

- SMIRNOV, A. I. (1955). The effect of mechanical agitation at different periods of development on eggs of autumn chum salmon. (*Oncorhynchus keta* Walb.). *Doklady Akademii Nauk SSSR* **105**, 873-876.
- SMITH, S. N., ARMSTRONG, R. A. & RIMMER, J. J. (1984). Influence of environmental factors on zoospores of *Saprolegnia diclina*. *Transactions of the British Mycological Society* **82**, 413-421.
- SNEDECOR, G. W. & COCHRAN, W. G. (1967). *Statistical Methods*. 6th edn. Ames: Iowa State University Press.
- TAUSSKY, H. H. & SHORR, E. (1953). A micro-colorimetric method for the determination of inorganic phosphorus. *Journal of Biological Chemistry* **202**, 675-685.
- WILLOUGHBY, L. G. (1978). Saprolegnias of salmonid fish in Windermere: a critical analysis. *Journal of Fish Diseases* **1**, 51-67.
- WILLOUGHBY, L. G. & PICKERING, A. D. (1977). Viable Saprolegniaceae spores on the epidermis of the salmonid fish *Salmo trutta* and *Salvelinus alpinus*. *Transactions of the British Mycological Society* **68**, 91-95.
- WILLOUGHBY, L. G., MCGRORY, C. B. & PICKERING, A. D. (1983). Zoospore germination of *Saprolegnia* pathogenic to fish. *Transactions of the British Mycological Society* **80**, 421-435.
- WINNICKI, A., BARTEL, R. & GORYCZKO, K. (1968). The effect of temperature on the uptake of water by the egg and on hardening of the egg membranes in the rainbow trout *Salmo gairdneri*. *Zoologica Polonica* **18**, 431-438.
- WOLKE, R. E. (1975). Pathology of bacteria and fungal disease affecting fish. In *The Pathology of Fishes* (ed. W. E. Ribelin & G. Migaki), pp. 33-116. Wisconsin: University of Wisconsin Press.
- YEMM, E. L. & COCKING, E. C. (1955). Determination of amino acids with ninhydrin. *Analyst* **80**, 209-213.

OCCURRENCE OF TWO MATING GROUPS IN *PHYTOPHTHORA MEADII* CAUSING ABNORMAL LEAF FALL DISEASE OF RUBBER IN SOUTH INDIA

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Heterothallism has been demonstrated in Indian isolates of *Phytophthora meadii* from rubber. Thirty-two isolates were mating type A₁, 24 were A₂, 2 were homothallic and 4 sterile.

Abnormal leaf fall caused by *Phytophthora* spp. is a very serious disease of rubber in India, and four taxa are reported as causing the disease. In South India the pathogen was identified as *Phytophthora meadii* by McRae (1919). Later, Petch suggested that two species of *Phytophthora*, *P. faberi* (synonymous with *P. palmivora*) and *P. meadii*, were involved (Petch, 1921), and he was of the opinion that the damage caused by *P. meadii* was greater than that caused by *P. faberi*. Studies carried out at the Rubber Research Institute of India also showed that both *P. palmivora* and *P. meadii* play an important role in causing the disease (Ramakrishnan & Radhakrishna Pillai, 1961; Thankamma, George & George, 1968). Subsequently *Phytophthora nicotianae* var. *parasitica* and *P. botryosa* were reported on rubber in India (Thomson & George, 1976, 1980). Present studies indicate that *P. meadii* is the dominant species mainly responsible for abnormal leaf fall in South India.

Of the four taxa mentioned, *P. palmivora*, *P. botryosa* and *P. nicotianae* var. *parasitica* are heterothallic (Ashby, 1922; Gadd, 1924; Turner, 1960; Zentmyer *et al.*, 1973; Suzuii, Kueprakone & Kamhangrid-Thirong, 1978; Shepherd, 1979) while *P. meadii* is considered to be homothallic (Waterhouse, 1974). The frequent failure to obtain

oospores in single sporangial cultures of many isolates of *P. meadii* led to the present study of their mating behaviour.

