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Oospore formation by Phytophthora infestans in host tissue after inoculation with isolates of opposite mating type found in the Netherlands

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In 1984, the A2 mating type of *Phytophthora infestans* (Mont.) de Bary was discovered in several European countries (Hohl and Iselin, 1984; Malcolmson, 1985). Before, this mating type was thought to be confined to Mexico, where it appears with the A1 mating type in a 1:1 proportion (Galleghy and Galindo, 1957). In the Netherlands the A2 mating type was first discovered among isolates collected in 1981, and in 1986 again several isolates were found mainly in allotment gardens, both on potato and tomato plants (Dr L.C. Davidse, pers. commun.). In Europe, several authors succeeded in producing oospores by mixing two mating types or by inducing (self)fertilization (Skidmore et al., 1984; Campbell et al., 1985). Tantius et al. (1986) reported the occurrence of completely self-fertile isolates in England. However, all experiments on the induction of oospores, reported up to date were conducted, using agar media in Petri dishes. None of these reports mentioned the formation of oospores in host tissue. Our research was aimed at answering the following questions: is production of oospores in plant tissue possible, and if so, in what plant organs are oospores likely to be present?

In experiments, all conducted in growth chambers at temperatures between 18 and 20 °C and a 16-h-day (light intensity 7 to 8 klx), 4-week-old tomato plants, cv. Moneymaker, and 3-week-old potato plants, cv. Eigenheimer, were used. Plants were inoculated till droplet run-off, using a DeVilbiss atomizer, with spore suspensions consisting of 1:1 mixtures of an A1 and an A2 mating type in demineralized water. The inoculum density used was 10^5 spores ml^{-1}. The tomato and potato A1 isolates had been obtained from the Research Institute for Plant Protection (IPO) at Wageningen. The A2 mating types had been collected from tomato (isolates 86061 and 86063) and potato (isolate 85027) plants (cultivars unknown) in allotment gardens near Wageningen. Both mating types had been multiplied on their original host species (cvs Moneymaker and Eigenheimer, respectively). After inoculation, plants were covered with a plastic sheet for 24 h to stimulate germination and penetration (Kröber, 1985). The r.h. under these covers was over 90%. Later, the plants were kept under conditions of water stress as describes previously for *Peronospora farinosa* f.sp. *spinaciae* (Frinking et al., 1985).

Observations were made 5, 9 and 15 days after inoculation. The epidermis at dif-

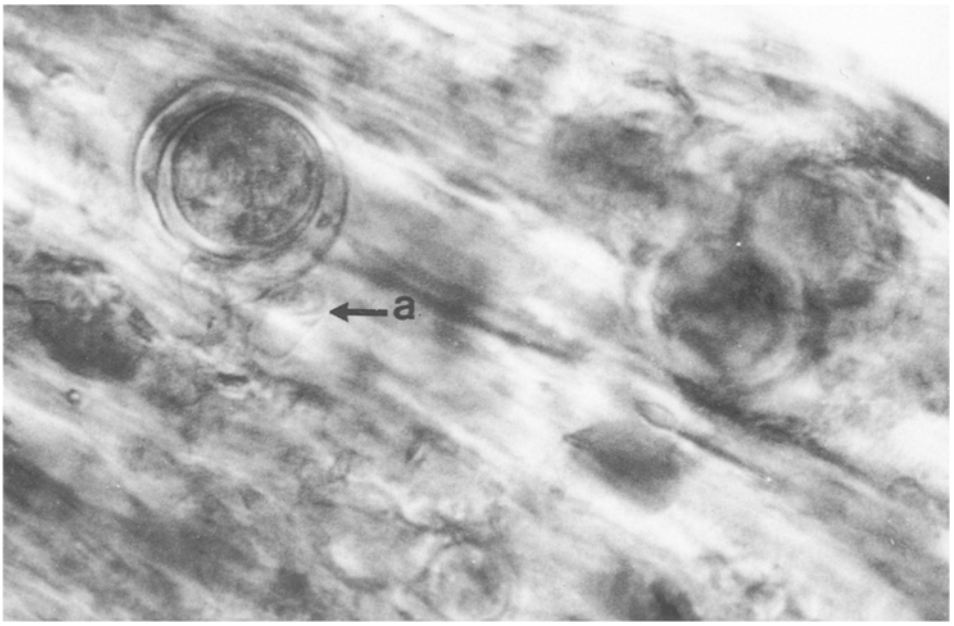

Fig. 1. Oospore with remnants of an amphigyne antheridium (a) of *Phytophthora infestans* produced in epidermal stem tissue of a tomato plant after inoculation with a mixture of an A1 and A2 mating type.

