FACTORS AFFECTING OOSPORE FORMATION IN PLASMOPARA HALSTEDII

F. Virányi, Plant Protection Institute, Hungarian Academy of Sciences, Budapest 114, Pf. 102, H-1525

SUMMARY

To investigate the location and timing of oospore development and to determine the sexual nature of Plasmopara halstedii, cotyledons and whole seedlings of sunflower /cv. GK-70/ were inoculated by dropping of, or immersing into a watery suspension of sporangia, respectively. Oospores formed predominantly in hypocotyl and root tissues of the host at least 10 days from inoculation. There was no correlation found between asexual and sexual sporulation. In a comparative test, 27 field isolates, as well as 17 single-sporangium lines were used. Oospores, although variable in appearance, occurred in all samples tested suggesting that P. halstedii may have a homothallic character.

INTRODUCTION

Oospores of Plasmopara halstedii are of greatest importance in inducing primary infection of sunflower crop. These fungal propagula are long-lived and also play an important role in the spread of this fungus worldwide /Sackston 1981/. Despite the potential risk they share in both disease development and intraspecific variation, very little is known of their biology.
Oospores of related fungi, produced during sexual reproduction, are generally assumed to form in response to nutritional or environmental stress /Frinking et al. 1985/, stimulatory substances and/or hormonal regulation /Ko 1980/. In addition, recent observations have implicated sexual reproduction as a consequence of outcrossing between complementary mating types /Michelmore and Ingram 1980/.
A detailed study has been initiated on the sexual reproduction of P. halstedii, and this report represents the first stages of investigations dealing with the location and timing of oospore formation and with the frequency of such spores in relation to fungal variability.

MATERIALS AND METHODS

The origin of isolates of P. halstedii examined for their ability to produce oospores is given in Table 1. The fungus was cultured on susceptible sunflower seedlings /cv. GK-70/ in the glasshouse and long-term stored at low temperature /Virányi 1985/. Prior to being used for experiments, the fungus was taken from deep-freezer, allowed to thaw and transferred onto glasshouse-grown sunflower seedlings by using the whole seedling inoculation technique according to Cohen and Sackston /1973/. To initiate the formation of oospores, detached, fully developed cotyledons or 3-day-old germlings of sunflower /cv. GK-70/ were inoculated. In the first case, single drops /approx. 32 µl/ of a sporangium suspension containing 10^5 ml^{-1} spores were placed on the adaxial surface of each cotyledon laid in a Petri dish lined with wetted filter paper; incubation followed at 16°C in the darkness for the first 24 h and then in a growth room lit for 15 h each day by daylight fluorescence tubes. From 5 days onwards, the cotyledons

were inspected for asexual sporulation. In the second case, sunflower germlings were inoculated as described earlier /Cohen and Sackston 1973/, planted into pots filled with a common soil mixture, and they were grown on glasshouse benches at 22 ± 3°C until assessment. Affected tissues were taken at intervals, washed thoroughly with tap water and, in case of hypocotyls, the lower part of these excised. Fixation and further procedures of the material were made by a method described elsewhere /Virányi 1974/. Cotyledons were observed as whole mountings, whereas hypocotyl pieces of 3 mm in length were squashed prior to microscopical observation. The incidence and density of oospores occurring in each sample were determined and a score from 0 through 3 was made as fllows: 0, nil; 1, rare; 2, frequent; 3, abundant. Based on this score, a calculation was made for each fungal sample, and the relative frequency for oospore production given by using the potency mapping technique /Lewi 1976/.

