Rubb Board bull. 12, 1; 1975; 7—10.

In Vivo Production of Oospores by Phytophthora palmivora and P. meadii

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Phythphthora palmivora and *P. meadii* have been reported to cause different diseases of rubber in India (6). The two species are distributed in an approximate ratio of 2 : 1 in the rubber growing tracts of India (7). They have been observed to occur frequently in the same locality. *P. meadii* is sexually compatible with *P. palmivora* (9). Under laboratory conditions compatible isolates of *P. palmivora* and *P. meadii* produce oospores at the meeting place readily and in abundance when grown together in artificial media at 22°C(7). Oospores have been observed in the infected host parts in nature (4 & 5). Ramakrishnan and Seethalakhmi (1956) have observed oospores in the pericarp of *Areca catechu* fruits (3). But other than these, reports on the occurrence of oospores in nature are lacking. So the sexual stage has very rarely been found in nature and the formation of oospores in the host under experimental conditions has been reported only in the case of *P. palmivora* on *Piper nigrum* leaves (1). Oospores produced in artificial media by pairing cultures *P. palmivora* and *P. meadii* have readily germinated in sterile water and agar media (8). But no report of oospores produced in vivo under experimental conditions by the two species and the resulting oospores germinating is available. Hence the role of the oospore as a resting stage and as a means of pathogenic variation has always been in doubt. The present study was undertaken with a view to determine whether *P palmivora* and *P. meadii* could produce oospores inside inoculated tissues of the rubber plant and the oospores thus produced are capable of germination.

Materials and Method

Phytophthora isolates No. 13 and 90 of the RRII culture collection were both isolated from infected rubber trees. Culture No. 13 is *P. palmivora* and 90 is *P meadii*. The cultures were grown in lima bean agar in petri dishes at 22°C. Eight day old cultures were used for inoculations.

Mature twigs of RRIM 701 collected from the budwood nursery were used for petiole inoculations. Inoculations were carried out in the method described by Pillai & Chee (2). Tender shoot bits of RRIM 701 and fruits of PB 86 were collected for inoculations. The twigs with cut ends immersed in water taken in 250 ml. Erlenmayer flasks were inoculated on the petioles with 0.6 cm. culture discs and covered with moist cotton. To facilitate easy and quick infection the inoculated sites were lightly punctured using a sharp needle. The inoculated twigs were thoroughly sprayed with water, and were kept inside polythene bags, the inside of which were also sprayed with water and incubated at 22°C. In the case of dual inoculations the petioels and midribs of leaves were inoculated with both cultures at a distance of 4 cm. Cut bits of tender shoots were also dual inoculated and kept inside petri dishes lined with moist blotting paper and were incubated at 22°C inside incubator. Inoculated fruits were incubated at 22°C inside moist chambers. Five twigs with 10petioles each were used for single inoculations and dual inoculations with cultures 13 and 90.

Examination of inoculated plant parts

In the case of petioles, midribs and shoot bits 1 to 1.5 cm. long bits cut from the central portion of the region where the two lesions had coalesced, were taken and cross secti ns and tangential longitudinal sections were cut and mounted in cotton blue lactophenol. One cm. square bits were cut from fruit pericarp at the region where the lesions have met, cross sections were taken and examined. In the case of all the above tissues, tissue macerations stained in cotton blue lactophenol were also examined

Results

Within 24 hours after inoculation, brownish black lesions appeared at the inoculated regions. In the case of fruit inoculations the lesions are circular in outline and in petioles and shoots they are elongated. Lesions formed on petioles and shoots met earlier than those on fruits, i. e.

within 48 to 96 hours. Observations were made on the 5th day onwards.

On the 8th day the dual inoculated shoot and petiole tissues showed the presence of oospores. The epidermal cells showed no oospores at all. The subepidermal collenchymatous layer showed oospores in plenty. In cross sections the oospores were seen as filling the cavity of the cell and the antheridia were not visible in most cases. The cells containing oospores are slightly enlarged in diameter. The sclerenchymatous cells below show no oospores at all. The phloem region and the cambial region show plenty of oospores (Fig. 1).

Fig. 1. Tangential Longitudinal Section of Petiole. Phloem region showing three oospores.

