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A virus disease of cultivated mushrooms in The Netherlands

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Gandy (1960) was the first to demonstrate that a serious disease of the cultivated mushroom, *Agaricus bisporus* (Lange) Sing., could be transmitted be means of mycelium. Hollings (1962) isolated three types of virus particles from diseased fruit bodies; isometric particles with a diameter of 25 and 29 mμ respectively, and elongated particles with rounded ends, size 19 × 50 mμ. By injecting a small quantity of a cell-free virus preparation into the base of young carpophores he could prove its infectivity. Some weeks after injection a pure culture was made of mycelium from mushrooms growing on the inoculated trays. It showed the slow, abnormal growth, characteristic of the disease (Gandy and Hollings, 1962). At spawning mycelium from the injected trays was added to trays with healthy mycelium. The mushrooms produced were mis-shapen and cropping was greatly reduced (Hollings et al., 1963). The demonstration of infectivity, however, was not completed by means of re-isolation of virus particles from carpophores harvested from inoculated trays.

In the United States of America particles were also found to be connected with a transmissible mushroom disease. The particles observed had a diameter of 25 mμ (Hollings, 1965; Schisler et al., 1967). Infectivity of these particles was not demonstrated.

The present study was carried out in order to investigate the possible correlation between a transmissible, crop-reducing disease of cultivated mushrooms in The Netherlands which showed certain similarities with Mushroom die-back (Gandy and Hollings, 1962), and the three kinds of virus-like particles which could be isolated from affected mushrooms.

Samples of fruit bodies, collected at mushroom farms where the disease was reported, were subjected to a combination of purification procedures based on those described by Hollings et al. (1965) and by Kitano et al. (1961). The latter method was originally applied to ECHO 7 virus, and was found to give very good results when used for purification of potato leafroll virus from its vector *Myzus persicae* (Peters, 1967).

The purification procedure was as follows: either fresh carpophores, or carpophores which were stored at 4 °C, were rinsed under running tap water and blotted with filter paper. After removing the lower parts of the stipes the fruit bodies were ground for 2 min in a Waring blendor in 30 ml of 0.033 M phosphate buffer containing 0.1 % thioglycollic acid, adjusted to pH 6.8, per 10 g of tissue. Fifty ml portions of the homogenate were subjected to ultrasonic treatment (Hollings et al., 1965) with a

