atic fungi but also some species of litter decay ngi. *Zelopelta* may well come within this category although the fungus was found on living leaves, *dera nepalensis* does occur in moist forests.

Zelopelta is mainly distinguished from other nera of the Pycnothyriales by its conidial rphology. The tri-radiate hyaline conidium, ough known in all other groups of the deutero-ycetes, has not yet been reported for the Pycno-hyriales. Indeed the only genus of this group proaching *Zelopelta* is *Characonidia* Batista & valcanti (1965) where the filiform septate nidiospores rather than being branched, anasto-ose midway along their length. There are several her genera of coelomycetes with pycnidial or omatic conidiomata in which hyaline radiate nidia have been described (Sutton, 1980), but ly in *Tetranacrium* Hudson & Sutton (1964), *laina* Batista & Peres (1961) and *Eriosporella* öhnel (1916) are the conidia of comparable rphology. The conidiomata in these genera are te different, being superficial and eustromatic h a distinct longitudinal line of dehiscence in *tranacrium*, superficial and cupulate in *Belaina*, d immersed, unilocular and eustromatic in *riosporella*. They also differ in conidiogenesis: *riosporella* is phialidic, *Tetranacrium* is holoblastic, d *Belaina* is holoblastic with sympodial regener-ion of the conidiogenous cell.

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DETECTION OF *GANODERMA LUCIDUM* IN BETELNUT BY THE FLUORESCENT ANTIBODY TECHNIQUE

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The presence of *Ganoderma lucidum* in roots of betelnut is detectable by the induction of fluorescent antibodies. This provides an interesting practical application of the technique.

aabe or foot rot of betelnut (*Areca catechu* L.) used by *Ganoderma lucidum* (Leys) Karst., is a rious disease in India (Koti Reddy, Kumar, raswathy & Roy, 1978) and is widespread in ill-ained, over-crowded and neglected plantations. sible symptoms appear five to six months after fection and such palms subsequently die; thus ere is a need to detect the disease during the cipient phases of infection to combat it more fectively. As the routine isolation of pathogen from roots is a tedious process, the use of the fluorescent antibody technique was investigated.

The pathogen was grown in Waksman's liquid medium for 30 days. 600 mg of dried mycelium was ground in physiological saline, Seitz filtered and the protein content estimated by the method of Lowry, Rosenbrough, Farr & Randall (1951). Antigen preparation containing 600 μg protein/ml was injected intravenously in the marginal ear veins of Albino male rabbits (1·0–1·2 kg wt) for 11 days. An immunization schedule starting with 0·25 ml, increasing by 0·25 ml/day to 2·0 ml (maximum) was used. On the 9th and 10th day, the antigen was mixed with an equal volume of Freud's complete adjuvant (Difco) prior to the injections. Seven days

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Table 1. *Antibody production by* Ganoderma lucidum *and its fluorescent reaction with different antigenic sources*

Source of antigen	Antibody titre with antiserum*									Fluorescence† with labelled antiserum*
	1:1	1:100	1:200	1:400	1:800	1:1600	1:3200	1:6400	1:12800	
Ganoderma lucidum (betelnut)	++++	++++	++++	+++	+++	++	++	+	–	++++
Ganoderma lucidum (coconut)	–	–	–	–	–	–	–	–	–	–
Trichoderma viride	–	–	–	–	–	–	–	–	–	–
Roots of anabe-affected betelnut palms	Not applicable									++++
Roots of apparently healthy betelnut palms	Not applicable									– to ++++
Roots of healthy betelnut palms	Not applicable									–

* Antiserum prepared against *Ganoderma lucidum* isolate of betelnut palm.
†–, No reaction; +, low; ++, moderate; +++, high and ++++, intense.

after the last injection, animals were bled and antisera obtained.

The antibody titre was determined by mixing 1 ml proportions of antigen with equal volumes of diluted antiserum, incubated at 37 °C for 24 h. The γ-globulins were separated from the antiserum by fractionation with ammonium sulphate and conjugated with Fluorescein isothiocyanate (Spendlove, 1967).

G. lucidum isolates from betelnut and coconut and also *Trichoderma viride* (a secondary invader in betelnut roots), were grown on glass slides as reported by Preece & Cooper (1969) and stained with the conjugated antiserum by the method of Nairn (1964). Thin hand sections of roots were made from the diseased, apparently healthy (those surrounding the diseased palms) and healthy betelnut palms and stained with the labelled reagent as detailed above. Prepared slides were mounted in buffered glycerol (Price, 1970) and examined by fluorescent microscopy, fluorescence being assessed from nil (−) to brilliant (++++) according to Heimer (1967).

Results showed that pre-immunization sera did not evince visible precipitate with the antigenic preparations. But the antisera developed from the *G. lucidum* isolate of betelnut exhibited a distinct precipitin reaction with its antigen. The antibody titre reached 1:6400 in eleven days. No such precipitin reaction could be detected when the above antiserum was mixed with the protein fraction of the *G. lucidum* isolate from coconut palm, or that of *T. viride*. The antiserum obtained thus appeared to be specific to the *G. lucidum* isolate of betelnut in this study, as observed with other organisms by Burrell, Clayton, Gallegly & Lilly (1966) and Paynter & Alconero (1979).

Stained preparations of the *G. lucidum* isolate of betelnut fluoresced brilliantly (++++) on glass slides. No such fluorescence (−) was observed with the other fungi studied (Table 1). Unstained mycelia of the latter, and those treated with conjugated normal serum (control) showed slight fluorescence, which can be distinguished from the brilliant yellowish green fluorescence of the betelnut pathogen treated with the labelled antiserum. Similar fluorescence was observed with other fungi (Preece & Cooper, 1969; Price, 1970).

Root sections of all the anabe affected palms and 5% of apparently healthy betelnut palms showed mycelium which emitted fluorescence (Table 1). Thus, some palms which appear healthy externally had their roots colonized by the pathogen, threatening their survival. Many other organisms have been reported to be easily detectable in the host by labelled antibodies (Coons, Creech, Jones & Berliner, 1942; Nagaraj & Black, 1961; Kumar & Patton, 1964; Auger & Shalla, 1975). The technique may therefore be useful for screening large numbers of root samples of betelnut palms for the presence of the anabe pathogen prior to the onset of visible symptoms. Efforts are also being made to determine post-infection substances in host plants.

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