Plant Pathology (1995) **44**, 86–94

Formation and survival of oospores of *Phytophthora infestans* under natural conditions

A. DRENTH*†‡ E. M. JANSSEN* and F GOVERS*
**Department of Phytopathology, Wageningen Agricultural University, Binnenhaven 9 and †Research Institute for Plant Protection (IPO-DLO), Binnenhaven 12, 6709 PD Wageningen, The Netherlands*

Phytophthora infestans is able to produce oospores in leaves of potato and tomato plants after inoculation with a mixture of A1 and A2 mating-type isolates. Various conditions for oospore formation were analysed. Under controlled conditions, oospores were produced in potato leaves at temperatures ranging from 5 to 25°C. In leaves of potato cultivar Bintje incubated at 15°C, oogonia and antheridia were observed 6 days after inoculation and thick-walled oospores appeared 3–4 days later. In field experiments oospores were found in leaves and stems of potato cultivars Bintje, Irene and Pimpernel and in leaves, stems and fruits of tomato cultivar Moneymaker within 2 weeks after inoculation. A bioassay was developed to test the survival of oospores in soil under various conditions. To determine whether late-blight infections derived from infectious soil were caused by oospores, DNA fingerprinting was performed. DNA fingerprint probe RG-57 was suitable for distinguishing asexual progeny from recombinant progeny arising from soil-borne oospores. We demonstrated survival of viable, infectious oospores of *P. infestans* in soil during the winter of 1992–93. Oospores were not infectious from soil exposed to temperatures of 40°C or higher but in the range 35°C to as low as −80°C for 48 h, oospores survived.

INTRODUCTION

Potato late blight caused by the oomycetous fungus *Phytophthora infestans* is one of the most devastating pathogens of potatoes worldwide. *P. infestans* is heterothallic with two known mating types, A1 and A2. Interaction between hyphae of opposite mating type can result in the formation of oospores. These were observed in infected potato leaves in central Mexico where A1 and A2 mating-type isolates were common (Gallegly & Galindo, 1958; Smoot *et al.*, 1958). Elsewhere, only A1 mating-type isolates were found. In these areas *P. infestans* reproduced exclusively asexually and overwintered as mycelium in potato seed tubers in storage or in potato tubers in cull piles and in volunteer potatoes.

In the early 1980s, however, A2 mating-type isolates were discovered in Switzerland (Hohl & Iselin, 1984) and subsequently in many other countries in Europe, Africa, Asia and the Americas (Spielman *et al.*, 1991; Drenth *et al.*, 1993b; Fry *et al.*, 1993). Population genetic studies, based on two polymorphic allozymes, revealed that since 1980 'new' A1 and A2 mating-type populations were established in Europe and replaced the 'old' A1 mating-type population present before 1980 (Fry *et al.*, 1991, 1992, 1993; Spielman *et al.*, 1991). Additional studies, based on DNA fingerprint analyses and virulence factors, demonstrated that after 1980 the level of genetic diversity in the Dutch *P. infestans* populations increased dramatically (Drenth *et al.*, 1994). Over 90% of the isolates identified each year had a unique genotype, suggesting that every year late blight was caused by different genotypes.

The production of oospores by *P. infestans* in the field and their survival under natural conditions has become an object of study since the introduction of A2 mating-type isolates. Oospores were observed in stems but rarely in leaves of potato and tomato plants grown in the greenhouse and inoculated with mixtures of A1 and A2 mating-type isolates of *P. infestans* (Frinking *et al.*, 1987; Mosa *et al.*, 1991). Small numbers of oospores were also found in tubers (Grinberger *et al.*, 1989). In Europe, oospore formation in potato leaves in the field was

‡Present address: CRC for Tropical Plant Pathology, The University of Queensland, Brisbane QLD 4072, Australia.

reported in Germany and in The Netherlands (Götz, 1991; L. J. Turkensteen, personal communication).

Oospores of oomycetous fungi are, in general, endogenously dormant, tolerant of adverse conditions, and capable of long-term survival (Ribeiro, 1983). Oospores from *Phytophthora fragariae*, the causal agent of strawberry red core, can survive for at least 3 years in soil (Duncan, 1980; Duncan & Cowan, 1980). Oospores of *Peronospora destructor*, the causal agent of downy mildew in onion, have remained infectious for up to 25 years in soil despite continuous exposure to natural weather conditions, including 1549 occurrences of frost (McKay, 1957).