Table 1 Origin of Plasmopara halstedii isolates used in oospore formation tests

Accession no.	Code no.	Place	Host[a]	Year
1	H1	Various	H	1976
2	H13	Bekecs	H	1982
3	H18	Kiszombor	H	1982
4	H19	Sásd	H	1982
5	H20	Nagyszénás	H	1982
6	H21	Nagyszénás	X	1982
7	H22	Nagyszénás	X	1982
8	H23	Nyíregyháza	H	1982
9	H24	Nyíregyháza	H	1982
10	H25	Nyíregyháza	H	1982
11	H26	Nyíregyháza	H	1982
12	H27	Nyíregyháza	H	1982
13	H28	Nyíregyháza	H	1982
14	H29	Bácsalmás	H	1982
15	H30	Dömsöd	X	1982
16	H31	Bácsalmás	H	1983
17	H32	Bekecs	H	1983
18	H33	Csákvár	H	1983
19	H34	Vál	H	1984
20	H36	Kőrösladány	H	1984
21	H37	Bicsérd	H	1985
22	H39	Bicsérd	H	1985
23	H40	Bicsérd	H	1985
24	H41	Bicsérd	H	1985
25	H42	Bicsérd	H	1985
26	H44	Szolnok	H	1985
27	H46	Szolnok	H	1987

[a] H = Helianthus annuus; X = Xanthium strumarium.

RESULTS

In our experimental system oospores of P. halstedii predominantly formed in hypocotyl and root tissues of sunflower, whereas cotyledons and true leaves hardly supported sexual reproduction. Sex organs started to develop after a week of incubation and the majority of oospores became visible 10-14 days from inoculation. No correlation was found between asexual and sexual sporulation. In many cases profuse sporangium formation could be seen on hypocotyl pieces containing considerable amount of oospores.
In a comparative test of 27 field isolates of the fungus, and of 17 single-sporangium lines derived from one of these, it was found that oospores, although variable in appearance, occurred in all the samples /isolates or lines/ tested /Tables 2 and 3/. Of the 27 isolates examined, H42 and H46 showed a capacity of intensive oospore production, and in contrast H29 and H34 exhibited poor formation of these fungal structures. A similar phenomenon occurred with single-sporangium lines derived from isolate H1.