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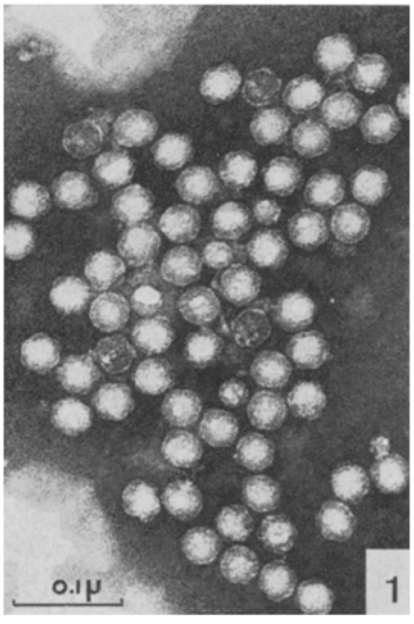
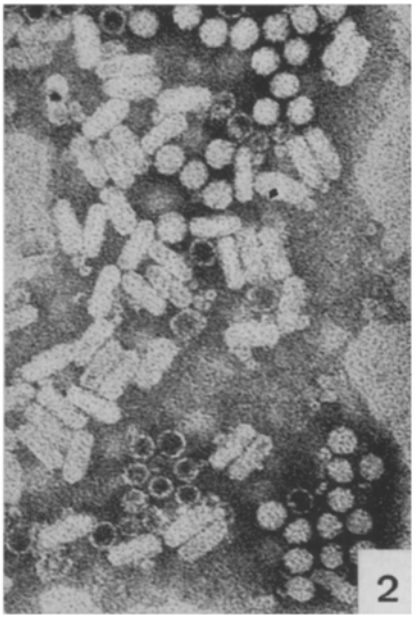
Kerry Vibrason cell disruptor (probe diameter 0.9 cm, output 50 W) for 10 min. During the treatment the thick-walled glass tube with an inner diameter of 3.5 cm, which contained the homogenate, was kept at 4 °C. A slightly modified version of the method of Kitano et al. (1961) was applied to the sonicated homogenate. To one volume of homogenate, an equal volume of 2.5 M potassium phosphate buffer pH 6.8 and 0.8 volume of a mixture of 2-butoxyethanol and 2-ethoxyethanol in the proportions 1:2 were added. The phosphate buffer was prepared by mixing hot solutions of 2.5 M KH_2PO_4 and 2.5 M K_2HPO_4 until a pH of 6.8 was reached.
The components were mixed gently by hand. Centrifugation at 1000 g for 5 min resulted in the partition of an aqueous bottom phase, an organic top phase and a gelatinous interphase. After decantation of the liquids the interphase was resuspended in 20 ml 0.033 M phosphate buffer pH 6.8 per 100 ml of crude homogenate. Centrifugation at 5000 g for 10 min gave a clear supernatant which was subjected to ultracentrifugation at 105,000 g for 60 min.
The pellet was resuspended in 0.5 ml 0.033 M phosphate buffer pH 6.8 per 100 ml crude homogenate. Centrifuging at 5000 g for 10 min gave a clear, slightly opalescent supernatant. The virus-like particles could be observed, after negative staining with 2% phosphotungstic acid pH 6.0, in a Siemens Elmiskop1 electron microscope. In most samples of diseased mushrooms three types of virus-like particles were detected in varying concentrations:
1. Isometric particles, diameter about 25 mμ (identical to Hollings's Mushroom virus 1?). Fig. 2 and 3.
2. Elongated particles with rounded ends, size 19 × 50 mμ (identical to Hollings's Mushroom virus 3?). Fig. 2.
3. Isometric particles with a distinct hexagonal outline, diameter about 34 mμ (Fig. 1). The diameter of these particles differed markedly from that of Hollings's Mushroom virus 2 (diameter 29 mμ).
No virus-like particles were present in healthy mushrooms.
To test the infectivity of the cell-free preparations containing all three types of particles carpophores were grown in steam-sterilized 30 × 30 × 25 cm trays on an approximately 20 cm high layer of horse manure-straw compost which was covered with a casing layer of soil with a depth of 5 cm. The trays were kept at 15 to 17 °C; the relative humidity was 70–80% and care was taken to keep the soil moist. The first crop (flush) of fruit bodies appeared five weeks after spawning.
Very young mushrooms of the first flush were, according to Gandy and Hollings (1962), inoculated with a purified preparation containing all types of particles by injection in the bases of the stipes with a hypodermic syringe and fine needle (27 G × 5/8″, Becton, Dickinson Cie.). The inoculated trays were observed daily and compared with the control trays, and once a week samples were collected from the trays which were tested for the presence of virus-like particles.
A few days after injection no particles could be detected in the inoculated mushrooms. Fruit bodies of the third flush, however, appearing two weeks after inoculation, showed long stipes and the off-white colour typical of the disease. These mushrooms contained the three types of virus particles, predominantly the 34 mμ "sphere" which also prevailed in the inoculum used in this experiment. Cropping ceased almost completely in the infected tray. Carpophores of the uninoculated control trays did not contain any particles.