Survival and infectivity of *P. infestans* oospores has not yet been studied in detail. Perches & Galindo (1969) showed that soil, collected from a Mexican field 2 years after that field was occupied by a severely blighted potato crop, was still infectious. In greenhouse experiments they showed that potatoes planted in this soil were infected on the lower part of the stems and on the leaves, close to or in contact with the soil. *P. infestans* could be isolated from this soil using selective media and the authors suggested that oospores were responsible for the infectivity of the soil.

Oospores of *Phytophthora* spp. are insensitive to fungicides (Duncan, 1985b) but sensitive to heat treatments of 45°C (Duncan, 1985a). Thus, some *Phytophthora* spp. (i.e. *P. cambivora*, *P. cryptogea*, *P. cinnamomi*) can be controlled by soil solarization (Juarez-Palacios *et al.*, 1991). Asynchronous germination is typical of oospores and most likely will enhance survival by ensuring a continuous supply of infective propagules in soil (Hord & Ristaino, 1991). Despite many studies (reviewed by Ribeiro, 1983), factors affecting germination of oospores are not well understood.

With the introduction of 'new' *P. infestans* A1 and A2 mating-type populations in Europe, together with evidence for the occurrence of sexual reproduction (Drenth *et al.*, 1994), there is a need to determine to what extent oospores contribute to late-blight epidemics and which factors influence production, survival, germination and infectivity of oospores of *P. infestans*. Here the effect of temperature on the development and production of oospores is described and oospore production in potato cultivars with different levels of resistance to foliar blight is compared. A bioassay has been developed to obtain late-blight infections from soil containing oospores. This bioassay was used to determine whether oospores, exposed to natural weather conditions in soil during the winter of 1992–93, remained viable and retained infectivity.

MATERIALS AND METHODS

Isolates

Two isolates of *P. infestans*, 80029 (A1 mating type) and 88133 (A2 mating type) collected from blighted potato crops in The Netherlands in 1980 and 1988, respectively, were used throughout this study. These two isolates were chosen from a large collection of isolates based on their unique RG-57 DNA fingerprint genotypes (Drenth *et al.*, 1994). Sexual progeny from a cross between 80029 and 88133 can be distinguished from the parental isolates and naturally occurring infections by RG-57 DNA fingerprint patterns (Goodwin *et al.*, 1992; Drenth & Govers, 1993).

Oospore formation

For studies on oospore formation in leaves under controlled conditions potato cultivars Bintje (susceptible), Irene (moderately susceptible) and Pimpernel (moderately resistant) were used. In all these experiments plants were inoculated with a mixture of sporangiospores (10^4spores/ml) containing equal amounts of both isolates 80029 and 88133. Ten potato leaf discs (18 mm diameter), cut with a cork borer, were floated upside down in Petri dishes (9 cm diameter) containing 20 ml of water, and inoculated in the middle with 10 μl of the sporangiospore mixture. To study the effect of temperature on the production of oospores the leaf discs were incubated at temperatures varying from 5 to 25°C at a light intensity of 120 mmol/m^2.s, 16 h per day. Every day the leaf discs from a single Petri dish were collected and frozen at −20°C. The frozen leaf discs were homogenized for 30 s (Polytron Homogenizer from Kinematica, CH-6010 Kriens LU, Switzerland) in 2 ml of water and the number of oospores counted using a haemocytometer.

To study oospore formation in the field, 40 small plots (1·2 × 1·2 m) containing either potato cultivar Bintje, Irene or Pimpernel, or tomato cultivar Moneymaker were inoculated in August 1992. Plants were inoculated in the evening by spraying the sporangiospore mixture (25 ml per

plot) with a rotary hand sprayer (DeVilbiss atomizer, DeVilbiss Health Care Division, Somerset, PA USA). Leaf and stem samples of potato plants and leaf, stem and fruit samples of tomato plants were collected 13 days after inoculation. In July 1993, two plots (6 × 6 m) of potato cultivar Bintje were inoculated in a similar way. Leaves with late-blight lesions were collected at day 7, 10 and 17 after inoculation.

Oospores in leaf tissue were detected after squashing the leaves on a microscope slide or clearing the leaf tissue. Leaves were cleared by autoclaving in 80% ethanol at 115°C for 5 min, incubation for 30 min in 1 M NaOH at 80°C and incubation for a few minutes in NaClO (4% available chlorine) at 60°C, until the leaves were clear, and then mounted on microscope slides.

Oospore survival

To study survival of oospores of *P. infestans*, soil was infested with oospores and pots containing the infested soil were exposed to natural weather conditions during the winter of 1992–93. In addition, field plots with potato or tomato plants were artificially inoculated and after 1 year soil samples were tested for the presence of oospores.