Phytophthora isolates for the study were made from petiole lesions of rubber leaves infected by abnormal leaf fall disease. Sixty-two isolates were used: 50 'regional' isolates, numbered R1 to R50, made from various parts of South India, and 12 'local' isolates, numbered L1 to L12, collected from different parts of the experimental station of the Rubber Research Institute of India. Single sporangial cultures of the above isolates were made and maintained on potato dextrose agar slants. 'Isolate 4' and 'isolate 7' from the Institute's culture collection produced oospores in abundance when inter-crossed; they were used as the standards for the study. Isolate 4 was sent to CMI, England, and identified as *P. meadii* (A₂ mating group, IMI 269757). Isolate 7 belonged to the A₁ group. All the 62 *Phytophthora* isolates included in the study were mated with both isolates 4 and 7 and examined for their mating behaviour. The pairing studies were made in Petri dishes of carrot extract agar medium. Each isolate was first inoculated singly on the medium and examined for oospore production. If oospores were not formed in single cultures after 10 days, the isolate was studied in paired culture. Inocula of the isolate under test and

Table 1. *Mating behaviour and oospore diameter of 'regional' isolates of P. meadii*

Isolate	Mating group	Oospore diam (μm)	
		Average	Range
R1	A ₁	34.48	24.0-50.0
R2	A ₁	35.50	22.0-50.0
R3	A ₁	34.16	22.0-48.0
R4	A ₁	38.58	30.0-50.0
R5	A ₂	39.22	26.0-50.0
R6	A ₁	34.56	28.0-44.0
R7	A ₂	32.96	22.0-44.0
R8	A ₂	33.22	26.0-44.0
R9	A ₁	38.86	26.0-50.0
R10	A ₂	36.00	26.0-42.0
R11	A ₁	32.50	26.0-40.0
R12	A ₁	44.70	34.0-62.0
R13	A ₁	34.22	26.0-40.0
R14	A ₂	33.06	26.0-40.0
R15	A ₂	35.20	28.0-40.0
R16	A ₁	36.74	28.0-44.0
R17	A ₁	34.64	28.0-40.0
R18	A ₁	31.72	28.0-38.0
R19	A ₁	36.82	28.0-52.0
R20	A ₁	37.06	26.0-46.0
R21	A ₁	40.88	30.0-60.0
R22	A ₁	32.66	24.0-42.0
R23	A ₁	37.12	28.0-64.0
R24	A ₁	36.54	26.0-46.0
R25	A ₂	34.84	28.0-44.0
R26	A ₁	28.74	24.0-36.0
R27	A ₂	36.44	28.0-46.0
R28	A ₁	30.52	24.0-46.0
R29	A ₂	37.24	30.0-50.0
R30	A ₁	41.50	28.0-44.0
R31	A ₁	36.92	28.0-44.0
*R32		36.22	22.0-44.0
*R33		35.14	30.0-40.0
R34	A ₂	34.46	24.0-40.0
R35	A ₁	34.00	28.0-40.0
R36	A ₂	33.78	26.0-42.0
R37	A ₂	35.90	24.0-48.0
R38	A ₁	33.70	24.0-44.0
R39	A ₁	39.90	30.0-44.0
R40	A ₁	33.64	26.0-42.0
R41	A ₁	32.62	26.0-42.0
†R42			
†R43			
R44	A ₂	39.94	30.0-52.0
†R45			
‡R46	A ₁		
R47	A ₁	32.36	26.0-44.0
‡R48	A ₁		
‡R49	A ₁		
‡R50	A ₂		

Fifty oospores of each isolate were measured. The pooled variance within isolates is 25.31.

* Oospores formed in single culture. † Oospores not formed. ‡ Oospore measurement not recorded.

of isolate 4 or isolate 7 were placed 3 cm apart on the medium. The culture plates, where dual inoculations were made, were examined on the sixth day after inoculation for their mating behaviour and oospore production. Based on oospore formation with either isolate 4 or isolate 7, the cultures were classified into A_1 or A_2 mating groups. Mating behaviour of the isolates of each group was also studied by selecting sets of two isolates from each group at random and crossing them. Ten sets of such matings were done from each group. Wherever oospore formation was not noticed the plates were examined again after ten days to confirm the absence of oospores. The size of the oospores was also recorded by measuring the diameter using a Visopan Lanameter. Oospore measurements were not recorded in the case of 4 regional isolates and 12 local isolates.

