SUSPECTED OCCURRENCE OF BYSSOCHLAMYS FULVA IN QUEENSLAND-GROWN CANNED STRAWBERRIES

During 1961 it was observed that the strawberries in some cans of commerically processed berries had completely disintegrated. Microscopic examination showed that the cells of the fruit had fallen apart, indicating that the cementing pectinous substance of the fruit had been attacked. In some cans white mould mycelium was reported to be seen on the fruit, but none of the cans submitted showed obvious fungal growth. The type of spoilage observed suggested that the fruit had been attacked in the can by the fungus *Byssochlamys fulva* (Olliver and Smith 1933). Isolation and identification of the fungus were therefore attempted and its heat resistance was investigated.

It was noticed that cans in which the fruit had disintegrated could be identified by the loose "sloppy" sound made when they were shaken. Such cans were opened aseptically and 1-ml samples were plated out in potato dextrose agar or on yeast-extract malt-extract agar. Microscopic examination of the syrup showed fragments of fungal mycelium even if mould growth was not obvious to the naked eye. Only one type of mould was isolated from the cans. It grew readily on both media mentioned above, and also on Czapek-Dox agar and potato sucrose agar (Olliver and Rendle 1934). The mould was isolated from one can in a batch showing high incidence of spoilage, although the fruit in the can had not been attacked. The mould grew rapidly over the surface of the syrup from this can when it was exposed to the air in a sterile flask stoppered with cotton wool. The mould grew well at 28°C but growth was more rapid at 32°C. The fungus produced conidia readily on all media, and this stage was identified as *Paecilomyces varioti* (Brown and Smith 1957). These authors stated that the conidial stage of *B. fulva* is practically indistinguishable from *P. varioti*.

Attempts to induce the formation of the perfect stage of the fungus failed. The methods used were: (*a*) inoculation of all isolations on to one plate to encourage the production of ascospores along the bordering margins of adjacent colonies; (*b*) inoculation of fresh fruit with conidia of the fungus isolated from canned material; (*c*) exposure of the petri dishes in which the fungus was growing to diffuse light in the laboratory; (*d*) exposure of colonies to direct sunlight for a few minutes on successive days; and (*e*) inoculation onto cornmeal agar and onto potato sucrose agar to which 2 ml of soil extract had been added.

The first heat-resistance test carried out was with a suspension of conidia from a colony grown on Czapek-Dox agar for 7 days. A heavy spore suspension was prepared in sterile distilled water and wetting agent. Czapek-Dox broths, prepared in 9-ml amounts in stoppered test-tubes, were brought to the required temperature in a water-bath; 1 ml of spore suspension was added to each and

the tubes were held at the required temperature for the required time, then removed from the water-bath. Duplicate 1-ml samples were taken from each tube and plated out with 9-ml melted Czapek-Dox agar. The plates were incubated at 35°C for several days and examined for mould development. A control culture was made, using unheated spore suspension. The results are set out in Table 1.

TABLE 1

HEAT RESISTANCE OF ISOLATE IN CZAPEK–DOX BROTH

Temperature (°C)	Time of Heat Treatment (min)			
	1	2	4	8
70	++	++	++	+−
75	++	−−	−−	−−
80	−−	−−	−−	−−

++ = both samples positive growth.

−− = both samples negative growth.

This experiment was later repeated, using a 30-day culture of the fungus grown on Czapek-Dox agar + soil extract and showing macrospores. The macrospores were formed only on hyphae submerged in the agar, so the whole contents of the petri dish were cut up finely with a sterile scalpel and suspended in 50 ml distilled water containing 0·5 ml "Teepol". Then 9-ml quantities of strawberry syrup from a sterile can of fruit were heated in stoppered test-tubes to the required temperature in a water-bath. Strawberry syrup was used instead of Czapek-Dox broth because it was found that destruction of resistant spores was more difficult when the spores were heated in the presence of sucrose (Hull 1934, pp. 64-73; 1935, pp. 68-73). To each, 1 ml of spore suspension was added and mixed in by inverting the stoppered tubes. Then the tubes were held at the required temperature for the required time, removed and plunged immediately into cold water. Duplicate 1-ml samples were taken from each tube and plated out with 9 ml melted Czapek-Dox agar. After incubation the plates were examined for mould development. Results are set out in Table 2.

TABLE 2

HEAT RESISTANCE OF MACROSPORES IN SYRUP

Temperature (°C)	Time of Heat Treatment (min)			
	1	2	4	8
70	++	++	++	++
75	++	++	++	++
80	++	++	++	++

++ = both samples positive growth.

To determine whether the presence of macrospores was responsible for this high heat resistance, a spore suspension was made from another mature (30-day) culture by washing conidia off the surface of the agar. The macrospores, being produced on submerged hyphae, would therefore not be included in the suspension. Heat-resistance tests were conducted as above. Results are set out in Table 3.

TABLE 3

HEAT RESISTANCE OF CONIDIA IN SYRUP

Temperature (°C)	Time of Heat Treatment (min)		
	1	2	4
80	*	++	++
85	*	++	++
87	*	++	++
90	++	++	++
95	−−	*	*
98	−−	*	*

++ = both samples positive growth.

−− = both samples negative growth.

* No tests performed.

Ascospores of *B. fulva* are stated to survive at least 84°C for 30 min (Olliver and Rendle 1934), so samples were taken from a tube of syrup heated to 84°C for 30 min. Samples were taken after 20 min and 30 min. There were no survivors after these treatments. However, in the tubes which had been subjected to 95°, 98° and 84°C for the above-mentioned times, and which had been kept sealed in the incubator for three days, mould growth appeared, indicating that a few remaining spores were viable.

The heat-resisting properties of this fungus indicate that it is almost identical with *B. fulva,* whose mature ascospores are said to be the portion of the fungus with heat-resistant properties. In the cultures examined, ascospores were not recognized, but the heat-resisting properties indicated their presence.

Considering the fungus as *B. fulva,* Gillespy (1938, p. 68) stated that natural infection of a can (i.e. from infected fruit) can be inactivated by an internal temperature of 82·2°C, whereas to destroy resistant spores 90·5°C at the centre of the can would be necessary (i.e. 90·5°C is considered to be a safe processing temperature). The fact that in the tests carried out spores resisted more severe heat treatments than this was due to the use of a very much higher concentration of spores in the tests than would ever be encountered under natural conditions.

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(Received for publication June 28, 1963)