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Sexual Compatibility of *Phytophthora* Isolates Causing Diseases of *Hevea* in India

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The fungus *Phytophthora* causes several diseases of *Hevea* in India. Among these, abnormal leaf fall, bark rot and canker are important. Two species, namely, *P. meadii* McRae and *P. palmivora* (Butl.) Butl. are reported to cause the abnormal leaf fall disease [7]. Sexual reproduction takes place in nature which is evidenced by the observation of oospores in the affected plant parts in the field [4, 5]. Homothallic and heterothallic species and interspecific sexual compatibility are also reported in *Phytophthora* spp [6]. Presence of two mating types [2] and the germination of oospores formed after the sexual phase were reported in *P. infestans* [1]. Interspecific crossing followed by germination of young oospores was recorded under laboratory conditions [8]. It has been reported that sexually compatible strains do occur within limited areas and even on the same tree [3]. Under these conditions it is possible that the sexual phase observed in nature and verified under the laboratory conditions may have some important consequence in the development, spread and severity of the diseases caused by the pathogen. Hence a study was made on the sexual compatibility behaviour of several isolates of the two species, *P. meadii* and *P. palmivora* causing various diseases of rubber.

Materials and Methods

Isolates for this study were obtained from infected leaf parts, shoot, fruit and bark of the rubber tree collected from different rubber growing tracts and maintained on potato dextrose agar medium. Eighty-nine isolates were used in the present study.

P. meadii is reported to be sexually compatible with *P. palmivora* [9]. It is also reported that *P. meadii* is compatible only with the rubber strain of *P. palmivora* and not with the cacao strain, which is considered an additional diagnostic character [3].

Isolates from rubber, serially numbered 2 and 36, provisionally identified as *P. palmivora* and *P. meadii* on the basis of morphological and sexual characteristics were paired with type cultures *P. palmivora* (cacao and rubber strains) obtained from and identified respectively at the Commonwealth Mycological Institute, Kew, Surrey, England. It was observed that isolate No. 36 produced plenty of oospores with *P. palmivora* rubber strains, whereas it showed repelling action with cacao strain. This compatibility behaviour together with flocculose type of aerial mycelium, abundant sporulation, production of oospores in single culture and absence of chlamydospores enabled us to identify culture No. 36 as *P. meadii*. Culture No. 2 which showed repelling action with *P. palmivora* rubber strain and observed to be sexually compatible with abundant oospore production when paired with a type culture of *P. meadii*, was identified as *P. palmivora*. Radiate type growth, sparse aerial mycelia, fewer number of large sporangia, absence of oospores in

single culture and presence of chlamydospores confirmed the identification of culture No. 2 as *P. palmivora*.

Based on this identification all isolates which were complementary to culture No. 2 (*P. palmivora*) were considered *P. meadii* and placed in Group I and those that were complementary to culture No. 36 (*P. meadii*) were considered *P. palmivora* and placed in Group II.

Pairing of isolates was carried out in leaf extract agar medium. The medium was prepared by mashing 200 gm of mature rubber leaves in 1000 ml distilled water, steaming for 30 minutes and filtering through muslin cloth. The filterate was made upto 1000 ml to which 20 gm of agar was added and autoclaved at 15 lb for 20 minutes.

Culture Nos. 2 and 36 were inoculated at opposite points near the periphery on leaf extract agar medium (15 ml poured into a 9 cm petridish). For inoculation 9 mm discs, removed from 7 day old culture plates with a sterile cork borer, were used. The isolates of unknown compatibility were inoculated in the centre of the petridish by adopting a similar procedure (Plate I). A sufficient number of replicates were also maintained and the pairing was repeated twice. All the inoculated plates were incubated at 22°C in a low temperature incubator. The lower surface of the plates was examined under a microscope for sexual compatibility, evidenced by production of oospores at the junction of the colonies in the case of compatible isolates, which normally took place after 2 days. In the case of incompatible isolates the colonies showed repelling action. By adopting this method the sexual compatibility behaviour of eightynine isolates was studied.

