Moulds that should be better known: 
*Thielaviopsis basicola* and *T. thielavioides*, two ubiquitous moulds on carrots sold in shops.

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Carrots, irrespective of their origin and whether they have been grown conventionally or organically, almost always harbour spores of *Thielaviopsis basicola* and *T. thielavioides* on their surface. Infections become visible as dark green mould patches upon prolonged incubation in polythene bags. These moulds produce beautiful asexual reproductive organs which are described here. We also present the first report of the previously unknown occurrence of repetitious conidial germination in both species.

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Much to the delight of mycologists but displeasure of those interested in their culinary value, carrots incubated under moist conditions in polythene bags at room temperature soon develop a dark greenish-grey or black mould on their surface. This phenomenon has been observed for many decades on conventionally produced and organic carrots (Lloyd & Lockwood, 1962; Punja et al., 1992). Almost all carrot samples carry inoculum, and infections occur especially if carrots have been washed prior to incubation in polythene. Development of infections seems to proceed optimally at room temperature (approx. 20-22°C), mould patches becoming visible after about 5 days (Fig 1). A well-known causal organism of post-harvest blackening on carrots is *Thielaviopsis basicola*. However, in our observations with carrots bought in English and German shops (Table 1), we found that the related species *Thielaviopsis thielavioides* (better known as *Chalaropsis thielavioides*) was at least as common. These two species can be easily distinguished as described below. Commercial damage caused by *T. basicola* during storage can be severe (e.g. Punja et al., 1992), but few literature data exist on *T. thielavioides*. The ubiquity of this species and its role as a storage rot organism do not seem to be widely appreciated.

![Fig 1](https://example.com/carrot-mould.jpg) Carrot sample stored for 10 d at room temperature after purchase. Infections of *T. thielavioides* are greenish-grey if conidia predominate, but turn black upon production of chlamydospores.
short, septate aerial hypha. Often further phialides arise from existing phialides or from the lower sterile cells of the aerial hypha, so that small clusters of phialides are formed (Fig 2). Upon closer inspection, the conidia are seen to arise well within the phialide body, i.e. they are fully delimited before they reach the phialide tip (Fig 3). Ingold (1981) has pointed out that the first-formed conidium has a slightly different shape from the later ones in being swollen at the top end and often carrying a little cap, the remnant of the phialide tip which broke off during its release.

The striking phialide defines the form-genus Chalara, and an alternative name for T. basicola is C. elegans Nag Raj & Kendrick 1975. An attempt was made by Nag Raj & Kendrick (1975) to group together all fungi which have the Chalara state but this state is found in some discomycetes as well as pyrenomycetes (Paulin & Harrington, 2000) and is therefore the product of convergent evolution.

In addition to phialoconidia, T. basicola also produces thick-walled chlamydospores, often basal to a phialide (Fig 4) or in clusters (Fig 5). Chlamydospores are formed from a swollen hypha which undergoes successive divisions followed by deposition of a thick melanized wall inside of the original wall (Riggs & Mims, 2000). About 3-6 thick-walled part-spores are formed. Ultimately, the composite chlamydospore fragments into its part-spores which are released into the soil. Chlamydospores are filled with lipid droplets (Figs 4 and 5) and are the main agent of survival in the soil (Tsao & Bricker, 1966). Thielaviopsis basicola is the only species in the Ceratocystis-Thielaviopsis complex with multicellular chlamydospores.

Chlamydospores are produced abundantly on oat meal agar whereas the phialidic state is best observed on weak clear media such as 0.2% malt-extract agar (MEA) or cornmeal agar. Thinly-poured plates are particularly suitable for microscopy. When squares of 0.2% MEA were cut from the margin of 14 d old colonies, washed with water and then mounted directly for microscopy, we observed that phialoconidia showed repetitious germination by producing a single phialide which gave rise to a new chain of phialoconidia (Figs 6 and 7). In our strain, these were identical in shape to, but smaller in size (8.0-11.0 _ 3.0-4.0 µm) than the primary phialoconidia (12.0-19.0 _ 4.5-5.5 µm). Production of secondary conidia was accompanied by the evacuation of the cytoplasmic contents, especially lipid droplets, from the primary conidium, and by their transfer into the secondary conidium (Figs 6 and 7). Consequently, each primary conidium produced only a limited number (<10) of new conidia until the spent cell lost its turgor pressure and collapsed (Fig 7).
contrast, phialides arising from the mycelium can produce very long chains of primary conidia because the septa at the base of the phialides are perforated (Fig

