Research Communication

Germination of Oospores Formed by Inter Specific Mating of *P. palmivora* (Butl.) Butl. and *P. meadii* McRae Causing Abnormal Leaf Fall Disease of Rubber in India

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Many species of *Phytophthora* are observed to produce oospores either in nature or in culture or in both. But the role of this sexual spore in the life history of the fungus is not clear [5]. Although it is considered to be a sexual spore it has yet to be confirmed that meiosis and segregation of genetic characters are associated with this spore [5]. Oospore germination of several species of *Phytophthora* is reported [1, 2, 5, 6 & 7] but no record of germination of the oospore of *P. meadii* or those formed by pairing of two sexually compatible species, is available. The present investigation was taken up to study the sexual behaviour of two species, *P. meadii* and *P. palmivora*, both reported to be causing abnormal leaf fall disease of rubber in India [8], with special reference to the possible hybridization taking place in nature resulting in the formation of new strains.

Materials and Methods

P. meadii and *P. palmivora* isolated from infected petioles of rubber from Kottayam District, Kerala, India, were used in this investigation. From five day old PDA cultures 0.6 cm discs of both species were removed and inoculated at opposite points in the periphery of 9 cm petridish, containing 15 ml leaf extract agar medium. The inoculated plates were incubated at 22°C. The colonies grew and met and profuse oospore formation was noticed, 48 hours after meeting of the colonies.

Leaf extract agar medium which was found to be favourable for oospore production was prepared by mashing 200 grams of mature rubber leaves in a Waring Blender with 1000 ml distilled water, filtered and boiled for 30 minutes. The extract was again filtered through muslin cloth and made to 1000 ml with distilled water. The pH of the medium was 5. 6. Further 12 grams of agar were added and the medium autoclaved for 20 minutes.

Culture in one petridish with very young oospores formed 96 hours after meeting of colonies was homogenised with 50 ml distilled sterile water in a Waring Blender for five minutes. The suspension was filtered through two sheets of sterile muslin cloth to remove mycelial bits. The filtrate with plenty of oospores was plated in PDA

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using dilution plate method and incubated at 22°C. The plates were examined at regular intervals and oospore germination was observed after 16 hours.

Results

Germination of very young oospores occurred after 16 hours incubation in PDA and after 24 hours in sterile distilled water under room temperature. Under these conditions mature oospores with thick reddish or yellowish brown walls did not germinate. Thin walled young oospores germinated by producing one to eight germ tubes in a manner similar to direct germination of sporangia. In some cases the bases of germ tubes were slightly swollen. Germ tubes were seen arising from the sides of the antheridium (Plate I), stalk of the oogonium or from the oospore wall (Plate II). The germinated oospores were partly or completely empty, the contents having passed into germ tubes (Plate II). The germ tubes branched soon and colonies were established (Plates III and IV). Some of the oospores, incubated in sterile distilled water under room temperature, produced germ sporangia at the tips of germ tubes which liberated zoospores in due course. Single germinated oospores transferred to PDA plates and incubated, were observed to grow normally into colonies, filling up the petridish in 96 to 120 hours.

Pathogenicity Tests

Culture grown from a single germinated oospore incubated for 120 hours at 22°C in bean extract agar to induce production of sporangia, was blended with 50 ml sterile water for five minutes in a Waring Blender and the suspension of sporangia thus obtained was used for artificial inoculation. Petioles on cut twigs of *Hevea* clone PB 86, kept in Erlenmeyer flasks half filled with water, were used for inoculation. Ten drops of sporangial suspension were used for inoculating each petiole under the method described by Pillai and Chee [3]. Inoculated twigs were incubated at 22°C for 60 hours. Typical *Phytophthora* infection lesions were obtained after 60 hours, thereby indicating that cultures obtained from germination of oospores are pathogenic to rubber. *Phytophthora* was reisolated from the lesions by tissue culture. This also indicates the possibility of new strains of the pathogen developing in nature.

Discussion

In many previous reports [6 and 7] it is recorded that germination of oospores occurred only after oospores have aged for 6–9 months. In *P. capsici* germination of oospores was reported to occur after 30 days or more [5], where during germination the thickness of the oospore wall decreased until it was approximately the same as that of the original oogonial wall. But in the present study two to four days old oospores with very thin wall germinated. None of the thick walled oospores was found to germinate. Mode of germination observed in the present study is similar to previously reported cases. However, oogonial germination as described by Romero and Gallegly [4] and the decrease in thickness of the oospore wall prior to germination and the production of a germ tube which is a continuation of oogonial wall have not been observed. Germination of oospore by the production of a sessile sporangium as described by Mokhtar and Edward [5] was also not observed.