To produce sufficient amounts of oospores for longevity trials, leaves of the potato cultivar Bintje floating upside down in large trays (37 × 57 cm) containing 2 l of water were inoculated with the sporangiospore mixture. The leaves were incubated at a light intensity of 120 mmol/m^2.s, 16 h per day at 10°C. Sixteen days after inoculation the leaves, containing approximately 5000 oospores/cm^2 were briefly homogenized in a blender. The homogenized oospore suspension was added to pots (volume 800 cm^3) containing field soil. Approximately $6{\cdot}6 \times 10^5$ oospores were added to each pot resulting in approximately 825 oospores/cm^3 soil. The pots were buried outside at ground level to expose the infested soil to natural weather conditions. The first soil sample was analysed for infectivity 1 week after the pots were buried. Four additional samples were taken after 23, 29, 30 and 35 weeks. The infectivity of the soil samples was assessed using the bioassay described below.

To study survival of oospores at extreme temperatures, soil from one pot was homogenized, and divided into nine portions of 100 g. The portions were incubated for 48 h at different temperatures ranging from −80°C to 50°C (Table 2). The infectivity of the treated soil was assessed using the bioassay described below.

To study survival of oospores under field conditions, eight plots (5 × 5 m, heavy clay soil) planted with potato cv. Bintje, Irene and Pimpernel (two rows of each cv., 75 cm distance between rows) and two plots (5 × 5 m) planted with tomato plants (cv. Moneymaker), were inoculated in early September 1992 with a sporangiospore mixture of isolates 80029 and 88133. (1 l containing $5{\cdot}4 \times 10^6$ spores per plot.) Late blight symptoms were visible 7 days after inoculation. All potato tubers were harvested in October 1992. The remaining blighted potato leaves and stems and the blighted tomato plants were mixed with the soil. In May 1993, all 10 plots were planted with potato cultivar Bintje. In July 1993, soil was randomly collected from each plot to assess infectivity of the soil using the bioassay described below. On 30 August, 1993 when late blight was present on most plants in all 10 plots, lesions from diseased plants were randomly collected (10 from each plot). The isolates obtained from these lesions were analysed by DNA fingerprinting to determine whether or not they originated from oospores produced by 80029 and 88133 the previous year.

Bioassay

The bioassay was based on the observation that the rate of oospore germination increases after incubation of oospores in large volumes of water and that zoospores of *Phytophthora* spp. have a negative geotaxis (Cameron & Carlile, 1977). Soil (1 kg) containing oospores was transferred to a 30 × 45 × 28 cm plastic container with a transparent lid and mixed with 2 l of water. After 2 days of incubation at 15°C, 20 leaves of the susceptible cv. Bintje were placed upside down on the water surface and incubated at 15°C at a light intensity of 120 mmol/m^2.s, 16 h per day. After 5 to 7 days, sporulating lesions appeared on the leaves. Spores from the lesions were transferred to potato tuber slices and after sporulating mycelium had grown through the tuber slices, sporangiospores were transferred to rye agar containing 200 μg/ml ampicillin, 10 μg/ml fenpliclonil, 10 μg/ml pimaricin and 30 μg/ml rifampicin to obtain pure cultures.

DNA fingerprinting

The moderately repetitive DNA probe RG-57 was used for DNA fingerprinting. This probe, obtained from a genomic *P. infestans* library,

hybridizes to several unlinked loci and provides an isolate-specific DNA fingerprint (Goodwin *et al.*, 1992). DNA isolation, *Eco*RI digestion, Southern blotting and hybridization with probe RG-57 were performed as described elsewhere (Drenth & Govers, 1993; Drenth *et al.*, 1993a).

RESULTS AND DISCUSSION

Oospore formation

In leaf discs of cv. Bintje, incubated at 15 °C, oogonia and antheridia were observed 5–6 days after inoculation. After 8 days, a few oospores were observed and at 9–10 days after inoculation, many thick-walled oospores were present in the leaf discs (Fig. 1).

Oospores of *P. infestans* were produced in leaf discs of cv. Bintje between 5 and 25 °C. A maximum of more than 6000 oospores per cm^2 leaf tissue was observed at 10 °C. Approximately 2000 occurred at 15 °C whereas at 20 °C less than 1000 oospores per cm^2 leaf tissue were observed (Fig. 2a). At 5 and 25 °C only limited numbers of oospores were observed (< 10 oospores/cm^2). At 25 °C leaf tissues of potato deteriorated rapidly whereas at 10 °C the growth of *P. infestans* mycelium in leaf discs of cv. Bintje appeared to be optimal for oospore formation.