These regions get disorganised soon and plenty of mycelial growth is visible there. The bigger xylem vessels contain plenty of mycelial growth and well formed large oospores. They project into the lumen of the xylem vessel with antheridium touching the wall and oogonium with oospore towards the centre (Fig. 2). The antheridia and oospores in the cortical zone are orientated parallel to the long axis of the petiole as the cells there are longer than broader. But the oospores in xylem are orientated at right angles to those in the cortical region and so antheridium and oogonium with oospore inside can be seen in one plane. As there is plenty of space inside the vessel the oospores develop freely inside and so are exactly spherical in shape whereas the shape of the oogonia and oospores in the outer cortical zone are dependent on the size and shape of the host cell and so are not exactly spherical. In TLS of the petioles the cells are longitudinally elongated parallel to the long axis of the petiole. The oogonia and oospores in some cases are also irregular or cylindrical. The oogonia are slightly elongated into oval or even rectangular

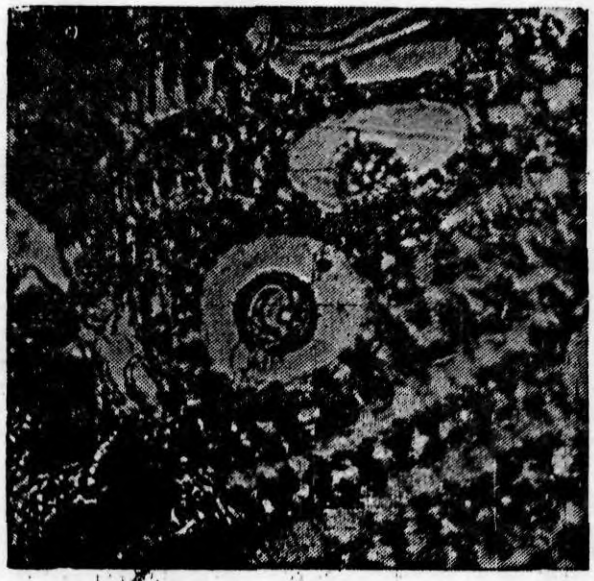

Fig. 2. A Mature Oospore within a Xylem Vessel Amphigynous Antheridium is clear below the Oospore. (Cross Section)

depending on the shape of the cells (Fig. 3). In few cases the oospores are slightly oval or elongated as the space inside the cells does not permit the normal growth of the oospore. Inside the pith cells also oospores were found

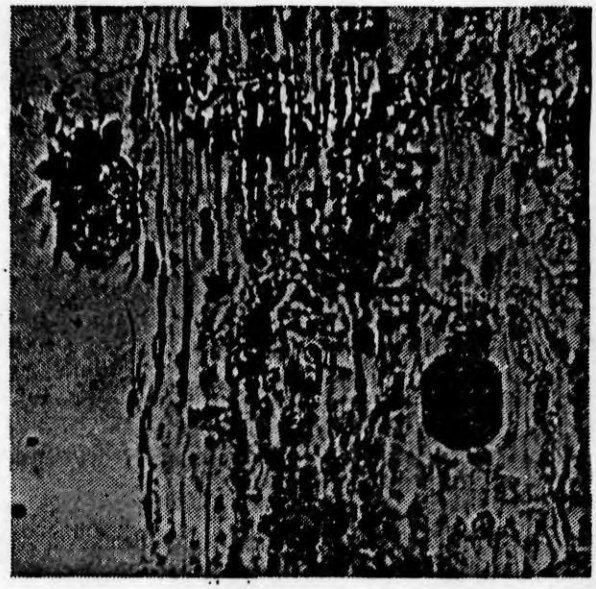

Fig. 3. T. L. S. of Petiole. Cambial region showing two oospores. Note the change in shape.

(Fig. 4) and they either fill the cavity of the host cell completely or not, depending on the size of the cell. The oospores formed towards

the outer cortical region are comparatively more mature and are reddish brown in colour whereas those towards the xylem and pith region are comparatively younger with thin walls. In the case of tender shoot bits and midribs also the formation of oospores in the tissues was similar to that described above In the fruit, the oospores were formed in the fleshy pericarp and the oospores are typical, formed inside the parenchyma cells which are rather isodiametric.

