

Filamentous and polyhedral virus-like particles in *Boletus edulis*

H. HUTTINGA¹, H. J. WICHERS and ANNEMARIE DIELEMAN-VAN ZAAZEN²

¹ Institute of Phytopathological Research (IPO), Wageningen

² Mushroom Experiment Station, Horst

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Abstract

In the north-east and east of the Netherlands a disease of *Boletus edulis* was noticed. Symptoms are described. The name 'little-cap disease' is proposed. In all cell-free preparations made from diseased, but also from healthy carpophores polyhedral virus-like particles about 33 nm and 42 nm in diameter were observed, the 33 nm particles prevailing. Occasionally a few polyhedral particles of about 28 nm were isolated from healthy and diseased carpophores. Some healthy and diseased mushroom samples yielded filamentous virus-like particles about 500 nm long and 13 nm wide, with an axial canal. To our knowledge no other filamentous virus-like particles have been isolated from fungi so far. The role of the virus-like particles in *B. edulis* and the cause of 'little-cap disease' are still unknown.

Introduction

In wild-growing but edible mushroom *Boletus edulis* Bull. ex Fr. a disease was observed which is characterized by the formation of relatively thick stipes and small caps (Fig. 1). Diseased mushrooms were more frequently found invaded by maggots and bacterial and fungal organisms than healthy ones. The disease was prevalent in the province of Drenthe where *B. edulis* is rather common and occurred to a lesser extent in the province of Gelderland. It was first noticed in 1969, but may have been present before. *B. edulis* was especially found under beeches and red oaks by the roadsides. Diseased carpophores occurred in enclosed places with a high humidity. Areas with diseased mushrooms varied in size from 1 to 100 m in diameter. After some years the production of mushrooms decreased or even ceased in such areas.

Caps of affected mushrooms remain small or are sometimes absent and carpophores may show a gradual transition between cap and stipe. Small caps being a prominent symptom, the name 'little-cap disease' is now proposed.

Small caps have also been mentioned for virus-diseased cultivated mushrooms (Schisler et al., 1967; Dieleman-van Zaayen, 1969) and for *Laccaria* species and *Cantharellus infundibuliformis* (Scop.) Fr. suspected to be virus-infected (Blattný and Pilát, 1957; Blattný, 1966). From microcephalic sporophores of *Laccaria laccata* (Scop. ex Fr.) Cooke virus-like particles 28 nm in diameter were isolated and healthy sporophores of this species did not yield virus-like particles (Blattný and Králík, 1968). Hollings and Stone (1971) found virus-like particles 50 nm in diameter in preparations of *Boletus* sp., showing no abnormal characters.

We have now studied little-cap diseased and healthy material of *B. edulis* for a possible association of the disease with virus particles.

Fig. 1. 'Little-cap disease' of *B. edulis*. Left, diseased mushroom; right, healthy.



Fig. 1. 'Kleine-hoedjesziekte' van *B. edulis*. Links, ziek; rechts, gezond.

Material and methods

Mushroom material. Diseased and healthy carpophores of *B. edulis* were collected in woods and in roadsides.

Purification. About 100 g of mushrooms were ground in a Waring blender in a mixture of 250 ml of 0.18 M phosphate-citric acid buffer pH 7 (PCA buffer) containing 0.1 % (v/v) thioglycolic acid, 20 ml of carbon tetrachloride, 20 ml of chloroform, and 10 ml of diethyl ether. The homogenate was centrifuged for 10 min at 8,000 g. The supernatant phase was decanted and centrifuged for 1½ h at 105,000 g. The resulting pellets were resuspended in about 38 ml of PCA buffer and after storing for 1–2 h at 4 °C, the suspension was centrifuged for 10 min at 8,000 g. After a final cycle of centrifuging at high and low speed (1½ h 105,000 g and 10 min 8,000 g) the material was suspended in 1 ml PCA buffer.

Sucrose-gradient centrifuging. 0.3 ml suspension was layered on a linear gradient of 10–40 % sucrose and centrifuged for 1½ h at 113,000 g (25,000 rpm, Beckman SW27

rotor). The opalescent zones were isolated from the sucrose gradients as described by Van Kammen (1967) and after dilution with PCA buffer concentrated by centrifuging for 3 h at 105,000 g and resuspending the pellets in 0.05–0.20 ml PCA buffer.