The mating behaviour of the isolates was quite characteristic. Isolates which formed oospores when mated with isolate 4 did not form oospores when mated with isolate 7 and vice versa. In the case of complementary strains there was an attraction between the isolates. When isolates belonging to the same mating type were paired there was repulsion between the isolates as they grew and approached each other. Of the 50 regional isolates studied, 31 formed oospores with isolate 4 and belonged to A_1 mating group, and 14 formed oospores with isolate 7 and belonged to the A_2 mating group. Two isolates were homothallic, producing oospores in single culture, and 3 isolates did not form oospores either in single culture or when mated with any of the standard types (Table 1). Among the 12 'local' isolates, 10 were A_2 and one (L 11) A_1 . One isolate (L 8) did not form oospores either in single culture or on pairing with the standard isolates. Isolates belonging to the same group did not form oospores among themselves.

Heterothallism in *Phytophthora* isolates from rubber was first observed by Gadd (1924) (cf. Zentmyer *et al.*, 1973). Two compatibility groups were distinguished among isolates of *P. palmivora* in Sri Lanka (Sachuthananthavale, 1963) but these isolates were later reported to include cultures of *P. meadii* (Peries & Dantanarayana, 1965). Sexual compatibility studies have been carried out in India between *P. meadii* and *P. palmivora* (Thankamma & Radhakrishna Pillai, 1970). In the present study it has been observed that *P. meadii* includes isolates which are homothallic as well as heterothallic, the latter being more common. Among the 'regional' isolates A_1 mating group was observed to be predominant, while among the 'local' isolates A_2 was predominant. The presence of two mating groups in *P. meadii* in South India and their

presence in the same locality provide ample chances for hybridization in nature and formation of new strains. This possibility is further supported by observations on oospore formation in nature on infected plant parts of the rubber tree and their germination (George & Thomson, 1975). Even interspecific hybridization and germination of oospores thus formed have been noticed among *Phytophthora* isolates affecting rubber (Thankamma, 1969; Thankamma & Radhakrishna Pillai, 1970). The variation in the size of oospores formed in matings of different combinations (Table 1) also suggests that the mating groups play a role in the variation observed within the species of *Phytophthora*.

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REFERENCES

- ASHBY, S. F. (1922). Oospores in cultures of *Phytophthora faberi*. *Bulletin of Miscellaneous Information, Kew* **9**, 257-262.
- GADD, C. H. (1924). *Phytophthora faberi* Maubl. *Annals of Royal Botanical Gardens, Peradeniya* **9**, 47-89.
- GEORGE, M. K. & THOMSON, T. E. (1975). Over-summering of *Phytophthora* causing abnormal leaf fall disease of rubber. *Rubber Board Bulletin* **12**, 112-114.
- MCRAE, W. (1919). A disease of the Para Rubber tree caused by *Phytophthora meadii* McR. *Agricultural Journal of India* **14**, 566-577.
- PERIES, O. S. & DANTANARAYANA, D. M. (1965). Compatibility and variation in *Phytophthora* cultures isolated from *Hevea brasiliensis* in Ceylon. *Transactions of the British Mycological Society* **48**, 631-637.
- PETCH, T. (1921). *Diseases and Pests of the Rubber Tree*. London: Macmillan.
- RAMAKRISHNAN, T. S. & RADHAKRISHNAN PILLAI, P. N. (1961). Abnormal leaf fall of rubber (*Hevea brasiliensis*) caused by *Phytophthora palmivora* (Butl.) Butl. in South India. I. *Rubber Board Bulletin* **5**, 11-20.
- SACHUTHANANTHAVAL, V. (1963). Complementary strains of *Phytophthora palmivora* from Ceylon rubber. *Phytopathology* **53**, 729.
- SHEPHERD, C. J. (1979). Mating behaviour of Australian isolates of *Phytophthora* species. I. Inter and intra specific mating. *Australian Journal of Botany* **26**, 123-138.
- SUZUI, T., KUEPRAKONE, M. & KAMHANGRID-THIRONG, T. (1978). Mating types of *Phytophthora palmivora*, *Phytophthora nicotianae* var. *parasitica* and *Phyto-*

