Effect of Polyoxin on Fungi IV Bursting of the Bulbous Germ Tubes of Polyoxin-treated *Alternaria kikuchiana* Tanaka in Pear and Fruit Extracts

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石崎 寛・栗田和明・久能 均：糸状菌に対するポリオキシンの影響 IV ナシ葉
または果実煎汁中におけるポリオキシン処理ナシ黒斑病菌胞子発芽管の破裂

In the previous papers^{1, 2, 3)}, we reported that polyoxin induced a characteristic bulbous structure on spore germ tubes and hyphae of *Alternaria kikuchiana* Tanaka, the causal fungus of the black spot disease of Japanese pear. In this paper, we report an interesting effect of pear extracts on the bulbous germ tubes induced by polyoxin; the bulbous germ tubes burst and released their contents.

The pear leaf extract was prepared as follows. Fifty grams of fresh Japanese pear leaf (var. Kikusui) was chopped and boiled in 500 ml of deionized water for 30 minutes. The extract was filtered through a layer of filter paper (Toyo, No. 2). Twenty grams of glucose was added to the filtrate, which was then diluted to a volume of 1000 ml with deionized water. Finally the extract was autoclaved at 1 atm. for 30 minutes. The pear fruit extract was prepared similarly. For comparison, slices of fresh pear fruit were used.

A polyoxin-sensitive isolate of *A. kikuchiana* Tanaka (Al-3, the stock number of Mie Univ.) was cultured on potato-glucose agar at 28 C for two weeks. Spores which were formed on the mycelial mat were floated on 5 ml of the pear leaf extract containing 10 ppm of law material of polyoxin-AL wettable powder (Nihon Noyaku Co. Ltd.) in a Petri dish (3cm in diameter), and incubated at 28 C for a fixed time. Control spores were floated on the pear leaf extract, potato-glucose medium, or potato-glucose medium containing 10 ppm polyoxin for the same time. At the fixed time, the spores were transferred onto a glass slide and observed with an Orthoplan microscope (Leitz).

Regardless of the presence of polyoxin in the incubation medium, spore germination was markedly accelerated by the pear leaf extract (Fig. 1). Ninety-one per cent of the spores germinated in the leaf extract containing polyoxin after 4 hours incubation, while only 19% did so in polyoxin-containing aqueous solution at the same time. The leaf extract also accelerated the appearance of bulbous germ tubes induced by polyoxin. As shown in Fig. 1, 86% of the germinating spores had bulbous germ tubes after 4 hours incubation when incubated in polyoxin-containing pear extract. On the other hand, 36% had such germ tubes in polyoxin aqueous solution at the same time. Cell walls of bulbous germ tubes increased in thickness with

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1) Ishizaki, H., Mitsuoka, K. and Kunoh, H. (1974). Ann. Phytopath. Soc. Japan 40 : 433-438.
2) Ishizaki, H., Mitsuoka, K., Kohno M. and Kunoh, H. (1975) *ibid.* 41 : 131-140. 3) Ishizaki, H., Mitsuoka, K., Kohno, M. and Kunoh, H. (1976) *ibid.* 42 : 35-41.

time when spores were incubated in the leaf extract with polyoxin. However, the wall thickness was not homogeneous; only parts of the cell wall appeared thick (Plate, Fig. 1). After a while, the cell wall split at a thin portion near the distal region of the bulbous germ tube and the contents of the germ tubes began to flow out through the openings (Plate, Fig. 2). Finally, a cytoplasmic strand appeared to remain along the inside of the cell wall and a large vacuole-like space occupied the center of the germ tube (Plate, Fig. 3). As shown in Fig. 1, about 8% of the bulbous germ tube burst after 8 hours incubation and 68% did so after 24 hours. Similar effects were observed also in the pear fruit extract with polyoxin. Such a phenomenon was never observed in polyoxin containing potato-glucose medium or in aqueous solutions.

When spores suspended in polyoxin aqueous solution were placed on a thin slice of pear fruit and incubated at 28 C, the bulbous germ tubes burst in the same manner. Although the substance remains unidentified, some constituent(s) of pear must be responsible for this interesting effect on polyoxin action. Since bursting bulbous germ tubes seen on fresh fruit slices were also observed in autoclaved leaf extract, the substance seemed heat-stable. Further studies should be done to identify the substance and to resolve a relationship between it and polyoxin action on this fungus.

We are indebted to Dr. J. R. Aist, Cornell University, for his critical reading of the manuscript and valuable suggestions.

(Received June 9, 1975)

Explanation of plate

Magnification : $\times 2900$

Fig. 1. A bulbous germ tube observed in polyoxin-containing pear leaf extract after 20 hours incubation. Note that the thickness of the cell wall is not homogeneous.

Fig. 2. A bulbous germ tube releasing its contents through a split in the cell wall.

Fig. 3. A vacuole-like space occupies the center of the germ tube and a cytoplasmic strand remains along the cell wall.