Electron microscopy. Purified samples were put on to 200 mesh grids and negatively stained with 1 % PTA pH 6.5 in water. In some cases mushroom material was crushed in 2 % PTA pH 6.5 in water. A drop of this mixture was then put on to a grid. Preparations were examined in a Philips EM 300.

Results

In crush preparations of diseased mushrooms polyhedral particles could be detected with the electron microscope, but their concentration was very low and identification seriously hampered by large amounts of normal host constituents. Particles could be found more readily in purified preparations and best results were obtained when these preparations had been additionally purified by sucrose-gradient centrifuging.

In all samples investigated, whether they consisted of healthy or of diseased mushrooms, two types of polyhedral particles were found: a small one with a diameter of about 33 nm (Fig. 2B), occurring in large numbers, and a large one of about 42 nm (Fig. 2A), which always was less numerous.

In one set of healthy and diseased mushroom samples, collected in the neighbourhood of Makkinga, filamentous particles were also found (Fig. 3A). They were about 500 nm long and about 13 nm wide. In the negatively stained particles a central canal could be seen (Fig. 3B).

Fig. 2. Virus-like particles of *B. edulis*. A, B, and C: polyhedral particles 42 nm, 33 nm, and 28 nm in diameter, respectively. Bar represents 100 nm.

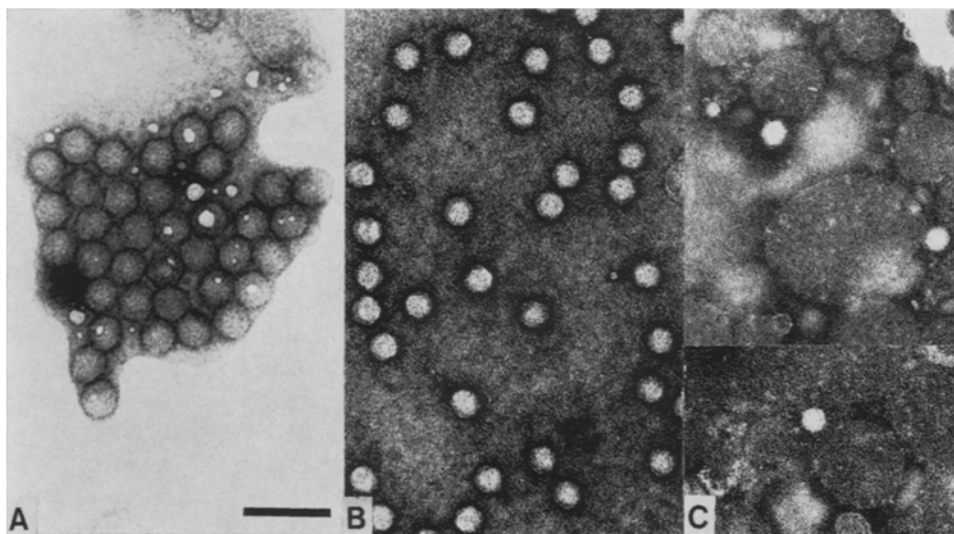


Fig. 2. Virusachtige deeltjes uit *B. edulis*. A, B en C: veelvlakige deeltjes met diameters van respectievelijk 42, 33 en 28 nm. De vergrotingsstreep geeft 100 nm weer.

Fig. 3. Virus-like particles in *B. edulis*. A: filamentous particles of 500×13 nm. Bar represents 1000 nm. B: filamentous particles showing a central canal. Bar represents 100 nm.

