Neth. J. Pl. Path. 87 (1981) 159-162 SHORT COMMUNICATION

A procedure to infect lettuce seedlings with oospores of Bremia lactucae

IDA BLOK

Research Institute for Plant Protection (IPO), Wageningen

Accepted 17 November 1980

Additional keywords: Lactuca sativa, induction of oospore formation.

After *Bremia lactucae* Regel was shown to exhibit heterothallism (Michelmore, pers. comm. 1978; Michelmore and Ingram, 1980), oospores of the lettuce downy mildew fungus could be readily obtained by inoculating leaf tissue with a suspension containing conidia of both compatibility types, called B_1 and B_2. Michelmore and Ingram (1980) examined the Dutch races of *Bremia lactucae* and they identified NL6 as a B_1 type, NL2, 3, 4, 5 and 7 as B_2 types, whereas NL1 was found to contain both compatibility types, with B_2 predominating.

For identification of the virulence phenotype of the progeny of a cross, it is necessary that the oospores germinate and infect lettuce plants. Morgan (1978) found that germination of oospores on water agar was stimulated by the presence of germinating lettuce seeds, but he did not mention infection of the seedling.

In our early experiments, sowing of seeds of a susceptible lettuce cultivar in soil, mixed with oospores, did not result in infected plants. Therefore a new technique was developped, which is described below.

Production of oospores

Suspensions of conidia of a B_1 and a B_2 type of *Bremia lactucae* are mixed and sprayed onto leaf discs of the lettuce cv. Olof (susceptible to all known races). Instead of leaf discs cotyledons may be used. The inoculated discs are incubated in a Petri dish on wet filter paper at approximately 17 °C under long day conditions. On some segments of the discs conidia will appear within one week. Some other segments turn brown and become necrotic within two weeks and usually contain many oospores (Fig. 1). Usually asexual sporulation and oospore formation do not take place in the same segment.

Germination of oospores and infection

Before infection from oospores can be obtained, the leaf discs containing the oospores have to be left in the Petri dish for about two months. The leaf tissue is then completely decayed and viable conidia are no longer present. The leaf discs together with the filter paper beneath them are cut out and placed on a layer of soil (approximately 2 cm) in a 5 cm high glass jar. No attempts are made to separate the

Fig. 1. Oospores of *Bremia lactucae* in lettuce leaf.

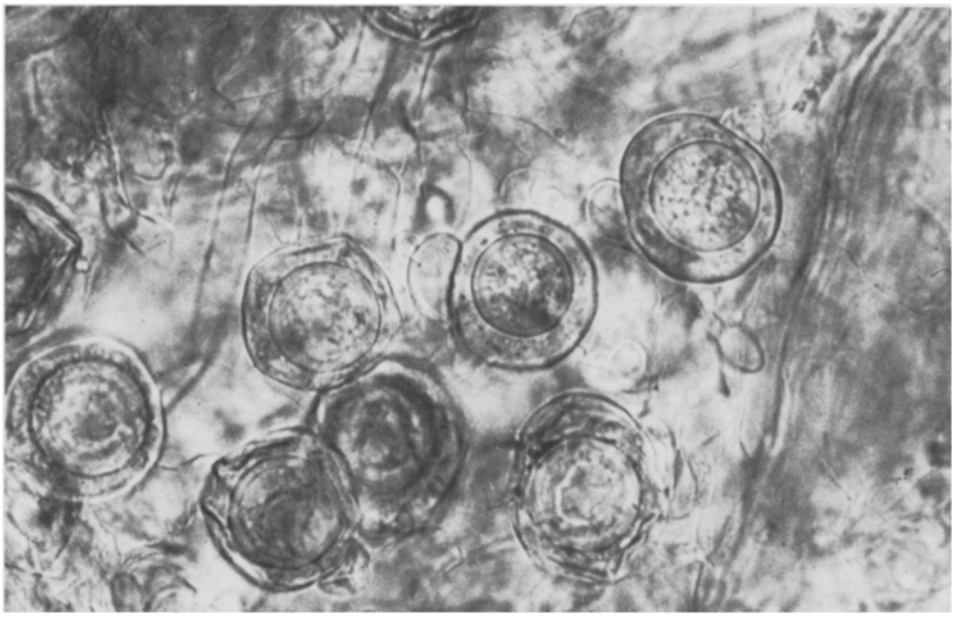

Fig. 1. Oösporen van Bremia lactucae in slablad.

Fig. 2. Filter paper with leaf discs, containing oospores, placed on soil and sown with lettuce seeds. Right jar: one week after sowing.