3), permitting the continual influx of material from mature hyphae.
**Thielaviopsis thielavioides** (Peyronel) Paulin, Harrington & McNew

The phialoconidia of *T. thielavioides* (Fig 8) and the *Chalara* state producing them (Figs 9, 10) are very similar to *T. basicola*. Depending on growth conditions and their age, the conidia may contain numerous lipid droplets which are often arranged into two groups, one at each pole of the spore, and separated by a central vacuole (Fig 8). Repetitious germination of conidia was observed in *T. thielavioides* but was not as frequent as in *T. basicola*. No sexual state is known for *T. thielavioides*, but like *T. basicola* this species is grouped in *Ceratocystis* on the basis of DNA sequence data (Paulin-Mahady et al., 2002).

The main microscopic difference between *T. thielavioides* (formerly *Chalaropsis thielavioides* Peyronel) and *T. basicola* is that the former produces chlamydospores singly rather than in chains (Figs 11-13). Further, these are not usually associated with the phialides but are formed by separate hyphae, often submerged in agar. In material taken directly from carrots, sympodial clusters of chlamydospores are often seen (Fig 13).

**Occurrence and ecology**

Both species are soil-borne and have been reported from the root surfaces of many different plants, including carrots (Nag Raj & Kendrick, 1975). *Thielaviopsis basicola* is best known as the cause of black root rot of tobacco in Europe and North America which can be serious (Delon et al., 1988). This species may also cause black root rots of varying severity in a number of other crops such as bean, citrus, cotton or soybean (Moore, 1959), and it is responsible for replant disorders in cherry and plum, i.e. the poor growth of trees in soils on which the same species has previously been grown.
(Sewell & Wilson, 1975). Many ornamental plants are susceptible and may be infected when, in the potted plant industry, the growth medium is made up with a field soil component. Such composts should be steamed or treated with fumigants (Daughtrey et al., 1995). By contrast, T. thielavioides is not known in these contexts.

Although typically present in field soils, T. basicola is rarely isolated on soil or dilution plates and the use of carrot tissue as an assay procedure was discovered by accident. Yarwood (1946) had reasoned that carrot tissue might well be a bait on which to isolate the destructive carrot pathogen, Sclerotinia sclerotiorum, from soils, but when he tried it out T. basicola was isolated instead. Subsequently, discs of carrot tissue were used extensively by many plant pathologists in baiting techniques for semi-quantitative estimation of the abundance of T. basicola in root rot soils. About 70% of all soil samples harbour T. basicola, whereby as few as four conidia per gram of soil are sufficient to give a positive result (McIlveen & Edgington, 1972). Using carrot disc baiting over a period of 33 years, Yarwood (1981) examined some 500 collections of soil and plant material worldwide and found that T. basicola was associated with the roots of some 137 genera of plants. These plants rarely showed symptoms of the disease. Yarwood (1981) also found that tissues of apple, pear and lemon fruit, sweet potato roots and celery petioles were suitable substrates for baiting T. basicola.

In agricultural situations, damage of carrot crops due to T. basicola in the field is slight or absent (except for damping-off of seedlings) because the fungus is unable to penetrate intact plant tissues (Punja et al., 1992). Extensive damage of stored carrots results because the fungus can infect the wounds caused by mechanized harvesting procedures. At 4°C, infections do not develop but the inoculum remains alive and can cause disease even weeks later when the carrots are placed at room temperature for sale. In nature, species of Thielaviopsis presumably grow on root exudates in the rhizosphere or degrade organic matter in the soil.

Lloyd and Lockwood (1962) found that the non-pathogenic T. thielavioides often occurred in place of T. basicola using carrot disk baiting, and they recommended identification by microscopic examination of the lesions developed on carrot disks. It is puzzling why T. thielavioides has not received the same attention as a storage carrot mould, given that in our observations it was more frequent than T. basicola (see Table 1). Possibly this species has been confused with T. basicola in the past, despite Lloyd & Lockwood's (1962) note of caution.

References