HELIA, **23**, Nr. 32, p.p. 19-26, (2000) UDC 633.854.78:632.4

HOMOTHALLIC SEXUAL REPRODUCTION IN *Plasmopara halstedii*, THE DOWNY MILDEW OF SUNFLOWER

Spring, O.*

University of Hohenheim, Institute of Botany, Garbenstr. 30, D-70593 Stuttgart, Germany

Received: November 01, 1999
Accepted: June 08, 2000

SUMMARY

Microscopic studies on single-spore-infected sunflower seedlings were carried out in order to study the homothallic or heterothallic nature of sexual reproduction in sunflower downy mildew, *Plasmopara halstedii*. The formation of oospores was found in the host tissue of all plants infected with single zoospores of three different pathotypes and originating from different geographic areas. This documents the homothallic nature of sunflower downy mildew and is of epidemiological importance, since a single infection in a field can lead to the contamination of the soil with oospores. Initiation of sexual reproduction started two to three weeks after inoculation of two-day-old seedlings with single, micromechanically selected zoospores. Oogonia and antheridia developed in close proximity to each other at the same hypha. The cytoplasm of the gametangia was separated from that of the adjacent hypha through the formation of a septum which consists of callose-like material according to histochemical staining with resorcin.

Key words: ***Helianthus annuus*, oospore formation, *Plasmopara halstedii*, single-spore infections, sunflower downy mildew**

INTRODUCTION

"The sexual breeding systems of most downy mildews are virtually unknown" was a general statement of Tommerup's review on the cytology and genetics of these organisms some twenty years ago (Tommerup, 1981). This is still true for large part of the downy mildews, particularly for obligate biotrophic taxa. For some model organisms like *Bremia lactucae* (Michelmore & Ingram, 1980) or *Phytophthora drechsleri* (Sansome, 1980), heterothallic distribution of oogonia and antheridia is known. Oospore formation in such cases depends on the contemporary infection of one host plant by two strains of the pathogen with opposite sexual com-

* phone (0)711-459 3811; fax (0)711-459 3355, e-mail: spring@uni-hohenheim.de

patibility (Norwood & Crute, 1983). But, homothallic production of oospores has also been reported for strains of the same species (Tommerup *et al.*, 1974).

For *Plasmopara halstedii*, the downy mildew of sunflower, the process of zygote formation in the oogonium and the exchange of nuclei from an adhering antheridium was cytologically studied by Nishimiura (1922), but without giving further insights in the location of the respective gametangia within the thallus. No clear information was yet available on the homo- or heterothallic mode of sexual reproduction. Viranyi (1988) assumed homothallic rather than heterothallic nature of *P.halstedii* due to the formation of oospores in plants infected with single sporangium-lines. However, since one sporangium may release up to about 30 zoospores, infections with single sporangia are no sufficient proof for homothallic sexual reproduction. Therefore, the aim of the current study was to use a recently described method for single-spore infections (Spring *et al.*, 1998) for the investigation of oospore generation in homothallicly infected sunflower seedlings.

MATERIAL AND METHODS

Sunflower seeds of HA 89 (a generally susceptible line for *P.halstedii*) were allowed to germinate for 36 h on wet filter paper in petri dishes. They were selected for single-spore inoculations when the radicle had reached the length of 1-2 cm and root hairs had developed.

Zoosporangia of the *P.halstedii* isolates "Leinfelden" (pathotype 330), "Hörblach" (pathotype 710) and "Bléré" (pathotype 730) were obtained from infected sunflower seedlings as described earlier (Spring *et al.*, 1997). For zoosporogenesis, they were spread on agar plates in dilute salt solution with rifampicin (10 μg per ml), and single zoospores were collected by means of microcapillary (Eppendorf transfer tips, Eppendorf Netheler-Hinz GmbH, Hamburg, 80 μm diameter, mounted on a micromanipulator) as previously reported (Spring *et al.*, 1998). Single zoospores were transferred onto the root of sunflower seedlings in the zone of root hairs. Inoculated seedlings were kept isolated on wet filter paper in a petri dish for 24 h in darkness at 16°C before they were planted in sterile soil. Plants were cultivated one by one in pots (8 cm diameter) for three to four weeks at 16°C with a 14 h photo period. Thereafter, plants were removed from the soil, washed thoroughly and incubated at 100% atmospheric humidity for 16 h in darkness. Infected plants were selected according to the development of sporangiophores and were checked microscopically for the formation of oospores by means of freehand sections through the hypocotyl and roots. Tissue sections were stained with resorcin.

RESULTS

Several series of sunflower plants were attempted to infect with single, micromechanically collected zoospores of *P.halstedii* from isolates of three pathotypes

(Table 1) which were differentiated according to the triplet code system suggested by Gulya (1995).

