated three times, were incubated in closed plastic bags at various controlled temperatures. Punctures were made through the skin with a 23-gauge (0.64-mm) hypodermic needle immediately before dipping.

Incidence of infection was determined by the proportion of berries parasitized in the bunch and the severity of symptoms assessed on an arbitrary scale of 0-5 where 0 = no visible fungal growth and 5 = abundant conidiophore and conidium production.

Meteorological data were collected from the weather station at the Harrow Research Station in Essex County, Ontario, and grape heat units (degree-days) were calculated from base 10 C (16).

RESULTS

Fungus. The fungus consistently isolated was identified as *A. aculeatus* (11), a member of the *A. niger* group with phialides only. Conidiophores on Czapek-Dox agar were 104-427 μ m long and 7.5-13 μ m wide; the vesicle subglobose, 35-61 μ m long, and 35-53 μ m wide; phialides 5-6 \times 3-4 μ m; and conidia globose to elliptical, 5-6.5 \times 4-4.5 μ m, and echinulate. On Czapek-Dox agar, growth was moderately fast; the mycelium mostly submerged, hyaline to pale ochreous on both surfaces of the colony; conidiophores scattered, but not densely, over the whole colony; and conidial heads at first spherical and hyaline soon turning grayish sepia, fuscous black in mass, and splitting into three to six angular columns. White and spherical sclerotia 1 mm in diameter formed partially submerged at the surface of the agar.

Pathogenicity. Only the white grape cultivars Aureole, Seyval Blanc, Dutchess, and Johannesburg Riesling were affected; Maréchal Foch, a black cultivar, was not affected. Nesting, the rapid growth of the fungus from berry to berry, such as occurs in gray mold, did not occur to any appreciable extent.

The disease was present in six of seven local vineyards visited, and up to about 20% of bunches of Seyval Blanc were affected in some vineyards. On individual vines, as many as 50% of the early bunches were affected. Other diseases present to a lesser extent were gray mold (*B. cinerea*) on rain-split berries and black rot (*G. bidwellii*). In addition to *A. aculeatus*, *Alternaria* spp., *Epicoccum nigrum* Link, *B. cinerea*, and various yeasts and bacteria were isolated from diseased tissues but relatively infrequently. Generally, early-maturing, insolated and tight bunches were the most affected. Fungal growth was concentrated at the pedicel, where pressure within the bunch had partially detached the berry, or at rain-induced splits or insect punctures. Mycelial growth was profuse on the exposed parenchyma, and black conidial heads were abundant. A ring of pale water-soaked tissue 2-4 mm in diameter was evident around the lesion, and the skin readily slipped off. The soft watery rot advanced throughout the berry, which collapsed and fell off. The infected bunch had a sour odor.

Grapes punctured artificially and inoculated by dipping in a suspension of spores of *A. aculeatus*, then incubated at 27 C, frequently developed radiating splits in which conidiophores formed, surrounded by the paler water-soaked lesion.

Bunches of punctured, dip-inoculated grapes of cultivars Seyval Blanc, Aureole, and Dutchess from one vineyard had, respectively, 11.7, 18.6, and 17.5% infection, significantly ($P \leq 0.05$) more than unwounded bunches, whereas in bunches of Seyval Blanc from another vineyard, there was no such difference.

Environmental factors. Some meteorological data are summarized in Table 1. The summer of 1983 was characterized by many days with temperatures exceeding 30 C and a 4-mo accumulation of 1,395 grape heat units compared with the 67-yr mean of 1,246.

Temperature relations. Tested over the range of 10-30 C, maximum radial extension of the colony on Czapek-Dox agar occurred at 30 C, with minimum growth at 10 C. Similarly, maximum parasitism of inoculated berries of Seyval Blanc occurred at 27.5-30 C as measured by both the proportion of parasitized berries in the bunch and the severity of symptoms (Table 2).

DISCUSSION

Aspergillus spp. are often secondarily associated with bunch rots of grapes

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Table 1. Meteorological data from the Harrow Research Station, Southwestern Ontario, 1983

Month	Mean temperature (C)		Days with temperatures		Grape heat units ^b		Rainfall (mm)		Mean RH ^c (%)
	Month	67-Yr ^a	≥25 C	≥30 C	Month	67-Yr ^a	Month	67-Yr ^a	
June	20.5	19.6	25	7	314	296	84	77	66
July	23.7	22.4	27	12	425	383	61	68	68
Aug.	22.9	21.4	29	7	398	352	67	67	74
Sept.	18.6	17.7	23	3	258	215	54	66	74

^a67-Yr mean.^bGrape heat units = degree-days to base 10 C (16).^cMean relative humidity from spot readings at 0800 and 1630 hours each day.**Table 2.** Effect of temperature on radial growth of *Aspergillus aculeatus* in agar and in grape berries

Temperature (C)	Mean colony diameter (mm at 3 days)	Percentage of affected grapes in inoculated bunches ^x	Severity of symptoms ^y
10.0	13.3 a ^z	0.0 a ^z	0
12.0	...	0.0 a	0
13.5	13.0 a	13.0 b	4
18.5	21.5 b	34.1 c	1
23.0	33.0 c	26.5 bc	2
24.0	32.0 c	72.7 de	2
25.0	33.0 cd	60.3 d	3
27.5	...	75.9 e	4
28.0	34.3 cd	85.0 ef	4
30.0	44.8 d	96.8 f	5

^xBunch size varied from 31 to 108 berries.^y0 = no symptoms; 5 = abundant conidiophore and conidium production.^zWithin each column values followed by the same letter are not significantly different ($P = 0.05$), t and χ^2 test, respectively.

(2-5,7,8,10,12-14,17), yielding the so-called "pourriture vulgaire" (12) that renders them useless for wine-making. This is the first record of *A. aculeatus* as a grape pathogen, but its effect on wine quality has not been tested.

Our isolate of *A. aculeatus* grows best at about 30 C, which agrees with previous reports on *A. aculeatus* (9) and on the possible synonymous species *A. violaceo-fuscus* (8). Its pathogenicity was also greatest at 30 C, which also agrees with observations on *A. violaceo-fuscus* (8). It is noteworthy that the grape disease appeared in a summer characterized by many days of high temperatures and in which accumulated grape heat units were almost 12% higher than the 67-yr mean.

As with other small fruits (15), it seems likely that the internal temperatures of grapes may be appreciably higher than ambient temperatures. Stevens and Wilcox (15) recorded internal temperatures up to 9 degrees higher in strawberries, gooseberries, red currants, cranberries, and blueberries, especially in insulated berries. The effects of internal fruit temperatures on pathological activities in grapes require further investigation.

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