At 15 °C more oospores were found in the leaf discs of cv. Pimpernel compared to leaf discs of cv. Bintje and Irene (Fig. 2b). A similar finding was reported by Estrada (1967) using cv. Bintje and cv. Atzimba, which was rated as moderately resistant. Abundant oospore production in moderately resistant potato cultivars can be explained by the fact that leaf discs of these potato cultivars show delayed deterioration resulting in extra time for production of oospores.

In 1992, plants of cv. Bintje, Irene and Pimpernel and plants of tomato cv. Moneymaker, all grown under natural field conditions, were found to contain oospores 13 days after inoculation with sporangial suspensions of both mating-type isolates. Oospores were found in leaves and stems of the potato plants and in leaves, stems and fruits of tomato plants. In the field experiment conducted in 1993, oogonia and antheridia were observed in the leaves of potato cultivar Bintje 10 days after inoculation, and numerous oospores were detected 7 days later. From these results it can be concluded that climatic conditions in The Netherlands allow formation of oospores in potato plants as well as in tomato plants.

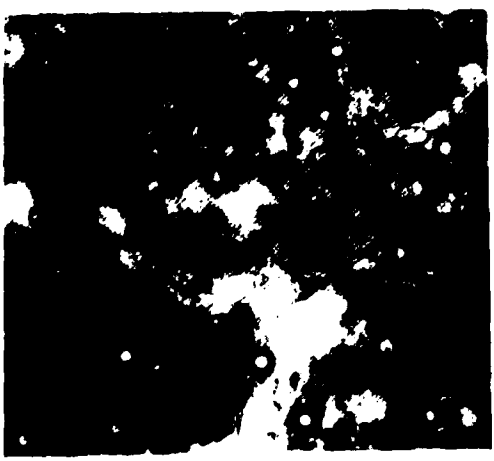

Fig. 1. Oospores of *Phytophthora infestans* formed in leaves of potato cultivar Bintje 10 days after inoculation with a mixture of equal amounts of sporangiospores (10^4 spores/ml) of *P. infestans* isolates 80029 (A1 mating type) and 88133 (A2 mating type). Leaves were incubated at 15 °C.

Oospore survival

One pot containing soil with oospores was exposed for 7 days to natural weather conditions. The soil was then tested for infectivity of oospores. DNA fingerprint analyses of 12 isolates that caused late-blight lesions in the bioassay revealed that 25% of the infections were caused by recombinant progeny from the parental isolates. The other lesions were caused by the parental isolates, 80029 or 88133 (Table 1). Thus, not only can vegetative spores or mycelium in plant debris survive for at least 1 week in soil but in addition, oospores are able to germinate and cause infections as soon as they are formed. Götz (1991) observed germinated oospores in potato leaf tissue. Hence, oospores not only act as a source of inoculum for the following season, but can also play a role in initiating new epidemics after a spell of unfavourable, e.g. dry, conditions within a season.

Soil containing oospores remained infectious for 23, 29, 30 and 35 weeks under natural weather conditions (Table 1). DNA fingerprint analyses revealed that all late-blight lesions in the bioassay were caused by recombinant progeny originating from oospores (Fig. 3) but neither asexual spores nor mycelium survived 23 weeks in soil (Table 1).

To test the sensitivity of the oospores for extreme temperatures, infested soil of the 35-week