- Phytophthora botryosa* in Thailand. *Transactions of the Mycological Society of Japan* **19**, 261-267.
- THANKAMMA, L., GEORGE, M. K. & GEORGE, K. V. (1968). Occurrence of two species of *Phytophthora* on *Hevea brasiliensis* in India. *Rubber Board Bulletin* **10**, 33.
- THANKAMMA, L. (1969). Germination of oospores formed by interspecific mating of *Phytophthora palmivora* (Butl.) Butl. and *P. meadii* McRae causing abnormal leaf fall disease of rubber in India. *Rubber Board Bulletin* **10**, 197-199.
- THANKAMMA, L. & RADHAKRISHNA PILLAI, P. N. (1970). Sexual compatibility of *Phytophthora* isolates causing diseases of *Hevea* in India. *Rubber Board Bulletin* **11**, 9-13.
- THOMSON, T. E. & GEORGE, M. K. (1976). *Phytophthora nicotianae* var. *parasitica* (Dastur) Waterhouse on *Hevea brasiliensis* in South India. *Rubber Board Bulletin* **13**, 1-2.
- THOMSON, T. E. & GEORGE, M. K. (1980). *Phytophthora botryosa* Chee causing abnormal leaf fall disease in South Andaman Island of India. *International Rubber Conference*, India, 1980.
- TURNER, P. D. (1960). Strains of *Phytophthora palmivora* (Butl.) Butl. from *Theobroma cacao* L. I. Isolates from West Africa. *Transactions of the British Mycological Society* **44**, 409-416.
- WATERHOUSE, G. M. (1974). In *Phytophthora Disease of Cacao* (ed. P. H. Gregory), pp. 71-79. London: Longman.
- ZENTMYER, G. A., MITCHELL, D. M., JEFFERSON, L., ROHEIM, J. & CARNES, D. (1973). Distribution of mating types of *Phytophthora palmivora*. *Phytopathology* **66**, 701-703.

OBSERVATIONS ON MARINE FUNGI IN SINGAPORE AND PENANG (MALAYSIA)

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Five marine higher fungi, *Aniptodera chesapeakensis*, *Clavariopsis bulbosa*, *Corollospora intermedia*, *Lignicola longirostris* and *Nia vibrissa* are reported from Singapore and Penang (Malaysia).

The mycogeographical distribution of marine fungi may be categorized into five littoral zones, namely arctic, temperate, subtropical, tropical and antarctic (Hughes, 1974). Data on geographical distribution, however, are scarce and incomplete, with most collections having been centred in Europe and North America (Kohlmeyer, 1983). Comparatively little information on the occurrence of marine fungi in the tropics is available and much less so around Singapore, an island situated 1° 20' north of the Equator between the Indian Ocean and the South China Sea.

In May 1984, intertidal sand samples were collected from the East Coast Park and Changi in Singapore and baited in the laboratory with pieces of sterilized balsa wood, feather and cotton cloth. Washed-up beach debris was also collected for incubation (Kohlmeyer & Kohlmeyer, 1979). Intertidal sand samples were also collected from Batu Ferringhi beach in Penang (about 774 km north of Singapore) and brought back to Singapore for baiting. Observations of the baits and incubated debris showed the presence of five species of marine fungi.

Aniptodera chesapeakensis Shearer & Miller. From Singapore (Changi), on twigs collected from a mangrove swamp. The immersed greyish brown

perithecia were detected by the white, tiny 'ribbons' of ascospores oozing from the ostioles. In the tropics, this fungus has been reported from Central America (Belize) and from Sri Lanka in the Indian Ocean (Kohlmeyer, 1984).

Clavariopsis bulbosa Anastasiou. Observed on incubated washed-up unidentified leaves on Singapore beach (East Coast Park). The teleomorph, *Corollospora pulchella*, has been observed in India (Kohlmeyer & Kohlmeyer, 1979) and Sri Lanka (Koch, 1982) in the Indian Ocean, the Philippines (Gacutan & Uyenco, 1983) and Hong Kong (Vrijmoed, Hodgekiss & Thrower, 1982) in the South China Sea, and in Thailand (Kohlmeyer, 1984).

Corollospora intermedia I. Schmidt. From Singapore (Changi) and Penang. The black ascocarps were abundantly formed on wood and cotton baits, on sand grains and incubated washed-up 'raffia' cords. Kohlmeyer & Kohlmeyer (1979) gave the Atlantic Ocean and the Baltic Sea as the distribution range of this species. Not much is known of its occurrence in this part of the tropics.

Lignicola longirostris (Cribb & Cribb) Kohlm. The brown long-necked ascocarps were abundant on incubated washed-up twigs collected from Singapore (Changi). This species has not been