Table 1: Oospore formation in sunflower plants after single-spore infections

Isolate/pathotype	No. of inoculated plants	No. of infected plants	No. of plants with oospores
"Leinfelden" / 330	57	2	2
"Hörblach" / 710	60	4	4
"Blere" / 730	31	1	1

About 5% of the seedlings revealed sporulation, mostly on roots and lower parts of the hypocotyl, when transferred into humid atmosphere ca. three to four weeks after inoculation. Sporulation was less profuse and general symptoms (chlorotic leaves, stunting) were much weaker in comparison with plants infected at high inoculum density. Cross sections of the hypocotyl and of the roots indicated the areas in which the mycelia had predominantly developed. Longitudinal sections through these areas revealed the formation of oospores in all infected plants. The use of geographically different isolates and of different pathotypes had no influence on the development of oospores in single-spore-infected plants.

Although usually developing in the intercellular space of the host's parenchyma (Figure 1a), some areas were detected where hyphae had occupied the lumen of a cell and formed oospores (Figure 1b). In areas of sexual reproduction hyphal growth became abnormal and intensive anastomosis was observed (Figure 1c). Oogonia and antheridia developed in close proximity to each other at the same hypha (Figure 2). Both gametangia were formed on short suspensor-like hyphae which either derived from a single ramification of the main axis (Figure 2a) or from individual branching points not far from each other (Figure 2b). When the cytoplasm was migrated into the gametangia, a septum separated the sexual organ from the supporting hypha. Treatment with resorcin resulted in a deep blue staining of the septum, thus implicating a callose-like constitution of this structure. This closely resembles the formation of similar septa in developing sporangiophores and in the germ tube of germinating oospores (Spring and Zipper, 1999).

DISCUSSION

Sexual reproduction appears to be a nonuniform feature in different taxa of the downy mildews and is still poorly understood (cf. Tommerup, 1981). The combination of compatible mating types is necessary for the production of oospores in some *Phytophthora* species (e.g. *P.infestans*). On the other hand, homothallic species such as *P.sojae* or *P.trifolii* are known from the same genus (Hansen, 1991). In *Bremia lactuca*, heterothallic reproduction was found in isolates of only one mating type. In addition, some isolates exhibited homothallic capacity when cultured from single-spores (Tommerup, 1981).

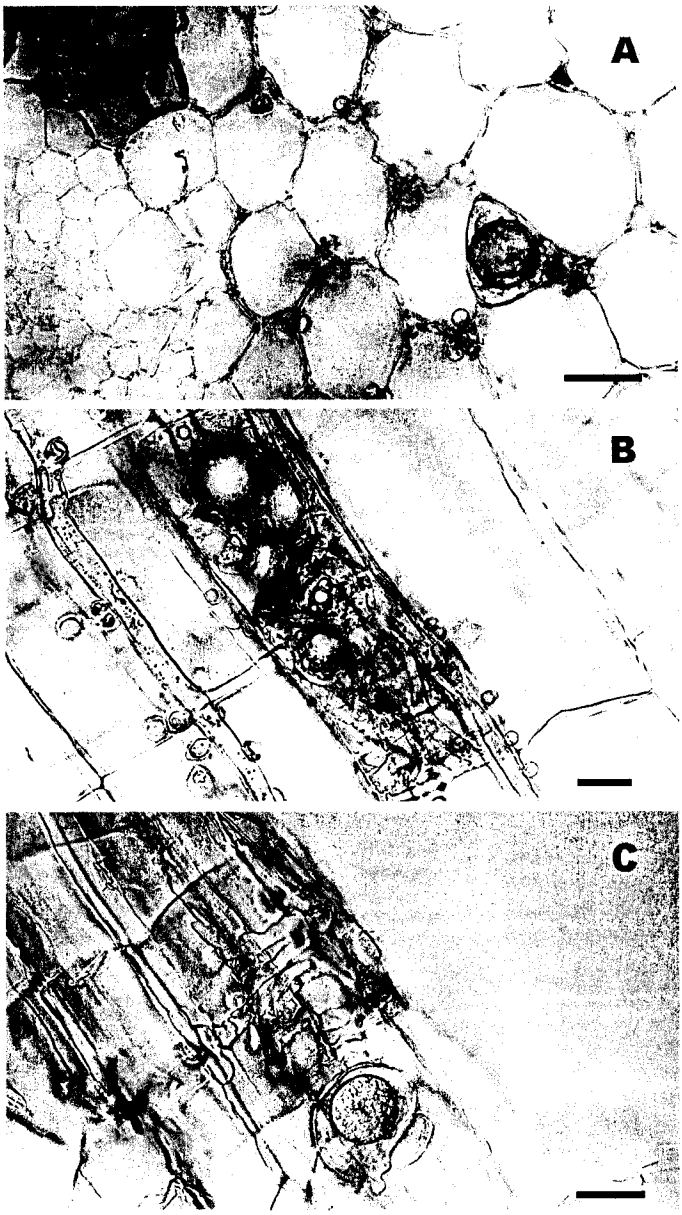

Figure 1: ***A:*** *Oospore in the intercellular space of the cortical parenchyma of the hypocotyl of a sunflower seedling, three weeks after inoculation with a single zoospore of P.halstedii.*
B: *Longitudinal section of sunflower hypocotyl with parenchymatic cell, filled with hyphae and oospores.*
C: *Unusual branching and anastomosis of hyphae during oospore formation. Bar equals 30 μm.*