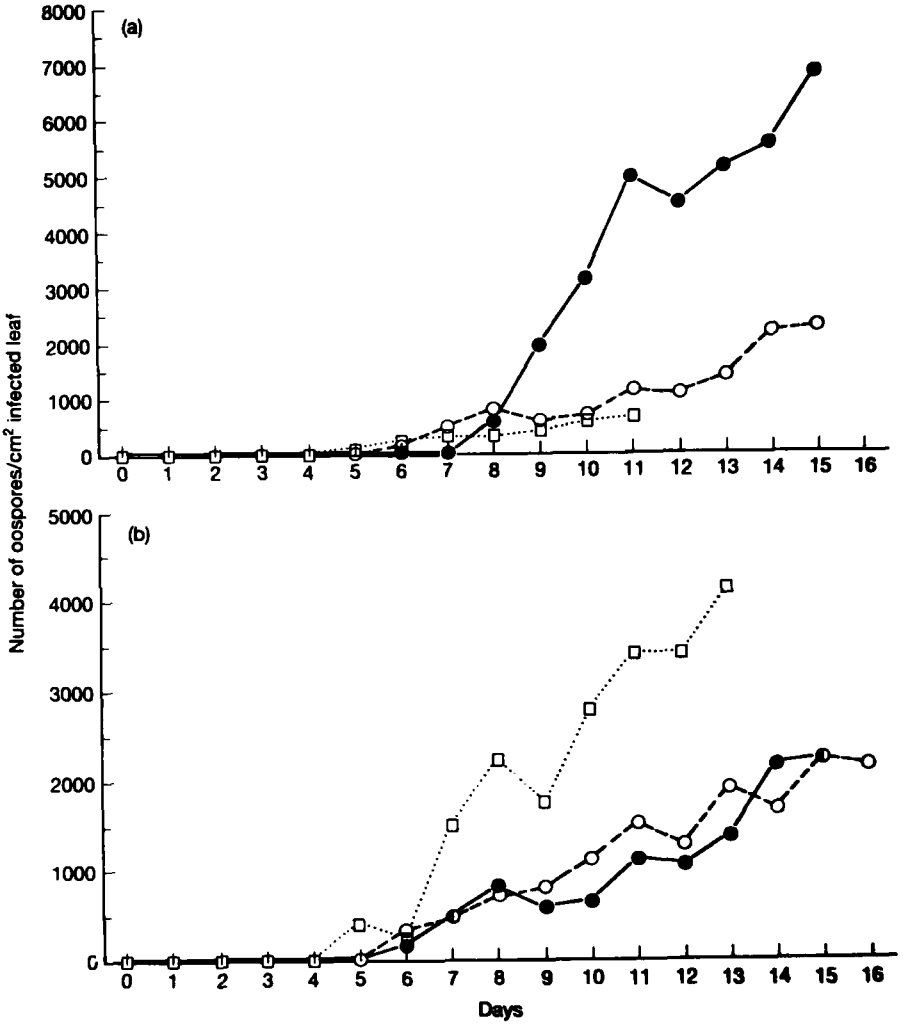

Fig. 2. (a) The number of oospores in leaf discs of potato cultivar Bintje (susceptible) plotted against days after inoculation. The leaf discs were incubated at different temperatures (●, 10°C; ○, 15°C; □, 20°C). (b) The number of oospores in leaf discs of potato cultivar Bintje (●, susceptible), Irene (○, moderately susceptible) and Pimpernel (□, moderately resistant) plotted against days after inoculation. The incubation temperature was 15°C.

treatment, was exposed to temperatures ranging from −80 to 50°C (Table 2). Soil exposed to temperatures of 40°C or higher for 48 h was not infectious but oospores could survive temperatures from 35°C to as low as −80°C for 48 h and they gave rise to late-blight lesions (Table 2). DNA fingerprint analyses confirmed that the lesions were caused by recombinant progeny.

Survival of oospores in plant debris, in soil collected from fields on which blighted potato plants were present 10 months before, was also tested. Soil samples gave rise to late-blight lesions on potato leaves in the bioassay. DNA fingerprint analyses of isolates obtained from three of these lesions revealed that one of the lesions was caused by a hybrid offspring originating from parental isolates 80029 and 88133. The other two were unrelated field isolates. The presence of unrelated isolates in the soil can be explained when infections in the previous year were not only caused by the inoculated isolates but also by other A1 and A2 mating-type isolates which produced oospores. It is also possible that in July 1993, when the soil samples were taken,

Table 1. Survival of asexual and sexual progeny of *Phytophthora infestans* in soil in The Netherlands during the winter of 1992–93.

Length of exposure in weeks[a]	Number of isolates tested	Percentage of hybrids[b]
1 (16 November 92)	12	25
23 (21 April 93)	25	100
29 (2 June 93)	23	100
30 (11 June 93)	15	100
35 (12 July 93)	21	100

[a] On 9 Nov. 1992 soil mixed with oospores was exposed to natural weather conditions. The sampling date is shown in brackets.
[b] Recombinant sexual progeny from isolates 80029 and 88133.

sporangiospores or zoospores from unrelated isolates were present in the field on the young potato plants which were planted there in May 1993. The spores might have been washed off from small lesions in the top of the leaf canopy. Because the genetic diversity in the *P. infestans* population is so high (Drenth *et al.*, 1994) it is impossible to trace the origin of unrelated isolates.

