Nematode-Trapping in Pleurotus tuberregium

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Mycologia, Vol. 86, No. 5. (Sep. - Oct., 1994), pp. 696-699.

Nematode-trapping in *Pleurotus tuberregium*

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Abstract: *Pleurotus tuberregium* is a common basidiomycete in parts of tropical Africa and Australia that fruits from a subterranean true sclerotium. Evidence from field collections suggests that the sclerotia are formed underground. Because its basidiocarps have intercalary skeletal hyphae, *P. tuberregium* has also been classified in both *Lentinus* and *Panus*. We investigated nematode-trapping in *P. tuberregium* in an attempt to resolve its generic placement. Aerial hyphae of *P. tuberregium* cultures on agar produced droplets of toxin on stalked secretory processes. Nematodes that came in contact with toxin droplets were paralyzed and then colonized by hyphae. This mode of nematode capture has been demonstrated previously only in *Pleurotus sensu stricto*, which supports classification of this species in *Pleurotus*.

   Key Words: *Lentinus*, nematophagy, *Panus*, *Pleurotus tuberregium*

Most major groups of fungi are known to contain species that attack and consume nematodes by means of adhesive or ingested spores or various structures on vegetative hyphae. Hyphal modifications for nematode attack or capture include adhesive knobs, nets, stephanocysts, and filaments, constricting and non-constricting rings, cells that secrete paralyzing toxin droplets, and mycelia that contain cytoplasmic toxins (Barron, 1977; Thorn and Barron, 1984; Barron and Thorn, 1987; Tzean and Liou, 1993). Clearly, nematode-trapping per se is a convergent character, but the particular modes and structures of capture may provide phylogenetically informative suites of characters. In this report we describe nematode-trapping

in *Pleurotus tuberregium* (Fr.) Singer and discuss its taxonomic significance.

   Pleurotus tuberregium occurs in Africa, India, and Australasia (Pegler, 1983). It is unusual among traditionally accepted *Pleurotus* species (e.g., Hilber, 1982) in that its fruiting bodies arise not from wood, but from underground true sclerotia. Young mushrooms of *P. tuberregium* are eaten, but with age they develop a tough, leathery consistency and are no longer palatable (Corner, 1981). The toughness is due to the presence of numerous thick-walled skeletal hyphae that are especially well-developed in the stipe. Sclerotia of *P. tuberregium* are used as food, medicine, and as a source of additional crops of fruiting bodies (Zoberi, 1973; Oso, 1977; Corner, 1981; Pegler, 1983).

   The taxonomic history of *P. tuberregium* reflects the lack of consensus regarding generic limits of *Pleurotus* (Fr.) Kummer, *Panus* Fr., and *Lentinus* Fr. Singer (1951, 1986), whose concept of *Pleurotus* we follow, placed *P. tuberregium* in *Pleurotus* primarily on the basis of anatomy of the hymenophoral trama and subhymenium. However, Pegler (1975, 1983) restricted *Pleurotus* to monomitic species (those lacking skeletal or binding hyphae) and therefore excluded *P. tuberregium*, which he placed in *Lentinus*. Corner (1981) accepted some dimitic species in *Pleurotus*, but only those with strictly terminal skeletal hyphae. *Pleurotus tuberregium*, which Corner placed in *Panus*, has intercalary and terminal skeletal hyphae.

   Information from studies of nucleic acids has helped refine the limits of *Pleurotus*, *Lentinus*, and *Panus*. The three genera are usually considered to be very closely related, but studies of ribosomal RNA gene sequence data have suggested that the lentinoid-pleurotoid fungi actually comprise at least four independent lineages (Hibbett and Vilgalys, 1993).