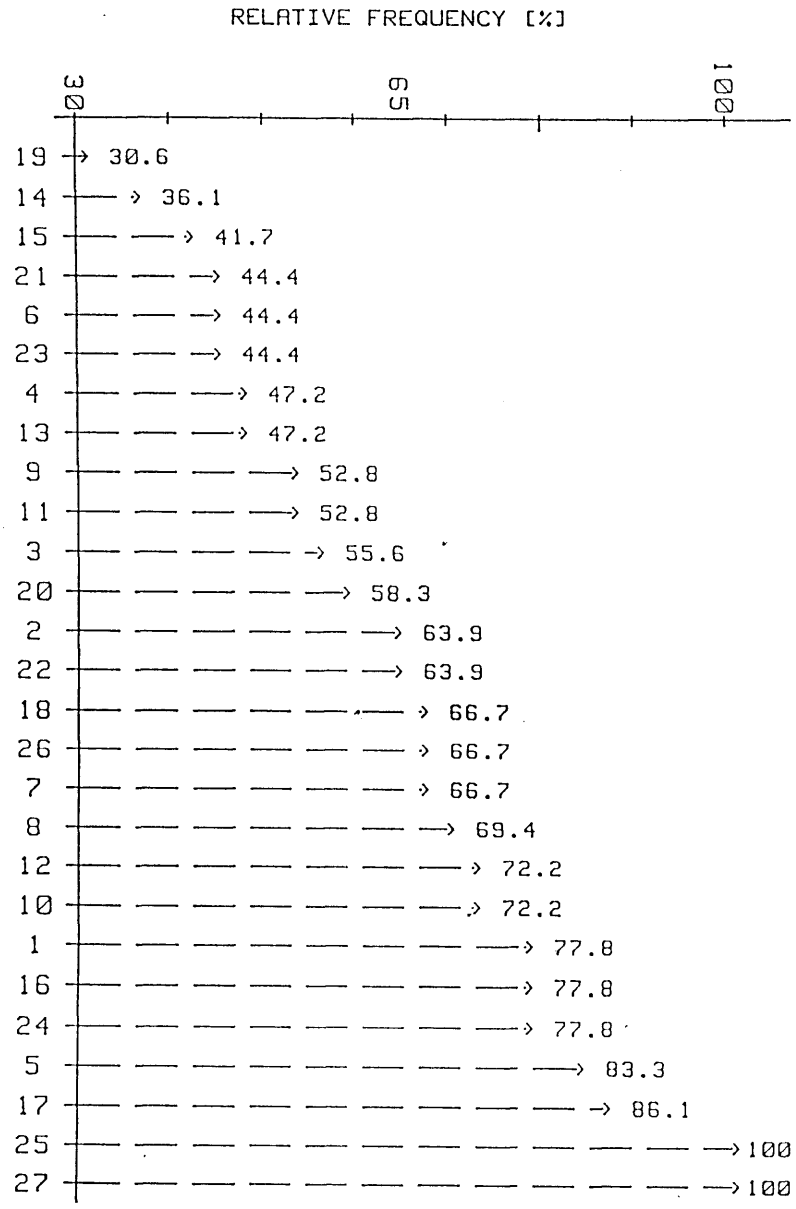
DISCUSSION

The predominant occurrence of oospores in the lower part of hypocotyl and root tissues of sunflower seedlings is not surprising and correlates well with previous findings /Sackston 1981/. The fact, however, that cotyledon leaves of systemically infected plants hardly supported sexual reproduction is quite interesting and contrasts with investigations on other related fungi, e.g. Bremia lactucae /Michelmore and Ingram 1980/ and Peronospora parasitica /Kluczewski and Lucas 1983/.
The duration of time required for oospore formation is similar to that described by Inaba and Morinaka /1983/ in soybean downy mildew, and indicates that under favourable conditions the fungus completes its sexual cycle very rapidly. It was interesting to see that in a number of cases both asexual and sexual sporulation was supported by the same host tissue. This finding confirms that of McMeekin /1960/, whilst contradicts with the results of others /Lehoczky 1965/.
As our field isolates were collected from different locations of Hungary /Table 1/ and they do not necessarily originate from a single infected plant, such isolates should be considered as populations. Consequently, their sexual reproduction may result from interactions between different mating types. On the other hand, the results obtained with single-sporangium lines do not support this hypothesis. Instead, it may suggest a homothallic nature /Michelmore and Ingram 1982/ of this fungus. Alternatively, secondary homothallism may also occur.

ACKNOWLEDGEMENTS

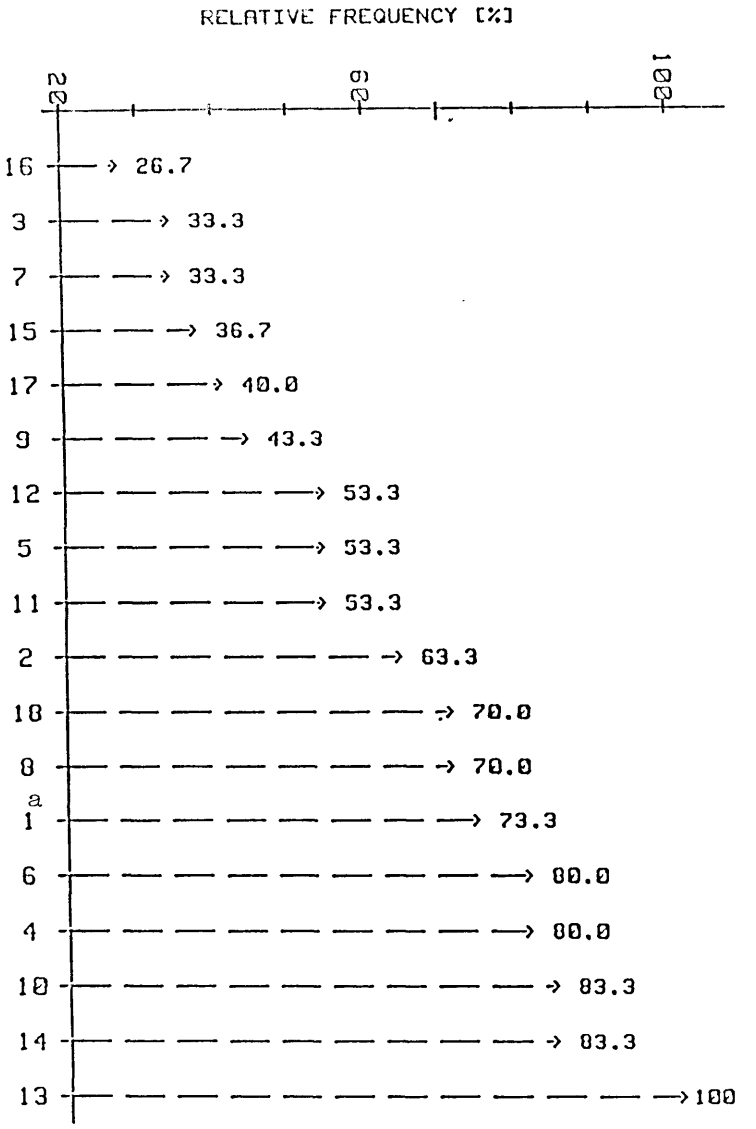
The author tahnks the Hungarian Academy of Sciences for a Research Support Grant /No. 13-86-191/, and he is greatful to Dr G. Oros for contributing in data analysis, and to Mrs Judit Égi for technical assistance.