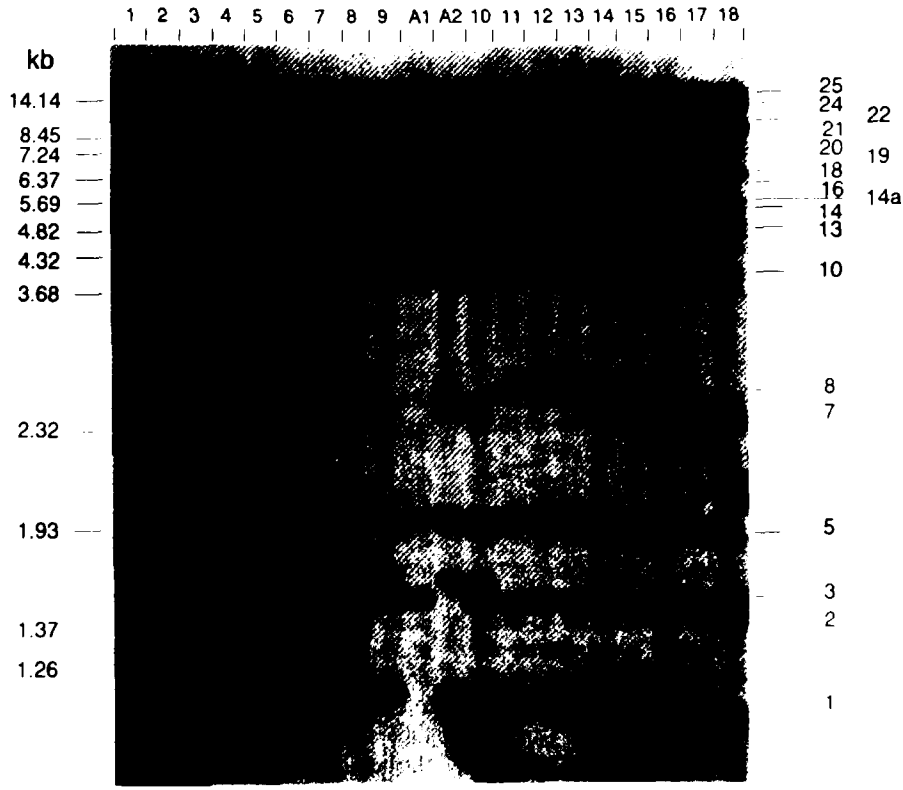

Fig. 3. Autoradiograph of a Southern blot hybridized with probe RG-57 and containing *Eco*RI-digested DNA isolated from the parental *Phytophthora infestans* isolates 80029 (A1) and 88133 (A2) and 18 isolates (1–18) grown from lesions on leaves which in the bioassay were brought in contact with soil containing oospores. The infested soil was exposed to natural weather conditions for 35 weeks. All 18 isolates appeared to be recombinant progeny. Size markers in kilobases (kb) are indicated on the left. Fragment numbers, corresponding to those used by Goodwin *et al.* (1992) and Drenth *et al.* (1993a) are indicated on the right.

Table 2. Survival of oospores of *P. infestans* maintained in soil at various temperatures.

Temperature[a] (°C)	Lesions[b]	Number of isolates tested by DNA fingerprinting[c]
−80	+	3 (100)
−20	+	3 (100)
15	+	8 (100)
30	+	4 (100)
35	+	3 (100)
40	−	NA
45	−	NA
50	−	NA

[a] The soil was first exposed to natural weather conditions for 35 weeks and subsequently treated at indicated temperatures for 48 h.
[b] Presence (+) and absence (−) of lesions in the bioassay.
[c] The percentage recombinant sexual progeny from isolates 80029 and 88133 is given in brackets.
NA, not applicable.

In the field plots numerous late-blight lesions appeared when the plants were 4 months old. Isolates were collected from lesions on stems and leaves and 76 of these were subjected to DNA fingerprint analyses. Although only one of the 76 single-lesion isolates obtained was a recombinant of the parental isolates, it can be concluded that oospores present in soil can infect potato plants and cause disease. The other 75 lesions were caused by unrelated *P. infestans* isolates. The cool, wet weather conditions in August 1993 resulted in potato late-blight epidemics throughout The Netherlands. It is obvious that spores were introduced into the field plots and caused infections but also the presence of oospores from other parental isolates cannot be excluded.

It is concluded that oospores of the 'new' *P. infestans* population can survive in soil during the winter in The Netherlands and are able to infect potato plants during the growing season. This conclusion is supported by the results of similar experiments conducted in the United Kingdom which showed that oospores of *P. infestans* produced *in vitro* were viable and able to germinate after exposure to natural weather conditions in soil for 8 months (Pittis & Shattock, 1994). It is expected that oospores of *P. infestans* can survive for many years in soil analogous to oospores of other oomycetous plant pathogens (McKay, 1957; Duncan, 1980; Duncan & Cowan, 1980). For the oospore survival experiment described here, many pots containing infested soil were buried outside and exposed to natural weather conditions. Regular sampling will provide a more detailed picture of the longevity of oospores of *P. infestans* in soil. In addition, the infested soil can be used to obtain a more precise picture of the conditions that influence survival, e.g. by exposing the soil to varying temperatures up to 35°C over several months or by incubating the infested soil in fields where crop rotation is practised. However, to be able to draw conclusions from this type of experiment, the bioassay decribed here must be modified to a quantitative form.