   Cultural characters have also provided taxonomic resolution. Most lentinoid-pleurotoid fungi produce a white-rot, but there are also some brown-rot species in *Lentinus s. lat.* and *Panus s. lat.* (Gilbertson, 1981). The brown-rot species have been segregated as *Neolentinus* Redhead & Ginns and *Heliocybe* Redhead & Ginns (Redhead and Ginns, 1985). The species removed to *Neolentinus* generally have weakly developed skeletal hyphae and are therefore anatomically intermediate between *Lentinus s. str.* and *Panus s. str.*, which are strongly dimitic, and *Pleurotus*, which is mostly

monomitic. Thus, segregation of *Neolentinus* and *Heliocybe,* which was supported by the RNA sequence data (Hibbett and Vilgalys, 1993), helped to differentiate *Pleurotus* from both *Lentinus* and *Panus* (Redhead and Ginns, 1985).

Members of *Pleurotus sensu* Singer (1975, 1986) display a method of nematode capture that appears to be unique to this genus (Thorn and Barron, 1984; Redhead and Ginns, 1985; Barron and Thorn, 1987; Petersen, 1993; Thorn and Tsuneda, 1993). In agar cultures, aerial hyphae of *Pleurotus* produce tiny droplets of nematode toxin on minute secretory processes. The toxin has been identified as trans-2-decenedioic acid (Kwok et al., 1992). Nematodes that come in contact with the toxin are paralyzed and later invaded by the *Pleurotus* hyphae. This mode of nematode attack has been observed in *P. cornucopiae* (Paulet) Rolland, *P. cystidiosus* O.K. Miller, *P. levis* (Berk. & Curt.) Sing., *P. ostreatus* (Jacq. : Fr.) Kummer, *P. populinus* Hilber & Vilgalys (as *P. ostreatus* p.p. and *P. subareolatus* Peck), and *P. pulmonarius* (Fr. : Fr.) Quél. (as *P. ostreatus* p.p.) (Thorn and Barron, 1984; Thorn and Tsuneda, 1993; redeterminations on the basis of mating studies, cf. Vilgalys et al., 1993). In the course of screening isolates for nematode-trapping ability, several other lentinoid-pleurotoid species have been tested for which the results are reported here for the first time. *Pleurotus dryinus* (Pers. : Fr.) Kummer (M. Tucic no. 2, TRTC), *P. euosmus* (Berk.) Sacc. (CBS 307.29), and *P. eryngii* (D.C. : Fr.) Quél. (CBS 100.82) demonstrated nematode-trapping ability typical of *Pleurotus,* but *Lentinus suavissimus* Fr. [DAOM 172542, as *Panus suavissimus* (Fr.) Singer], *L. tigrinus* (Bull. : Fr.) Fr. (DAOM 3048), *Panus lecomtei* (Fr.) Corner (DAOM 21964, RGT 840910/02, as *P. rudis* Fr.), *Lentinula edodes* (Berk.) Pegler (DAOM 185574) and *Neolentinus lepideus* (Fr. : Fr.) Redhead & Ginns (DAOM 155904) were all negative. The present study was intended to demonstrate whether *Pleurotus tuberregium* produces nematode-trapping toxin droplets, which we suggest may be a synapomorphy and diagnostic character for *Pleurotus.*

The isolate of *P. tuberregium* used in this study (DSH 92-155) was collected in October, 1992, in lowland rainforest at Amron, Madang Province, Papua New Guinea. The collection consists of a single basidiocarp that arises from a sclerotium 8 cm in diameter. The basidiocarp and sclerotium were separated and dried for approximately 12 h.

In August, 1993, a portion of the sclerotium was excised and fragments were placed on MEA (1.25% malt extract, 2% agar) plates and incubated at room temperature. Clamped hyphae grew out from the sclerotial tissue within 2 days. MEA blocks with hyphal tips were transferred to WA (2% agar) plates and grown for 7–14 days, after which copious toxin droplet-pro-

ducing secretory processes were visible (FIGS. 1–3). These structures have been called secretory cells (Barron and Thorn, 1987), but we have avoided this term because we could not see septa at their bases. Secretory processes were borne on aerial hyphae and usually were pointed more or less straight up, so that from the top only the round outlines of the toxin droplets were visible (FIG. 1). Viewed from the side, the secretory processes were composed of a slender stalk, leading to a rounded head about 1 μm broad (FIGS. 2, 3).