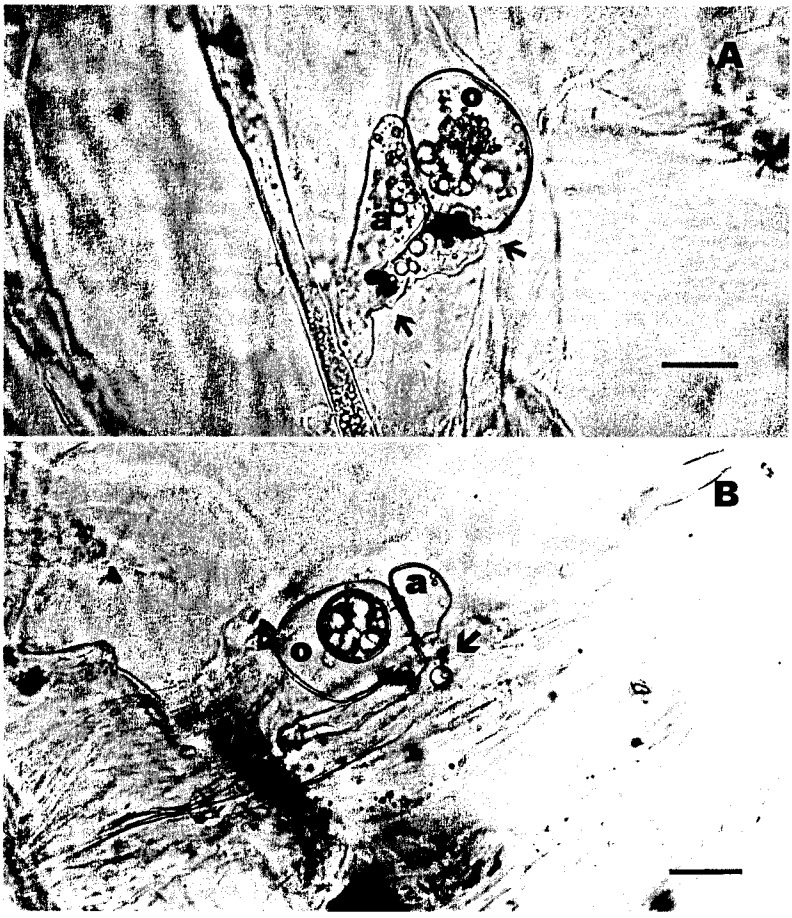

Figure 2: ***A:*** *Antheridium (a) and oogonium (o) developing from a single hyphae; arrows mark resorcin-stainable septa.*
B: *Antheridium and oogonium (with oospore) developing from separate ramifications (arrows) of the same hyphae. Bar equals 30 μm.*

For *Plasmopara halstedii* no such information were yet available. The observation of oogonia and antheridia formed on different hyphae (Zahka and Viranyi, 1991) could lend support to the assumption of a heterothallic nature of sunflower downy mildew. In contrast, successful sexual reproduction in plants infected with single sporangium-lines led to the suggestion of homothallism rather than a heterothallism for the pathogen (Viranyi, 1988). Although the use of zoosporangia instead of single zoospores for the infection could not exclude heterothallism, the experiment made it unlikely that different mating types were required for successful oospore production.

Due to the recent progress in single-spore infections (Spring *et al.*, 1998), it was now possible to show homothallic sexuality of *P.halstedii* by antheridia and oogonia formed at the same hypha. Homothallism occurred in isolates of different geographical origin and was not correlated with a specific pathotype. This may support the view that sunflower downy mildew generally reproduces through selfing. However, further investigation will be necessary in order to prove whether this is the exclusive mode of sexual reproduction or it just occurs in the absence of a mating partner. In any case, from the epidemiological point of view it appears relevant that a single infection event, as it frequently may occur in nature *via* so-called secondary infections, can lead to a long lasting source of infection in the soil of a sunflower field.

ACKNOWLEDGEMENTS

The author thanks R. Zipper, Institut für Botanik, Universität Hohenheim, for excellent technical assistance in single-spore infections.

REFERENCES

Gulya, T.J., 1995. Proposal for a revised system of classifying races of sunflower downy mildew. Proc. 17th Sunflower Res. Workshop, Fargo, pp. 76-77.