Summary

Very young 2–4 days old oospores of *Phytophthora* formed by pairing of *P. palmivora* and *P. meadii* both isolated from rubber, were observed to germinate after 16 hours in PDA when incubated at 22°C and after 24 hours in distilled sterile water under room temperature. The germinating oospores produced germ tubes from the sides of the antheridium, oogonial stalk or oospore wall. Germ sporangia were also produced at the tips of germ tubes. The

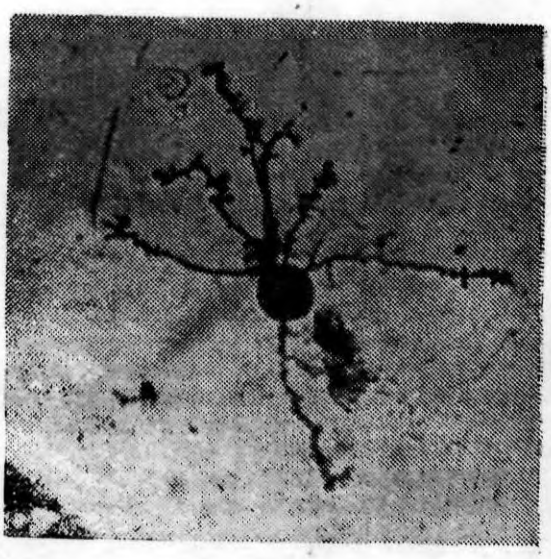

PLATE I

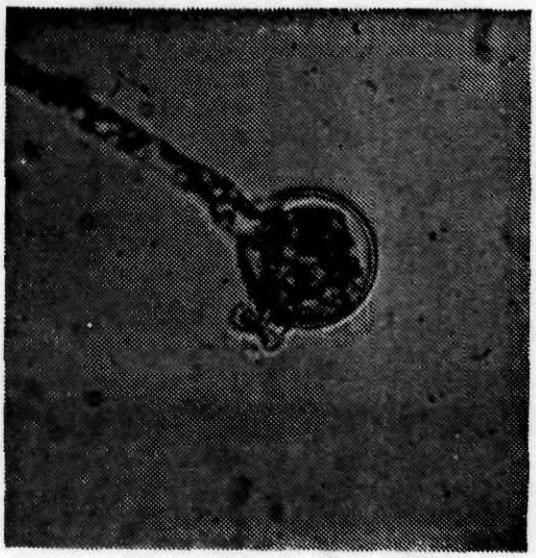

PLATE II

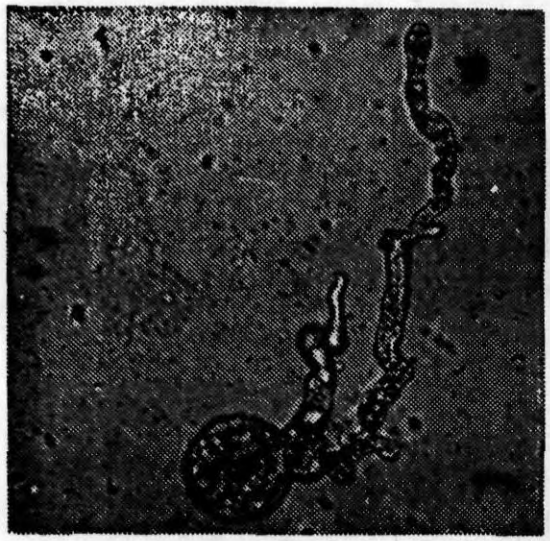

PLATE III

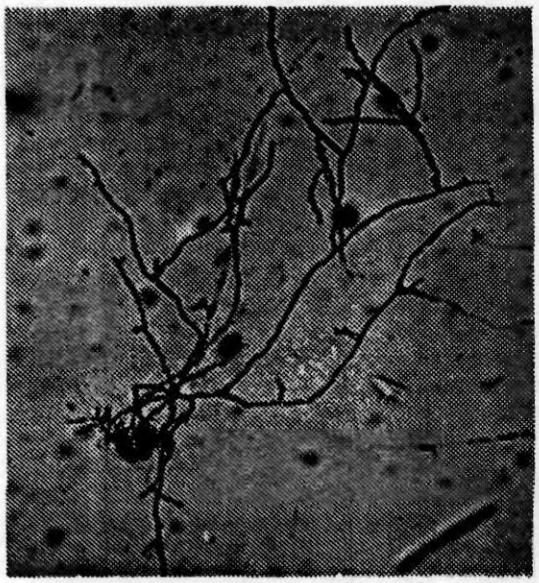

PLATE IV

cultures developed from single germinated oospores were found to be pathogenic to rubber.

Acknowledgment

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