Live nematodes (*Rhabditis* sp., Carolina Biological Supply Co., catalog no. L268) were transferred directly to the mycelial mat or to the periphery of the mat. Nematodes swam easily over hyphae at the very periphery of the colony, where there were no toxin droplets. However, when nematodes struck toxin droplets on the older parts of the mat they usually recoiled immediately. Smaller nematodes were affected most profoundly; they quickly became sluggish, and the sinuous, energetic swimming movements were replaced by slow wagging of the head back and forth, ineffectual flexing of the entire body, and eventual paralysis, with only occasional weak movements of the body. As reported previously with *P. ostreatus* (Barron and Thorn, 1987), the nematode's oesophagus became contorted and the shape of the head changed from elongate and gradually tapering to bluntly rounded after contact with toxin droplets (FIGS. 4, 5). Larger nematodes were not as susceptible to the toxin and some appeared to be completely unaffected. Nematodes that were paralyzed by the toxin droplets were colonized by hyphae after about 12 h.

The mode of nematode capture exhibited by *P. tuberregium* is essentially identical to that previously reported in other *Pleurotus* species (Thorn and Barron, 1984; Barron and Thorn, 1987), which suggests that this fungus is correctly classified in *Pleurotus.* There are also corroborating anatomical features. *Pleurotus tuberregium* has large, smooth, cylindric, hyaline spores, that are typical of *Pleurotus.* Many *Pleurotus* species are monomitic, but skeletal hyphae are found in *P. dryinus* and *P. levis.* Perhaps the most unusual feature of *P. tuberregium* is the sclerotium from which fruiting bodies are produced. No other species presently classified in *Pleurotus* produces such a structure. However, sclerotia are produced by some species of *Lentinus s. lat.,* including *Lentinus dactyloides* Clel., which is an Australian fungus that is dimitic with skeletal hyphae and that fruits from a subterranean, digitate sclerotium (Cleland, 1935; Pegler, 1983). *Lentinus dactyloides* has been shown to be closely related to *Pleurotus eryngii* in cladistic analyses of ribosomal RNA sequence data (Hibbett and Vilgalys, 1993). Taken together, the molecular and morphological characters suggest that *L. dactyloides* may be properly classified in *Pleurotus.* Cul-
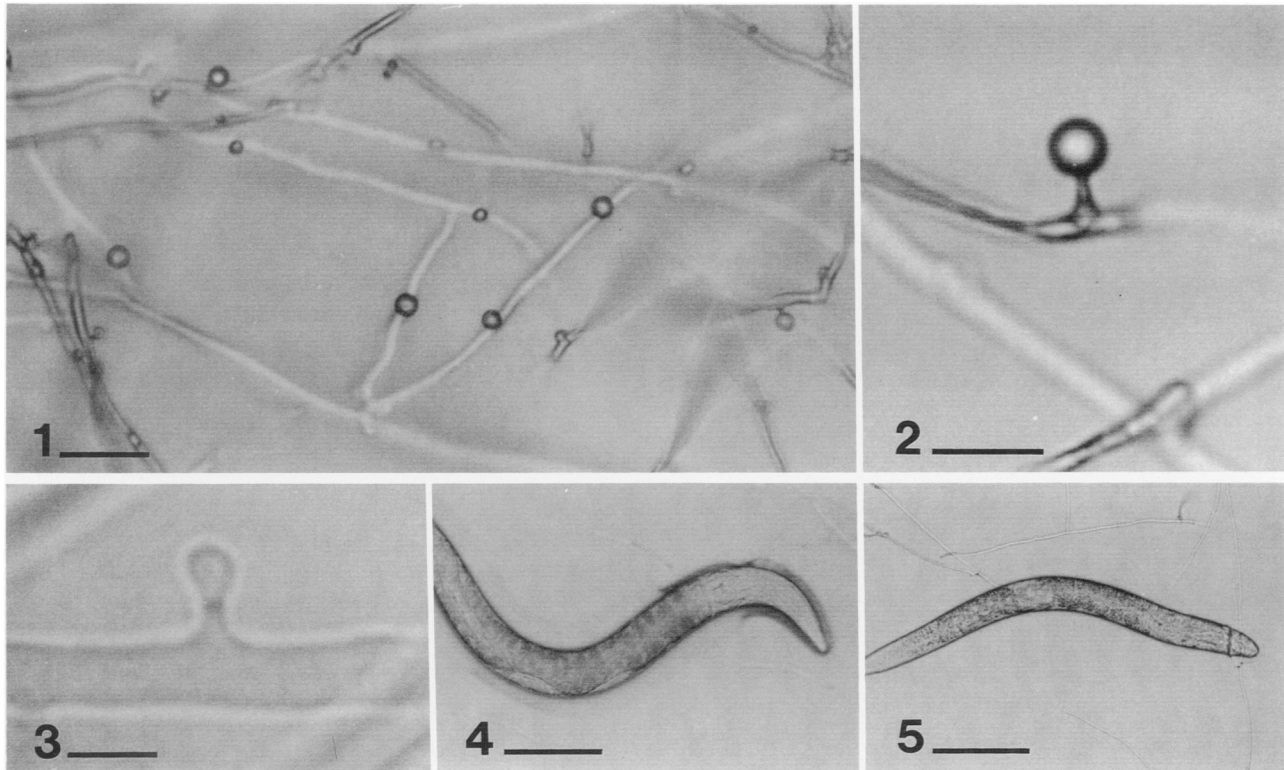
FIGS. 1–5. Nematode-trapping structures of *Pleurotus tuberregium* and nematodes on agar cultures of *P. tuberregium*. 1. Toxin droplets on aerial hyphae, viewed without coverslip. 2. Side view of toxin droplet on stalked appendage, viewed without coverslip. 3. Secretory process viewed in water mount under coverslip. The extracellular toxin droplet has been disrupted or dissolved. 4. Healthy, swimming nematode. 5. Nematode that has been paralyzed by contact with toxin droplet. Note that the head of the nematode has become bluntly rounded as compared to the head of the unaffected nematode in FIG. 4. Scale bars = 20 μm in FIG. 1; 10 μm in FIG. 2; 2 μm in FIG. 3; 50 μm in FIGS. 4 and 5.