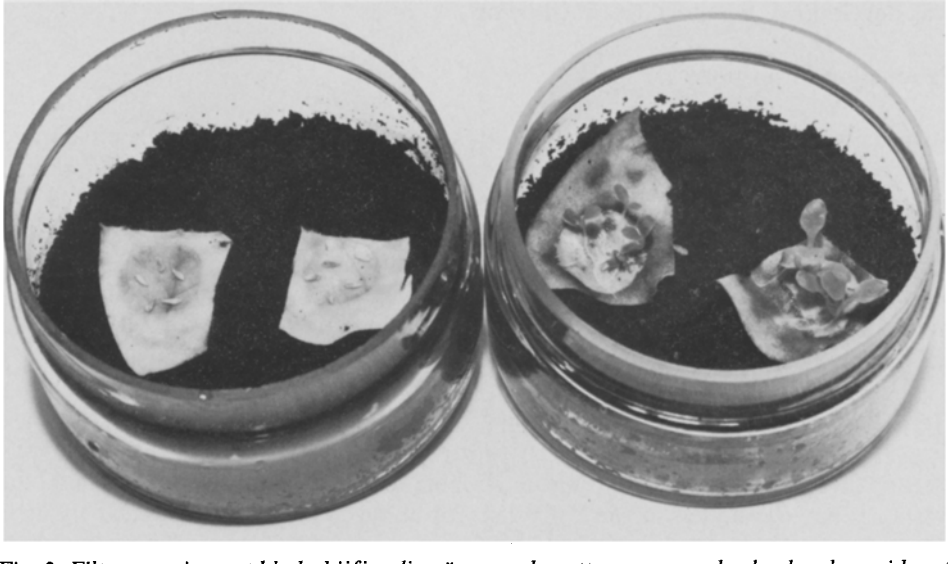

Fig. 2. Filtreerpapier met bladschijfjes die oösporen bevatten, op grond gelegd en bezaaid met slazaad. Rechter schaaltje: een week na het zaaien.

oospores from the tissue. Five seeds of the lettuce cv. Olof are placed on each disc (Fig. 2). The jar is closed and incubated at approximately 17 °C. Conidia appear on infected seedlings after three to five weeks, usually first on the cotyledons. These conidia are washed off and the suspension is sprayed onto a differential set of lettuce cultivars for identification of the virulence phenotype of the isolate (race identification).

Preliminary results

When leaf discs with oospores were used at least two months but no more than three months following inoculation, often all five seedlings placed on them became infected. A total of 166 isolates was examined. The conidia from one seedling were considered as one isolate because, except in one case, no segregation could be noticed after re-testing on the differential set. The progeny had usually more restricted virulence phenotypes than the parents. This is in agreement with the findings of Michelmore and Ingram (1981). As an example the results of the cross NL6 × NL5 (B_1 × B_2) are given in Table 1.

Further back-crossing of the progeny with the parents will be carried out in order to elucidate the genetics of virulence in *B. lactucae*.

Samenvatting

Een manier om sla-zaailingen met oösporen van Bremia lactucae te infecteren

Oösporen van *Bremia lactucae* kunnen worden verkregen door bladschijfjes van sla te inoculeren met een gemengde conidiënsuspensie van twee fysio's van de schimmel, die de twee compatibiliteitstypen (B_1 en B_2) vertegenwoordigen. Wanneer het bladweefsel geheel vergaan is, worden de schijfjes met oösporen samen met het

Table 1. Reaction of different lettuce cultivars upon two Dutch races of *Bremia lactucae* and their progeny (isolate I and II). + = susceptible, − = resistant.

Cultivar		Race of *B. lactucae*			
	R-genes	NL6 (= B_1)	NL5 (= B_2)	NL6 × NL5 I	NL6 × NL5 II
	v-genes	1,2,(4),5,8, (9),10,11	1,3,4,7	?	?
Olof	0	+	+	+	+
Deciso	2,4	+	−	−	−
Solito	3,7	−	+	−	−
Pallas	2,3,7	−	−	−	−
Hilde × L.serr.	11	+	−	−	+

Tabel 1. Reactie van enkele slarassen op twee Nederlandse fysio's van Bremia lactucae en hun nakomelingen (isolaat I en II). + = vatbaar, − = resistent.

onderliggende filtreerpapier op een dunne laag grond in een glazen schaaltje gebracht. Hierop worden vijf zaden van een vatbaar slaras te kiemen gelegd. Zaailingen die worden aangetast vertonen na drie tot vijf weken sporulatie op de cotylen. De conidiën worden op een toetssortiment van slarassen gespoten om het aantastingspatroon van het isolaat te bepalen. De eerste resultaten toonden aan dat de nakomelingen van een kruising gewoonlijk een beperkter aantastingspatroon hebben dan de ouders.

References

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

Michelmore, R.W. & Ingram, D.S., 1981. Recovery of progeny following sexual reproduction of *Bremia lactucae*. Trans. Br. mycol. Soc. 77: 131-137.

Morgan, W.M., 1978. Germination of *Bremia lactucae* oospores. Trans. Br. mycol. Soc. 71: 337-340.

Address

Instituut voor Plantenziektenkundig Onderzoek (IPO), Binnenhaven 12, P.O. Box 42, 6700AA Wageningen, the Netherlands.