SEROLOGICAL TEST FOR THE DIAGNOSIS OF *GANODERMA LUCIDUM*

T. V. ANANTHANARAYANAN*[a] and
M. KOTI REDDY[b]
[a] *Biochemistry Section,* [b] *Plant Pathology Section,*
Central Plantation Crops Research Institute
Regional Station, Vittal 574 243, India.

**Present address: Indian Institute of Horticultural Research (ICAR) Bangalore 560 080, India.*

SEROLOGICAL methods as a tool for differentiating species or intraspecific strains of fungi parasitising man or animals have been commonly employed, but the reports of their application to plant pathogenic fungi are scanty. A number of reports have emanated in the past elucidating the serological relationship between viruses[1-3] as also against different strains of micro-organisms[4,5]. With the advances in sero-diagnostic techniques, their application to plant pathogenic fungi has been found to be useful[6-10]

In perennial plants like arecanut and coconut, early detection of the disease at the incipient stage assumes overwhelming importance, in view of the long incubation (gestation) period of the fungal pathogen like *Ganoderma lucidum*, before visible symptoms develop on the host plant. In India, a number of hosts are infected by *G. lucidum*[11]; however, no information is available on the variability of the pathogen. Hence, an attempt is made to raise antibodies against *G. lucidum*, infecting the roots of arecanut palms and causing the 'Anabe' disease so as to evolve a suitable early diagnostic technique.

Two cultures of *G. lucidum* used in the study (one was isolated from infected arecanut gardens around Hirehalli, Karnataka, India, and the other was obtained from the Forest Research Institute, Dehradun, India) were grown for 30 days in Waksman's liquid medium, in still conditions, containing 0.025% each of biotin and thiamine at room temperature (30° ± 2°C). The mycelium was filtered through Buchner funnel, dried in folds of filter paper at 40°C in a hot air oven. Dry mycelium (600 mg) was ground in physiological saline for 15–20 min with chilled acid-washed sand and filtered through Seitz filter. The filtrate was made upto 20 ml and stored in the refrigerator at 4°C.

The protein content of the culture filtrate was estimated by Lowry's method[12] using Folin's reagent. A preparation containing 600 μg protein/ml was used for the immunisation of rabbits as described below:

Albino rabbits weighing 1–1.25 kg were used for raising the antiserum against *G. lucidum.* Intravenous injections were given daily to the rabbits (through the marginal ear vein), increasing the dosage gradually. Two ml of the antigen were injected on the 8th, 9th, 10th and 11th days respectively. The antigen was emulsified with an equal volume of Freund's complete adjuvant (Difco) on the 9th and 10th day and administered intravenously. The animals were rested for 7 days. Bleeding was done for 4 days by puncturing the marginal ear vein and 10 ml of the blood was collected. The serum was obtained by centrifugation at 5,000 g and the microprecipitin test was carried out as elucidated below:

Serial dilutions of the antiserum were made in the range 1:100 to 1:25 600. One ml of the antigen was added to an equal volume of the diluted antiserum in test tubes. The tubes were shaken and incubated at 37°C in a water-bath and the precipitation recorded after 24 hr.

The pre-immunisation sera showed no visible precipitate when treated with the corresponding antigen. As compared to this, the antisera obtained after the completion of immunisation schedule showed visible signs of the antigen-anti-body precipitin reaction when treated with the corresponding antigen. The results presented in table 1 show that the immune response is

Table 1 *Precipitin reaction profile of G. Lucidum antigen*

Area	Total protein administered (mg)	Intensity of precipitation* after *24hr incubation with antiserum* Dilution of antiserum									
		1/50	1/100	1/200	1/400	1/800	1/1600	1/3200	1/6400	1/12800	1/25600
Dehradun	13.20	d	d	d	c	c	c	b	b	a	a
Hirehalli	12.00	d	d	d	c	c	b	b	a	e	e

*: No reaction; a: low; b: moderate; c: high; d: intense; e: Undiluted antigen was employed.

highly sensitive to the presence of the fungal antigen. Since an axenic culture of the fungus was used for the experiment, the immune response was caused due to the fungus alone, and not due to any other component. The specificity of the reaction is evident from these observations.