tures of *L. dactyloides* have not been tested for nematode-trapping ability.

Both Corner (1981) and Pegler (1983) have suggested that *P. tuberregium* is part of a group of closely related species with an Australasian-African distribution. Species that are putatively closely related to *P. tuberregium* include *Lentinus connatus* Berk., *L. anthocephalus* (Lév.) Pegler, and *L. fusipes* Cooke & Massee, all of which are in *Lentinus* sect. *Tuberregium* (Singer) Pegler (Pegler, 1983). Another species of *Lentinus s. lat.* that may in fact be a *Pleurotus* is *L. giganteus* Berk. which occurs in tropical Asia and Australia (Corner, 1981; Pegler, 1983). *Lentinus giganteus* fruits on the ground and has a deeply radicating stipe that originates from buried wood (Corner, 1981; Pegler, 1983). Similarities of *L. giganteus* to *Pleurotus*, including marcescent fruiting bodies and the presence of capitate, lecythiform cystidia, were discussed by Corner (1981) and Pegler (1983). Future studies aimed at understanding the monophyletic limits of *Pleurotus* should consider these species as well as *L. dactyloides*, and should test their cultures for nematode-trapping ability.

Our final note concerns formation of the sclerotium in *P. tuberregium*. Corner (1981) and Pegler (1983) stated that the sclerotia of *P. tuberregium* develop above ground in logs, then fall to the ground and become covered with soil. Our observations of *P. tuberregium*, which was abundant at Amron, do not support this view. The sclerotium that we collected was buried approximately 10 cm underground. All of the fruiting bodies that we saw grew out of densely packed soil with a thin litter layer and no evidence of disturbance. It is most probable that the sclerotia were formed underground.

and GLB, and G. L. Barron, Dennis Desjardin and an anonymous reviewer for their comments.

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