Results

Sexually compatible isolates grew rapidly and the colonies met quickly, and oospore formation was noticed after two to four days from inoculation (Plate II). In the case of incompatible strains a definite repelling behaviour, by mycelial growth becoming thinner and thinner towards each colony, was noticed (Plate III). Some complementary strains produced oospores quickly in abundance, whereas in others oospore production was slow and sparse. In the case of some isolates with strong mating reactions the meeting region was visible to the naked eye from the reddish brown colour developed due to the presence of large number ofmature oospores (PlateIV). During the period of observation sporangial production was not observed in any of the cultures. Similarly oospore production in single colonies was also not observed.

All the isolates tested showed compatibility either to one or other type culture and none showed compatibility to both. Among the eightynine isolates studied, thirtythree were observed to come under Group I (*P. meadii*) and fiftysix in Group II (*P. palmivora*).

Sexual Compatibility of 89 Phytophthora Isolates

Infected plant organ	Total No. of isolates	Group I Compatible to type culture 2	Group II. Compatible to type culture 36
Leaf	66	23	43
Shoot	8	4	4
Fruit	13	5	8
Bark (Canker)	1	—	1
Bark (Rot)	1	1	—
Total :	89	33	56

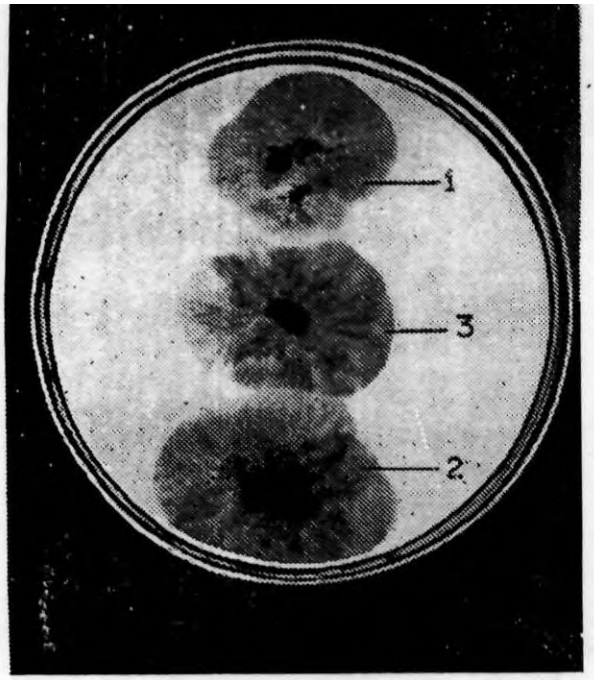

PLATE I Cultures 2 (1) & 36 (2) at the periphery and culture of unknown compatibility (3) in the centre.

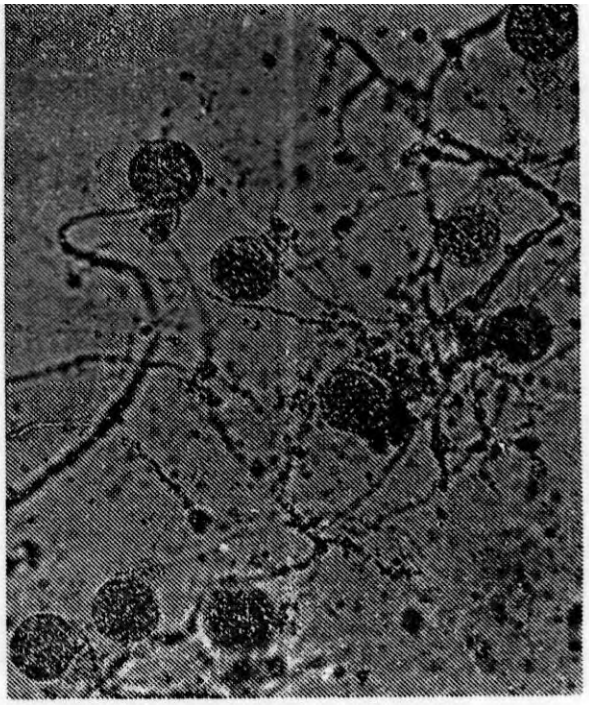

PLATE II Young oospores formed at the junction of compatible cultures.