ferent places of the stems and leaves was stripped with a pair of tweezers and cleared for 24 h in a 3:1 (v/v) mixture of 96% ethanol and glacial acetic acid (c. 100%) at room temperature.

Five days after inoculation with isolates of opposite mating type, microscopic examination of the strips showed the presence of oogonia and antheridia. Control inoculations, with one isolate only, did not result into the formation of oogonia nor antheridia. After 9 days fertilization could be established. Completely developed oospores were found 15 days after inoculation (Fig. 1). Oospores were found exclusively in the epidermis of the stems of both tomato and potato plants. No oospores were observed in leaves, even when both, adaxial and abaxial leaf surfaces, had been inoculated. These results suggest that, under the experimental conditions, the fungus encounters difficulties in producing oospores in leaf tissue due to rapid necrotisation and decay of infected tissue after infection. Stem tissue, which decays less rapidly, evidently sustains growth of the fungus for a period long enough to allow oospores being formed. A preliminary study on the use of lower inoculum densities, e.g. 10^2 spores ml^{-1}, gave similar results. Further investigations are in progress to identify the conditions necessary for oospore production in the field.

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Samenvatting

Oösporenvorming in tomate- en aardappelplanten na inoculatie met in Nederland gevonden A1- en A2- paringstypen van Phytophthora infestans

Uit combinaties van A1- en A2- paringstypen van *Phytophthora infestans* werden zowel bij tomaat als bij aardappel oösporen verkregen in de plant. Oösporen werden niet in de bladeren gevormd, maar werden veelvuldig aangetroffen in de epidermis van de stengels. De proeven werden uitgevoerd in de klimaatkamer; de veldsituatie moet nog onderzocht worden.

References

Campbell, A.M., Duncan, J.M. & Malcolmson, J.F., 1985. Production of oospores in vitro by self fertilization in single isolates of A1 mating types of *Phytophthora infestans*. Transactions of the British Mycological Society 84: 533-535.

Frinking, H.D., Harrewijn, J.L. & Geerds, C.F., 1985. Factors governing oospore production by *Peronospora farinosa* f. sp. *spinaciae*. Netherlands Journal of Plant Pathology 91: 215-223.

Galleghy, M.E. & Galindo, J., 1957. The sexual stage of *Phytophthora infestans* in Mexico (Abstr.). Phytopathology 47: 13.

Hohl, H.R. & Iselin, K., 1984. Strains of Phytophthora infestans from Switzerland with A2 mating type behaviour. Transactions of the British Mycological Society 83: 529-530.

Kröber, H., 1985. Erfahrungen mit *Phytophthora* de Bary und *Pythium* Pringsheim. Mitteilungen aus der biologischen Bundesanstalt für Land- und Forstwirtschaft Berlin-Dahlem 225, 175 pp.

Malcolmson, J.F., 1985. *Phytophthora infestans* A2 compatibility type recorded in Great Britain. Transactions of the British Mycological Society 85: 531.

Skidmore, D.I., Shattock, R.C. & Shaw, D.S., 1984. Oospores in cultures of *Phytophthora infestans* resulting from selfing induced by the presence of *P. drechsleri* isolated from blighted potato foliage. Plant Pathology 33: 173-183.

Tantius, P.H., Fyfe, A.M., Shaw, D.S. & Shattock, R.C., 1986. Occurrence of the A2 mating type and self-fertile isolates of *Phytophthora infestans* in England and Wales. Plant Pathology 35: 578-581.