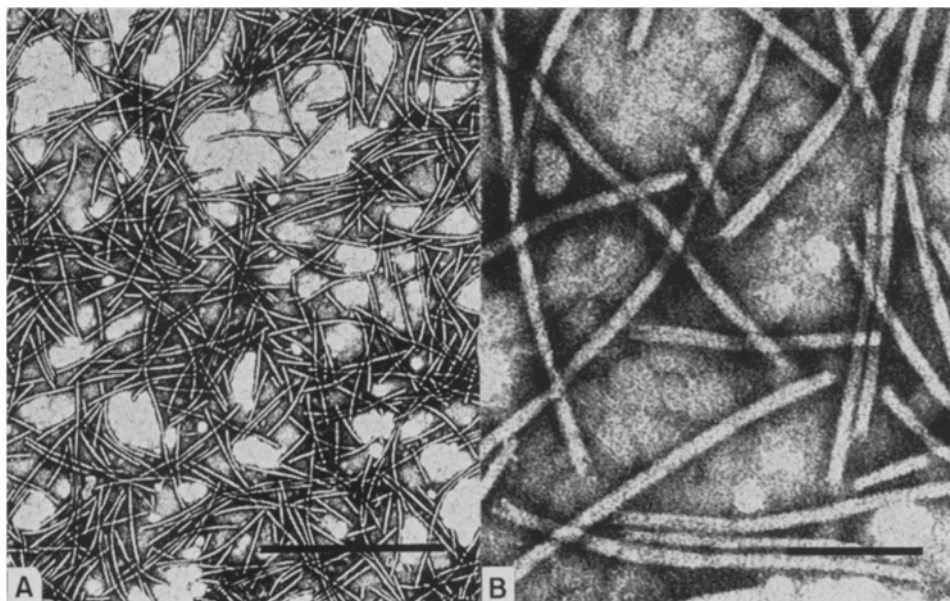


Fig. 3. Virusachtige deeltjes uit *B. edulis*. A: draadvormige deeltjes van 500×13 nm. De vergrotingsstreep geeft 1000 nm weer. B: draadvormige deeltjes met een centrale holte. De vergrotingsstreep geeft 100 nm weer.

Apart from these three types of particles which occurred in easily detectable amounts when present, in a few cases both in healthy and diseased mushrooms very small numbers of polyhedral particles of about 28 nm were found (Fig. 2C).

Discussion

All three types of polyhedral particles and the flexuous ones were detected in cell-free preparations from healthy as well as from diseased *B. edulis* carpophores. Hence, the 'little-cap disease' in *B. edulis* cannot be attributed to one or more of these types of particles. However, in size and shape they greatly resemble viruses. Since no infectivity could be demonstrated they are described as virus-like. Their role is unknown.

Most viruses reported from fungi are polyhedral or bacilliform (Lemke and Nash, 1974). Rod-shaped virus-like particles were first detected in 1967 by Dieleman-van Zaayen. To our knowledge filamentous particles have not earlier been isolated from a fungus, although a certain relationship has been claimed between the filamentous potato virus X and its vector *Synchytrium endobioticum* (Schilb.) Pers. (Nienhaus and Stille, 1965). In this respect it is interesting to note that the filamentous particles from *B. edulis* morphologically resemble the members of the potexvirus group.

Samenvatting

Draadvormige en veelvlakkige virusachtige deeltjes in eekhoorntjesbrood, Boletus edulis

In het noordoosten en het oosten van Nederland werd een ziekte waargenomen in eekhoorntjesbrood, *Boletus edulis*, waarvan de symptomen worden beschreven. Naar het meest karakteristieke symptoom wordt de naam 'kleine-hoedjesziekte' voorgesteld. Aangezien een analoog ziektebeeld voorkomt bij viruszieke champignons en ook beschreven is voor enkele wilde paddestoelen, die waarschijnlijk virusziek zijn, werden zieke boleten onderzocht op de aanwezigheid van virus. In celvrije preparaten van zieke, maar ook van gezonde boleten werden steeds veelvlakkige virusachtige deeltjes gevonden met doorsneden van 33 en 42 nm; de 33 nm-deeltjes waren het talrijkste. Af en toe werden uit gezond en ziek materiaal weinige veelvlakkige deeltjes van 28 nm geïsoleerd. Enkele monsters van gezonde zowel als van zieke boleten, leverden preparaten op met draadvormige deeltjes van ongeveer 500 nm lang en 13 nm dik, voorzien van een centrale holte. De betekenis van de virusachtige deeltjes in *B. edulis* en de oorzaak van de 'kleine-hoedjesziekte' zijn nog duister.

Uit schimmels werden nog niet eerder draadvormige virusachtige deeltjes geïsoleerd. De draadvormige deeltjes uit *B. edulis* vertonen uiterlijk overeenkomst met virussen uit de aardappelvirus X-groep (Potexvirus groep).

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Addresses

- H. Huttinga: Instituut voor Plantenziektenkundig Onderzoek, Binnenhaven 12, Wageningen, the Netherlands.
H. J. Wichers: Kokstraat 4, Eefde, the Netherlands.
A. Dieleman-van Zaayen: Proefstation voor de Champignoncultuur, Peelheideweg 1, Horst-America, the Netherlands.