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Observations on Oospore Production by Plasmopara viticola in Floating Leaf Discs in Artifii cal Culture

by

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Introduction

Considerable difficulty may be experienced in making biological examinations of obligate parasitic fungi, which cannot be grown in axenic culture. Strains of the fungus must be maintained on living plants and frequently microscopic examinations can be made only on excised parts of the infected plant. This may render the conducting of continuing observations of the same tissue or fungal colony over a period of weeks difficult or impossible. Where prolonged observations are desired, the leaf disc method may be successfully employed.

The leaf disc method is widely employed at present. IZARD (4) placed tobacco leaf discs on filter paper moistened with a special nutrient solution and then inoculated them with conidia of *Peronospora tabacina* ADAM. OORT and DEKKER (5) used floating leaf discs for the examination of systemic fungicide preparations.

Floating leaf discs cut from grape leaves were inoculated with the downy mildew fungus, *Plasmopara viticola* (BERK. et CURT.) BERL. et DE TONI in our studies. We were surprised to find that oospores developed in the tissues of the infected discs.

Methods

Fully developed leaves of the glabrous varieties Leányka and Feri hybrid were throroughly washed in running water, rinsed several times in chemically pure*) water and dried on filter paper. Discs 18 mm in diameter were cut from the leaves with a cork borer which had been sterilized in alcohol. The discs were placed, lower side up, in 100 mm Petri dishes which contained 50 ml of chemically pure water. Ten leaf discs were placed in each Petri dish (Fig. 1). The leaf discs were then inoculated in 5 places using a conidium zoosporangium suspension of $5 \cdot 10^{-3}$ density, the inoculum all coming from a common site. After inoculation the Petri dishes were placed in diffused light in a room where the temperature fluctuated between 19 and 25° C.

The Petri dishes and leaf discs were examined regularly on a small transillumination table using a Zeiss type SM XX binocular microscope. For observations at higher magnifications the leaf discs were placed on slides in drops of distilled water, without a cover slip, and examined under a microscope in normal light. The photomicrographs were taken in this way using a ROW-Microphot camera.

*) The "chemically pure" water was provided by a "Hyderit-Zweisäulen-Vollentsalzer" (ion exchange apparatus) manufactured by L. Siebold, Vienna.

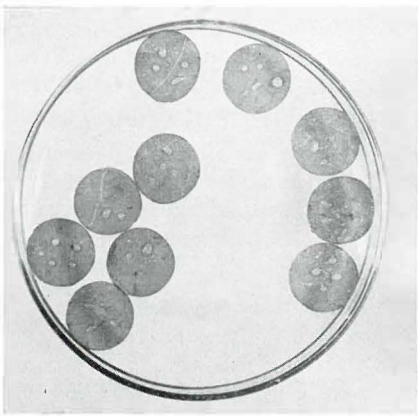

Fig. 1: Leaf-discs with drops of a conidial suspension in chemically pure water on their surface.

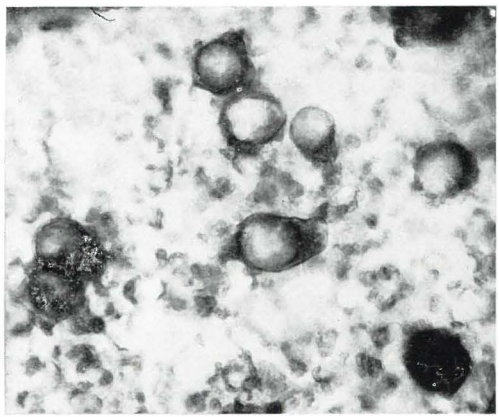

Fig. 2: Oospores, magnified about 300 x.

Results

Conidiophores always appeared within 5 to 7 days following inoculation. Positive results were obtained in 12 to 36 per cent of the inoculations in a series based on inoculation sites.

The lesions which developed on the leaf discs were always small and mosaic-like, typical of those formed on normal mature leaves in the field.

The oogonium or developing young oospore(s) could be observed as soon as 9 days after inoculation, or 2 days following the appearance of conidiophores. In other instances oospores were observed a few days later, 12 days following inoculation. As many as 86 oospores have been counted on a 4.8 sq.mm field of the small interveinal areas. Their size varied from 24.5 to 45.5 microns, including the wall thickness. The most prevalent size was 31.5 microns (Fig. 2). The thickness of the oospore wall was as much as 10.5 microns. GRÜNZEL (2) concluded that the lower temperatures of late summer and autumn did not play a primary role in the initiation of oospore formation. This conclusion is supported by observations of the formation of oospores under laboratory conditions as reported here. This conclusion is also confirmed by earlier observations. ISTVÁNFFI and PÁLINKÁS (3) found oospores outdoors after a spontaneous infection in mid-July. ARENS (1) found oospores even earlier, in mid-June.

We found that conidiophores are very seldom observed in the small intercostal areas in which oospores have developed. The area containing oospores shows a slight brown discoloration (Fig. 3 b, area B). while the surrounding and similarly infected intercostal areas remained a yellowish green. This phenomenon was also frequently observed on naturally infected leaves collected outdoors.

Areas in which the formation of conidiophores was abundant (Fig. 3 b, areas A and C) were adjacent to areas containing oospores. This could often be observed on naturally infected leaves collected outdoors. The assumption of GRÜNZEL (2) that the oospores of the downy mildew fungus are formed heterothallically seems to be supported by this phenomenon. Presumably the hyphae of sexually different lines had been developing in two distinct areas (Fig. 3 a and b, areas A and C) with

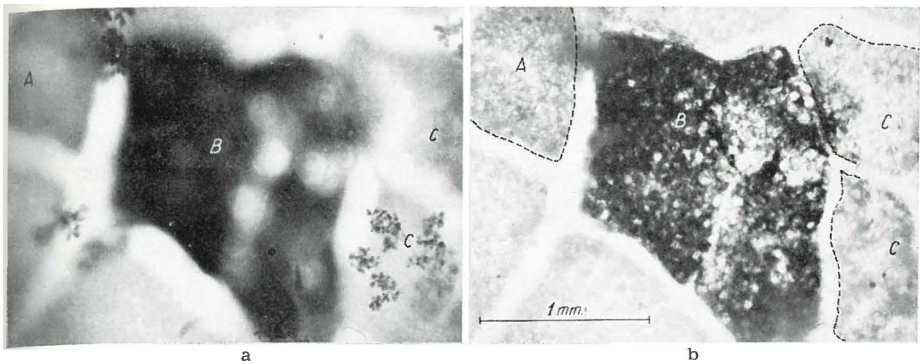

Fig. 3: Conidiophores present only in the lighter coloured A and C areas (a). Large numbers of oospores, present only in the darker coloured B areas (b).

abundant conidiophore formation. The hyphae of these two lines simultaneously entered the intercostal area separating them (Fig. 3 a and b, area B). Oogamy was initiated upon their meeting, with the subsequent development of sexual organs and then the production of oospores.

The floating leaf-discs as used in these inoculation studies may be kept alive for as long as 4 to 5 months.

Summary

Oospore formation by the grape downy mildew fungus, *Plasmopara viticola* (Berk. et Curt.) Berl. et de Toni was observed for the first time in inoculated leaf-discs floated on chemically pure water. The technique described here should prove useful in the resolution of problems concerning oospore formation which are presently not clearly understood.

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