Results were obtained on the basis of three different sets of experiments.

The results clearly showed that the antibody titre for the Dehradun and Hirehalli cultures were 1:12800 and 1:6400 respectively. These are fairly high values and antisera of high titre can therefore be obtained in a short period, hence, the methodology would be of immense benefit in raising antisera for early detection of the disease perhaps using the fluorescent antibody technique.

The authors express their grateful thanks to Mr. S. N. Sampath Kumar of CPCRI, Research Centre, Hirehalli for providing the Hirehalli cultures used in this investigation. Thanks are also due to Scientist-in-charge, CPCRI, Regional Station, Vittal, for providing facilities.

31 December 1982; Revised 19 September 1983

1. Gingery, R. E., *Phytopathology*, 1978, **68**, 1526.
2. Mink, G. I., Lizuka, N. and Kiriyama, K., *Phytopathology*, 1975, **65**, 65.
3. Anand, G. P. S. and Sahambi, H. S., *Indian Phytopathol.*, 1965, **18**, 204.
4. Sarma, C. R. and Sen, A. N., *Curr. Sci.*, 1979, **48**, 173.
5. Coons, A. H., Creech, H. J., Jones, R. N. and Berliner, J., *Immunology*, 1942, **45**, 159.
6. Sayed, A. K. M. and Sen Gupta, P. K., *Trans. Br. Mycol. Soc.*, 1969, **53**, 99.
7. Preece, T. F. and Cooper, D. J., *Trans. Br. Mycol. Soc.*, 1969, **53**, 99.
8. Amos, R. E. and Burrell, R. G., *Phytopathology*, 1967, **7**, 32.
9. Burrell, R. G., Clayton, C. W., Gallegly, M. E. and Lilly, V. G. *Phytopathology*, 1966, **56**, 422.
10. Price, T. V., *Trans. Br. Mycol. Soc.*, 1970, **55**, 327.
11. Bakshi, B. K. *Indian Polyporaceae (on trees and timber)*, ICAR, New Delhi, 1971, 246.
12. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, **193**, 265.

DISPERSION OF MALE AND FEMALE PLANTS OF *DIOECIOUS EUPHORBIACEAE* ALONG A MOISTURE GRADIENT

N. C. NAIR and V. S. RAJU
Botanical Survey of India, Southern Circle, Coimbatore 641 003, India.

THE spatial distribution of male and female plants of dioecious species within a population is likely to influence the foraging behaviour of pollinators and consequently the level of pollination[1]. Spatial segregation of male and female plants due to ecological differentiation was observed in the case of *Chamaelirium luteum* (L.) Gray[2]. Moreover, studies of sex ratios in tropical trees contribute significantly towards an understanding of deviant sex ratios in flowering plants[3]. The question is whether staminate and pistillate plants show any segregation or not along an ecological gradient since a particular sex is capable of exploiting that ecological niche better than its counterpart. Testing by either the nearest-neighbour method or by examining the distribution of males and females along with an environmental gradient would seem to give an answer[1]. The latter method is employed here and the gradient considered is soil moisture.

Three dioecious species and longlived perennials of Euphorbiaceae occurring on the banks of streams were chosen from the wet evergreen forests of Idukki, Kerala. The species studied were *Agrostistachys meeboldii* Pax & Hoffm., *Antidesma menasu* (Tul.) Miq. ex Muell.-Arg. and *Aporusa acuminata* Thw. in 'islands' of natural forests amongst grasslands, savannas and partially or highly disturbed moist deciduous forests. The areas examined were confined to the forests associated with the river Meenmutti and its adjacent streams. The study was conducted when these species were in flower and the plants of both sexes were easily identifiable.

It was found that while the number of plants of *Antidesma menasu* gradually decreased from the river banks spreading upto 100 m, the trend was reverse and the plants dispersed only upto 30 m in the case of *Aporusa acuminata*. Neither of these trends was exhibited by *Agrostistachys meeboldii*. This may be due to its narrow range of extension from the river edge (it was found occurring either in the islands of the streams or confined to their banks, *i.e.* upto 6 m only—figure 1). However, in all the three cases, no segregation of male and female plants was evident along a moisture gradient (figure 1). In other words, the probability of a