Fig. 4. Oospore within Pith Cell : Oogonial and Oospore Wall are clear. (C. S.)

The single inculated petioles with 13 and 90 when observed for the presence of oospores ; petioles inoculated with culture No. 13 showed only mycelia and no oospores whereas in the case of 90, few oospores were observed.

In order to find out whether the oospores thus produced in vivo germinate, thin cross sections of petioles with plenty of oospores inside the tissues were taken, mounted in distilled water on glass slides and were kept inside moist chambers at 22°C. They were examined after 24 hours and it was observed that many of the oospores have germinated in situ. In the case of oospores inside the xylem vessels the germination was clearly visible (Fig. 5). The germ tubes grew to some extent and produced sporangia at their tips in some cases. By frequent examination at different intervals the beginning of germ tube growth from the oospore through the oogonial stalk, sides of the antheridium or from the sides of the oospore wall and its growth and corresponding gradual emptying of contents of the oospores were observed. Along with thin walled young oospores few mature oospores with reddish brown thick walls also have germinated in the tissues. Tissue macerations with plenty of oospores suspended in sterile water, was also incubated at 22°C, at moist conditions, but they showed no oospore germination at all, perhaps due to some toxic substances produced in the tissues due to *Phytophthora* infection which might have inhibited germination of oospores.

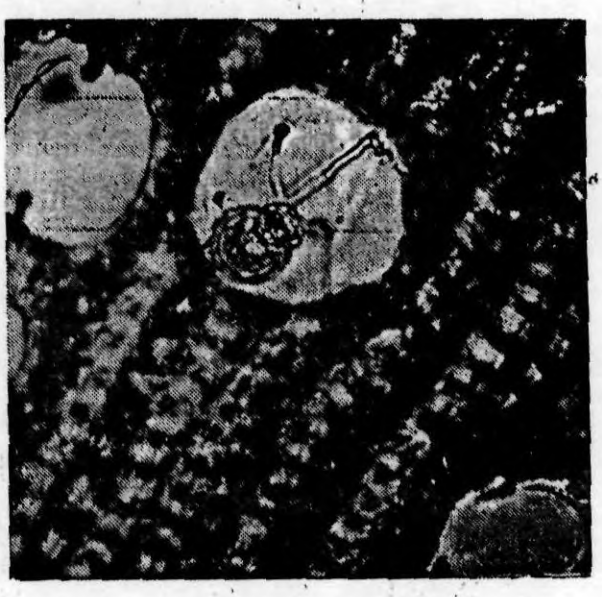

Fig. 5. Germination of Oospore within a Xylem Vessel.

Nine month old potted seedlings of Tjir 1 when inoculated on the petioles with both cultures after wounding and incubated at 22°C, produced oospores at the junction of the two lesions after 15 days from the date of inoculation.

Discussion

P. palmivora and *P. meadii* both causing diseases of *Hevea* in India are sexually compatible and some paired isolates produce oospores readily and in abundance when inoculated in suitable agar media and incubated at low temperature (20 ± 2°C). Though the sexual stage has been observed in the infected host tissues its formation in vivo under experimental conditions has not been reported. The results obtained in the present work show that the two species are well able to produce sexual spores in host tissues and the oospores thus formed are capable of germination. In the light of the present result it can reasonably be understood that genetic recombination with regard to pathogenic variation may be taking place in nature due to sexual reproduction, the ultimate result of which is the formation of physiologic strains.

Summary

Host tissues when dual inoculated with *P. palmivora* and *P. meadii* and incubated at low temperature produced oospores in abundance. Oospores were formed inside the cortical region, phloem, cambium, xylem and also the pith. The shape of oogonia and oospores varied

slightly depending on the size and shape of the host cell. Comparatively young oospores germinated in situ by the production of germ tubes passing through the oogonial stalk, antheridium or the oogonial wall Potted plants when dual inoculated also produced oospores.

Acknowledgement

The author wishes to express sincere thanks to Shri P. N. Radhakrishna Pillai, Deputy Director (Mycology/Plant Pathology), for giving guidance in the study. Sincere thanks are also due to Shri V. K. Bhaskaran Nair, Director of Research, Rubber Research Institute of India, for his interest in the work.

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