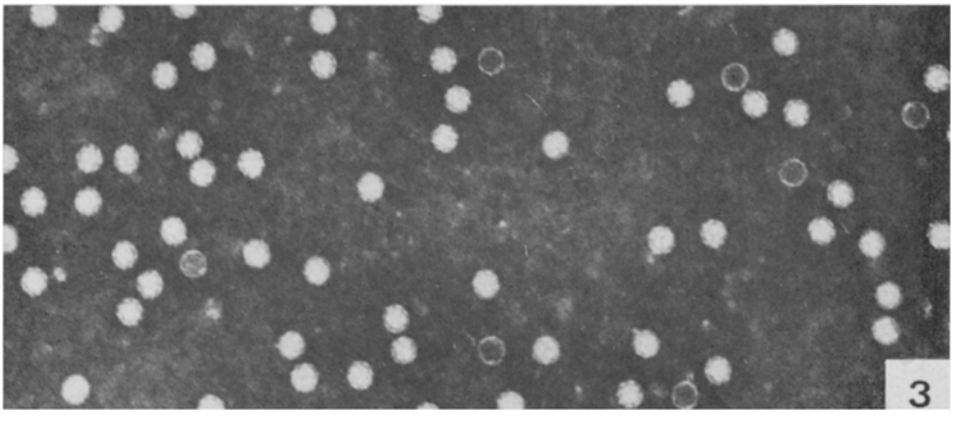


Fig. 1–3 are all at the same magnification (×160,000)
Fig. 1. Virus particles isolated from cultivated mushrooms, diameter 34 mμ. Some 25 mμ particles are visible. The preparation was negatively stained with phosphotungstic acid.
Fig. 2. Elongated virus particles with rounded ends, size 19 × 50 mμ, and 25 mμ particles
Fig. 3. Mushroom virus particles with a diameter of 25 mμ

Fig. 1–3 hebben dezelfde vergrotingsfactor (160.000×)
Fig. 1. Uit champignons geïsoleerde virusdeeltjes met een diameter van 34 mμ. Enkele 25 mμ-deeltjes zijn zichtbaar. Negatief contrast met fosforwolfraamzuur.
Fig. 2. Langwerpige virusdeeltjes met afgeronde einden, grootte 19 × 50 mμ, en 25 mμ-deeltjes
Fig. 3. Champignon-virusdeeltjes met een diameter van 25 mμ

Electron micrographs were taken by Mr. S. Henstra of the Service Institute for Applied Mechanics and Technical Physics in Agriculture at Wageningen.
De elektronenmicroscopische opnamen werden gemaakt door de heer S. Henstra van de Technische en Fysische Dienst voor de Landbouw te Wageningen.

Thus it was demonstrated that a purified preparation containing the three kinds of virus particles was able to infect healthy mushroom cultures and that all types of virus particles could be re-isolated. The infection procedure did not always lead to success, probably due to the age of carpophores used in virus purification. Nevertheless, mechanical transmission will be an important tool in future infection experiments in which the infectivity of the three types of virus particles will be investigated separately and which may lead to the correlation of symptom expression with type of virus particle(s) involved.

Samenvatting

Een virusziekte van champignons in Nederland

In Nederland treedt een ziekte op in de champignoncultuur, die gelijkenis vertoont met de "Die-back disease" in Engeland. Uit zieke champignons werden drie soorten virusdeeltjes geïsoleerd: bolvormige met een diameter van 25 mμ (Fig. 2 en 3), respectievelijk 34 mμ (Fig. 1), en langwerpige deeltjes met afgeronde einden van 19 × 50 mμ (Fig. 2).
Inoculatie met een celvrij preparaat, waarin naast enige virusdeeltjes van 25 mμ en 19 × 50 mμ voornamelijk deeltjes van 34 mμ voorkwamen, bracht voor de ziekte karakteristieke symptomen teweeg. Herisolatie van de drie soorten virusdeeltjes uit deze kunstmatig geïnfecteerde culture bleek mogelijk te zijn. Mechanische inoculatie kan een belangrijk hulpmiddel zijn bij het onderzoek naar infectievermogen van de verschillende soorten virusdeeltjes afzonderlijk.

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