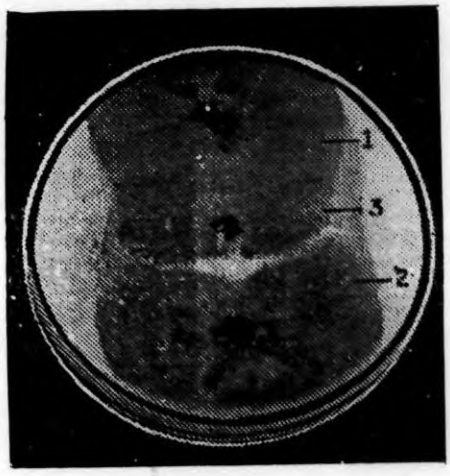

PLATE III Isolate of unknown compatibility (3)showing attraction to culture No. 2 (1) and repulsion to culture No. 36 (2).

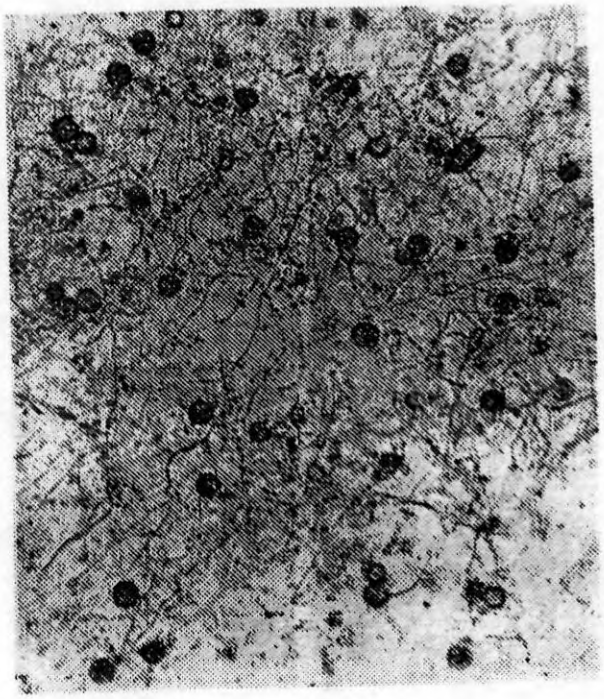

PLATE IV Mature oospores formed at the junction of compatible cultures.

This was further confirmed by the observation that 24 isolates in the Group I produced oospores in single cultures within a period of 3 weeks when grown in leaf extract agar at 22°C. But none of the isolates under Group II produced oospores under similar conditions.

Discussion

P. meadii and *P. palmivora* causing different diseases of rubber in India are sexually compatible. Under laboratory conditions these two are observed to produce oospores when paired on an artificial medium. It is observed that oospores are produced in the living host tissue when both species are artifically inoculated on the petioles of potted plants and incubated at 22°C under high humidity. Oospore formation was observed on shoot rot, bark rot and pod rot affected specimens in nature. Germination of young oospores produced after pairing of the two species under laboratory conditions was also reported. Hence it is reasonable to believe that sexual combination may be taking place in nature which could ultimately result in physiologic strain formation. It was revealed in the present study that the two species, *P. meadii* and *P. palmivora*, causing diseases of rubber are distributed approximately in 1 : 2 ratio in the different rubber growing tracts of this country.

Summary

Eightynine isolates of *Phytophthora* obtained from infected parts of the leaf, shoot, fruit and bark of the rubber tree, collected from different rubber growing tracts in India, were studied for their sexual compatibility behaviour. Among the eightynine isolates studied, thirty three belong to Group I (*P. meadii*) and fiftysix belong to Group II (*P. palmivora*). The distribution of the two species in the different rubber growing tracts is approximately in the ratio of 1 : 2. Sexual reproduction noticed in artificial pairing and oospore formation observed in living host tissues under laboratory conditions, observation of oospores in nature and reported germination of young oospores formed by interspecific mating of the two species under laboratory conditions, suggest the possibility of physiologic strain formation of the pathogen.

Acknowledgement

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