Table 2 Oospore production of field isolates[a] of Plasmopara halstedii



[a] Isolate numbers in this table correspond to accession numbers in Table 1

Table 3 Oospore production of single-sporangium lines of Plasmopara halstedii



[a] Isolate H1 from which all the lines derived

REFERENCES

COHEN Y. and SACKSTON W.E. 1973. Factors affecting infection of sunflowers by Plasmopara halstedii. Canadian Journal of Botany 51, 15-22.

FRINKING H.D., HARREWIJN J.L. and GEERDS C.F. 1985. Factors governing oospore production by Peronospora farinosa f. sp. spinaciae in cotyledons of spinach. Netherlands Journal of Plant Pathology 91, 215-223.

INABA T. and MORINAKA T. 1983. Effect of conidium formation on oospore formation in lesions of soybean downy mildew caused by Peronospora manshurica. Annals of the Phytopathological Society of Japan 49, 252-255.

KLUCZEWSKI S.M. and LUCAS J.A. 1983. Host infection and oospore formation by Peronospora parasitica in agricultural and horticultural Brassica species. Transactions of the British Mycological Society 81, 591-596.

KO W.H. 1980. Sexuality, evolution and origin of Phytophthora. Plant Protection Bulletin /Taiwan R.O.C./ 22, 141-151.

LEHOCZKY J. 1965. Observations on oospore production by Plasmopara viticola in floating leaf discs in artificial culture. Vitis 5, 17-19.

LEWI P.J. 1976. Spectral mapping, a technique for classifying biological activity profiles of chemical compounds. Arzneimittel-Forschung 26, 1295-1300.

McMEEKIN Dorothy 1960. The role of the oospores of Peronospora parasitica in downy mildew in Crucifers. Phytopathology 50, 93-97.

MICHELMORE R.W. 1981. Sexual and asexual sporulation in the downy mildews. In: D.M. Spencer /Ed./, The Downy Mildews. Academic Press, London, 165-181.

MICHELMORE R.W. and INGRAM D.S. 1980. Heterothallism in Bremia lactucae. Transactions of the British Mycological Society 75, 47-56.

MICHELMORE R.W. and INGRAM D.S. 1982. Secondary homothallism in Bremia lactucae. Transactions of the British Mycological Society 78, 1-9.

SACKSTON W.E. 1981. Downy mildew of sunflower. In: D.M. Spencer /Ed./, The Downy Mildews. Academic Press, London, 545-575.

VIRÁNYI F. 1974. Studies on the biology and ecology of onion downy mildew /Peronospora destructor /Berk./ Fries / in Hungary. Acta Phytopathologica Academiae Scientiarum Hungaricae 9, 311-314.

VIRÁNYI F. 1985. A simple technique for long-term storage of Plasmopara halstedii sporangia at low temperature. Transactions of the British Mycological Society 85, 529-531.