Hansen, E.M., 1991. Variation in the species of the *Phytophthora* megasperma complex. In: Lucas, J.A., Shattock, R.C., Shaw, D.S. and Cooke, L.R. (eds.), *Phytophthora*, Cambridge Univ. Press, pp. 148-163.

Michelmore, R.W. and Ingram, D.S., 1980. Heterothallism in *Bremia lactucae* Regel. Trans. Br. Mycol. Soc., 75: 47-56.

Nishimura, M., 1922. Studies in *Plasmopara halstedii*. J. Coll. Agric., XI/3: 185-210.

Norwood, J.M. and Crude, I.R., 1983. Infection of lettuce by oospores of *Bremia lactucae*. Trans. Br. Mycol. Soc., 81: 144-147.

Sansome, E., 1980. Reciprocal translocation heterozygosity in heterothallic species of *Phytophthora* and its significance. Trans. Br. Mycol. Soc., 74: 175-185.

Spring, O., Rozynek, B. and Zipper, R., 1997. Leaf disk inoculation - a useful tool for selecting infections of sunflower downy mildew at low inoculum concentration, but inappropriate to pathotype characterization. J. Phytopathology, 145: 189-191.

Spring, O., Rozynek, B. and Zipper, R., 1998. Single-spore infections with sunflower downy mildew. J. Phytopathology, 146: 577-579.

Spring, O. and Zipper, R., 1999. Isolation of oospores of sunflower downy mildew, *Plasmopara halstedii*, and microscopical studies on oospore germination. J. Phytopathology (in press).

Tommerup, C., 1974. Oospores of *Bremia lactucae*. Trans. Brit. Mycol. Soc., 62: 145-150.

Tommerup, C., 1981. Cytology and genetics of downy mildews. In: Spencer, D.M. (ed.), The Downy Mildews, Academic Press, London, pp. 121-142.

Viranyi, F., 1988. Factors affecting oospore fromation in *Plasmopara halstedii*. Proc. 12th Int. Sunflower Conf., Novi Sad, 1988, Vol. 2, pp. 32-37.

Zahka, G.A. and Viranyi, F., 1991. Axenic culture of the downy mildew fungus *Plasmopara halstedii* in *Agrobacterium* rhizogenes-induced roots of sunflower (*Helianthus annuus*). Can. J. Bot., 69: 2709-2715.

LA REPRODUCCIÓN SEXUAL HOMOTÁLICO DEL HONGO *Plasmopara halstedii*, AGENTE DEL MILDIU DE GIRASOL

RESUMEN

El estudio microscópico de plántulas infectadas por esporas singulares del patógeno ha sido efectuado para determinar la natura homotálica o heterotálica de la reproducción sexual del hongo *Plasmopara halstedii*, agente del mildiu de girasol. Fué constatada la formación de oosporos en el tejido de las plantas huéspedes infectado por zoosporas singulares de tres patotipos que provienen de diversas áreas geográficas. Eso confirma la natura homotálica del mildiu de girasol. Esta constatación es de importancia epidemiológica porque la aparición de la infección solo en un lugar del campo puede causar la infección del suelo por oospores. El proceso de la reproducción sexual empezó en dos a tres semanas después de la infección artificial de las plántulas de dos dias por zoosporas singulares, micro-mecanicamente escogidas. Los oogonios y las anteridias se desarrollaban unos próximos a otros a la misma hifa. El citoplasma de gametangia fué separado del citoplasma de la hifa vecina por la barrera que consistia, como la coloración histoquimica por resorcina 10 ha mostrado, en el material semejante a ese calloso.

HOMOTHALLIE DE LA REPRODUCTION SEXUELLE DE *Plasmopara halstedii*, AGENT DU MILDIOU CHEZ LE TOURNESOL

RÉSUMÉ

L'étude microscopique de germes de tournesol infectés par des spores individuels pathogènes a été effectuée dans le but d'établir l'homothallie ou l'hétérothallie de la reproduction sexuelle de *Plasmopara halstedii*, agent du mildiou chez le tournesol. La formation d'oospores a été établie dans le tissu porteur de toutes les plantes infectées par des zoospores individuels de trois différents types pathogènes et originaires de différentes régions géographiques. Ceci confirme l'homothallie du mildiou du tournesol et revêt une importance épidémiologique car une seule infection dans un champ peut conduire à la contamination du sol par des oospores. Le processus de reproduction sexuelle a commencé deux à trois semaines après l'inoculation micromécanique des zoospores individuels sélectionnés dans des germes de deux jours. Les oogènes et anthéridies se sont développées à proximité les unes des autres sur les mêmes hyphes. La coloration histochimique à la résorcine a montré que le cytoplasme du gamètange était séparé de l'hyphe adjacente par la formation d'un septum constitué d'une matière semblable à la callose.