Oospores as inoculum source

There is limited information concerning the mechanism by which potato plants are infected by oospores under field conditions. It is suggested that during periods of high rainfall, oospores present on and in soil germinate, and the motile zoospores move to the surface where they infect leaves and stems of potato and tomato plants in contact with the soil surface. Splash dispersal during rainfall and overhead sprinkling irrigation may even lead to infections higher up in the leaf canopy.

We have shown that late-blight affected leaves can contain up to 6500 oospores/cm^2 When plant debris containing clumps of oospores is mixed with soil, the germinating oospores will produce mixtures of A1 and A2 zoospores. In case the oospores germinate synchronously it is more likely that lesions resulting from infections by these zoospores give rise to oospores than lesions caused by infection by air-borne sporangia.

Currently, little is known of the relative efficiency of oospores as inoculum source compared to other sources. However, the observation that the genetic diversity in the *P. infestans* population increased dramatically in the last decade shows that reproduction via oospores occurs (Drenth *et al.*, 1994). Sexual reproduction of *P. infestans* and the presence of oospores in soil acting as an inoculum source, in addition to overwintering mycelium in potato tubers, must surely influence the epidemiology of the late-blight disease and this, in turn, has many consequences for the control of late blight on potatoes. First, oospores in soil can start an epidemic whenever weather conditions are favourable for the fungus and where potato plants (planted or volunteer) are present. This may lead to earlier and more widely dispersed

foci of late-blight. Second, oospores will most probably infect leaves in the lower part of the leaf canopy and may escape protectant fungicides, mainly applied to the top of the canopy. Third, sexual reproduction will result in genetically more diverse *P. infestans* populations. Fourth, oospores present in potato leaves and stems may permit the fungus to survive unfavourable weather conditions within the season, from where they can start colonizing the remaining healthy foliage as soon as conditions become conducive for the disease again. Thus, the change from exclusively asexual reproduction, before 1980, to mixed sexual/asexual reproduction after 1980 (Drenth *et al.*, 1994) is likely to affect the strategies for controlling *P. infestans* in The Netherlands and other countries where 'new' populations have become established.

ACKNOWLEDGEMENTS

We thank Professor Dr J. C. Zadoks, Professor Dr Ir P. J. G. M. de Wit and Dr Ir L. J. Turkensteen for critical evaluation of the manuscript.

REFERENCES

Cameron JN, Carlile MJ. 1977. Negative geotaxis of zoospores of the fungus *Phytophthora*. *Journal of General Microbiology* **98**, 599–602.

Drenth A, Govers F. 1993. DNA fingerprinting of the potato late blight fungus, *Phytophthora infestans*. In: Schots A, Dewey FM, Oliver R, eds. *Modern Assays for Plant Pathogenic Fungi: Identification, Detection and Quantification*. Wallingford, UK: CAB International, 223–30.

Drenth A, Goodwin SB, Fry WE, Davidse LC. 1993a. Genotypic diversity of *Phytophthora infestans* in The Netherlands revealed by DNA polymorphisms. *Phytopathology* **83**, 1087–92.

Drenth A, Turkensteen LJ, Govers F. 1993b. The occurrence of the A2 mating type of *Phytophthora infestans* in The Netherlands; significance and consequences. *Netherlands Journal of Plant Pathology* **99** (Suppl. 3), 57–67.

Drenth A, Tas ICQ, Govers F. 1994. DNA fingerprinting uncovers a new sexually reproducing population of *Phytophthora infestans* in The Netherlands. *European Journal of Plant Pathology* **100**, 97–107.

Duncan JM. 1980. Persistence of mycelium of *Phytophthora fragariae* in soil. *Transactions of the British Mycological Society* **75**, 383–7.

Duncan JM. 1985a. Effect of temperature and other factors on in vitro germination of *Phytophthora fragariae* oospores. *Transactions of the British Mycological Society* **85**, 455–62.

Duncan JM. 1985b. Effect of fungicides on survival, infectivity and germination of *Phytophthora fragariae* oospores. *Transactions of the British Mycological Society* **85**, 585–93.

Duncan JM, Cowan JB. 1980. Effect of temperature and soil moisture content of persistence of infectivity of *Phytophthora fragariae* in naturally infested field soil. *Transactions of the British Mycological Society* **75**, 133–9.

Estrada SP. 1967. *Importancia de las Oosporas de* Phytophthora infestans *(Mont.) de Bary como Inoculo Primario del 'Tizon Tardio' de la Papa*. Chapingo, Mexico: Escuela Nacional de Agricultura Colegio de Postgraduados. M.Sc. thesis.

Frinking HD, Davidse LC, Limburg H. 1987. Oospore formation by *Phytophthora infestans* in host tissue after inoculation with isolates of opposite mating type found in the Netherlands. *Netherlands Journal of Plant Pathology* **93**, 147–9.

Fry WE, Drenth A, Spielman LJ, Mantel BC, Davidse LC, Goodwin SB. 1991. Population genetic structure of *Phytophthora infestans* in the Netherlands. *Phytopathology* **81**, 1330–6.

Fry WE, Goodwin SB, Matuszak JM, Spielman LJ, Milgroom MG, Drenth A. 1992. Population genetics and intercontinental migrations of *Phytophthora infestans*. *Annual Review of Phytopathology* **30**, 107–29.

Fry WE, Goodwin SB, Dyer AT, Matuszak JM, Drenth A, Tooley PW, Sujkowski LS, Koh YJ, Cohen BA, Spielman LJ, Deahl KL, Inglis DA, Sandlan KP. 1993. Historical and recent migrations of *Phytophthora infestans*: chronology, pathways and implications. *Plant Disease* **77**, 653–61.

Gallegly ME, Galindo J. 1958. Mating types and oospores of *Phytophthora infestans* in nature in Mexico. *Phytopathology* **48**, 274–7.

Goodwin SB, Drenth A, Fry WE. 1992. Cloning and genetic analyses of two highly polymorphic, moderately repetitive nuclear DNA's from *Phytophthora infestans*. *Current Genetics* **22**, 107–15.

Götz E. 1991. Untersuchungen zum auftreten des A2-paarungstyps bei *Phytophthora infestans* (Mont.) de Bary in Ostdeutschland. *Potato Research* **34**, 233–7.

Grinberger M, Kadish D, Cohen Y. 1989. Occurrence of the A2 mating type and oospores of *Phytophthora infestans* in potato crops in Israel. *Phytoparasitica* **17**, 197–204.

Hohl HR, Iselin K. 1984. Strains of *Phytophthora infestans* with A2 mating type behaviour. *Transactions of the British Mycological Society* **83**, 529–30.

Hord MJ, Ristaino JB. 1991. Effects of physical and chemical factors on the germination of oospores of *Phytophthora capsici in vitro*. *Phytopathology* **81**, 1541–6.

Juarez-Palacios C, Felix-Gastelum R, Wakeman RJ, Paplomatas EJ Devay JE. 1991. Thermal sensitivity of three species of *Phytophthora* and the effect of soil solarization on their survival. *Plant Disease* 75, 1160–4.

McKay R. 1957. The longevity of the oospores of onion downy mildew *Peronospora destructor* (Berk.) Casp. *Scientific Proceedings of the Royal Dublin Society. New Series* **27**, 295–307.

Mosa AA, Kobayashi K, Ogoshi A, Kato M, Sato N. 1991. Formation of oospores by *Phytophthora infestans* in inoculated potato tissues. *Annals of the Phytopathological Society of Japan* **57**, 334–8.

Perches ES, Galindo AJ. 1969. Supervivencia del *Phytophthora infestans* (Mont) de Bary del Tizón tardio de la papa y jitomate. *Agrociencia* **5**, 92–8.

Pittis JE, Shattock RC. 1994. Viability, germination and infection potential of oospores of *Phytophthora infestans*. *Plant Pathology* **43**, 387–96.

Ribeiro OK. 1983. Physiology of asexual sporulation and spore germination in *Phytophthora*. In: Erwin DC, Bartnicki-Garcia S, Tsao PH, eds. *Phytophthora, its Biology, Taxonomy, Ecology and Pathology*. St Paul, MN, USA: APS Press, 55–70.

Smoot AJ, Gough FJ, Lamey HA, Eichenmuller JJ, Gallegly ME. 1958. Production and germination of oospores of *Phytophthora infestans*. *Phytopathology* **48**, 165–71.

Spielman LJ, Drenth A, Davidse LC, Sujkowski LJ, Gu W, Tooley PW, Fry WE. 1991. A second worldwide migration and population displacement of *Phytophthora infestans*? *Plant Pathology* **40**, 422–30.