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Production, survival and infectivity of oospores of *Phytophthora infestans*

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The formation of oospores of *Phytophthora infestans* was studied in tomato and potato crops and volunteer plants under field conditions, and in laboratory tests with leaf discs of potato cultivars differing in their level of race-nonspecific resistance. Oospores were readily detected in blight-affected tomato leaflets and fruits, and in leaflets of field crops and volunteer potato plants. Oospores extracted from blighted potato leaflets yielded 13 oospore-derived progeny. Oospores were also produced following inoculation of leaf discs of eight potato cultivars expressing different levels of race-nonspecific resistance with a mixture of sporangia of A1 and A2 isolates. The highest numbers of oospores were produced in cvs Bintje (susceptible) and Pimpernel (resistant), and the lowest in Nicola (intermediate resistance). The relationship between lesions per leaflet and oospore incidence, affected by varying A1 : A2 ratios, was explored using a simple mathematical model, and validated by comparing actual oospore production in leaflets with multiple lesions of the race-nonspecific-resistant potato clone Lan 22-21 with the predictions generated by the model. Survival of oospores was investigated after their incorporation in either a sandy or a light clay soil in buried clay pots exposed to the local weather conditions. Over 6 years these soils were regularly assessed for their infection potential using floating leaflets in a spore-baiting bioassay. Sandy and clay soils contaminated with oospores remained infectious for 48 and 34 months, respectively, when flooded. Infections of floating potato leaflets occurred within 84–92 h and ceased after 11 days. Soil samples remained infective if dried and re-flooded on two, but not more, occasions.

Keywords: epidemiology, late blight, potato, race-nonspecific resistance, sources of inoculum, tomato

Introduction

Late blight is considered to be a disease of re-emerging importance in potato and tomato crops worldwide (Fry & Goodwin, 1997). *Phytophthora infestans*, the causal organism of potato late blight, is heterothallic and produces oospores when compatible strains of the opposite mating types A1 and A2 interact (Galindo & Gallegly, 1960). In Central Mexico, the presumed centre of diversity of *P. infestans*, oospores are abundantly formed in the field and were found to be infective for up to 2 years (Niederhauser, 1991).

The A2 mating type was not found outside Mexico until 1984, despite an extensive survey coordinated by the International Potato Center (CIP) from 1974–77 on isolates from Costa Rica, Germany, Japan, Mexico, Peru, Sweden, the Netherlands and the USA (L. J. Turkensteen, unpublished results). All European isolates tested were collected before 1976, the year in which a new population of *P. infestans* may have been introduced to Europe (Niederhauser, 1991).

Recent introductions of new strains into the USA and western Europe have included the introduction of the A2 mating type (Spielman *et al.*, 1991; Drenth *et al.*, 1993), which has enabled the pathogen to reproduce sexually and, as a consequence, to produce oospores. However, the role of oospores in the epidemiology of late blight is poorly understood (Andrivon, 1995). Since the work of Drenth *et al.* (1995), only few efforts have been made to clarify the role of oospores in initiating late blight epidemics (Pittis & Shattock, 1994; Andersson *et al.*, 1998). For example, it was not known whether oospore germination was confined to a single 'burst' of zoospore release, or whether germination took place throughout a period of favourable environmental conditions.

This study describes observational and experimental studies on the presence of oospores in field crops, on the

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formation of oospores in relation to field resistance and, over a 6 year period, survival and infection potential of oospores of *P. infestans* under field conditions.

Materials and methods

Sampling and sources of *P. infestans*

From 1986 to 1994, small experimental plots with *R*-gene differentials of potato and tomato were laid out to monitor the race structure of the *P. infestans* population at sites near Zeewolde, Renkum and Wageningen (all in the central part of the Netherlands). In September 1992, green fruits of tomato accession West Virginia (W.Va.) 63 (Gallegly, 1964) showing two or more lesions were collected from these plots and examined for the presence of oospores using several techniques, described below. Blight-affected leaflets and stems of potato cv. Bintje and tomato W.Va. 63 with two or more lesions were also collected at these sites. In addition, blighted tomato fruits of various, unidentified cultivars were sampled at an allotment garden complex near Ede (Turkensteen *et al.*, 1996).

In September 1993 and 1994, late blight was commonly found in the starch potato-growing area in the north-eastern Netherlands. From each of 20 commercial potato crops, five leaflets, each with two lesions, were collected each year. The leaflets were incubated and nondestructively examined for the presence of oospores, and oospore-derived offspring were established.

In 1994 the experimental potato clone Lan 22-21 (Turkensteen, 1993) was grown in an allotment garden complex near Ede. Lan 22-21 is an experimental CIP clone with a high level of race-nonspecific resistance (see Table 2). At the end of September, leaflets of this clone with two or more distinctly visible lesions per leaflet were collected and examined for the presence of oospores.

In 1998, extremely high incidences of volunteer potato plants were observed in farmers' fields. High levels of late blight developed on these unprotected plants during the growing season. During July, August and September, six such fields (three wheat, two sugar beet and flax) were visited in the starch potato-growing area. Leaflets with two or more lesions were collected and examined for the presence of oospores.

Detection and viability of oospores in leaf tissues

Immediately after collection, every leaflet was placed in a 9 cm diameter Petri dish containing 10 mL 2% water agar. Leaflets were incubated in a climate chamber at 15°C with a 16 h photoperiod at a light intensity of 12 W m^{-2} for 10–14 days. After a further 2 week incubation period at 20°C in the light, the leaflets were kept for 2 weeks in the dark at ambient temperature to decompose. Leaf tissues were cleared by boiling in 96% ethanol for 10 min, mounted in glycerine, and examined microscopically for the presence of oospores.

Where oospore viability was to be maintained, the leaflets were left to decompose for 2 weeks at 15°C in the dark before microscopic observation. Oospore-derived offspring were obtained from these leaflets. For this purpose, 12 oospore-containing leaflets were frozen overnight at −20°C to avoid infection from mycelial fragments or sporangia, and each leaflet subsequently homogenized for 60 s at 20 000 r.p.m. using an IKA T20 homogenizer with S20 probe (IKA, Staufen, Germany). The homogenate was separated by means of 100 and 50 μm filters, and the oospore-containing filtrate spread on 0·8% water agar plates containing ampicillin (200 mg l^{-1}) and PCNB (75% WP, 67 mg l^{-1}) and incubated at 15°C in the dark for 10 days. The viability of oospores was determined by collecting germinating oospores and transferring them to tuber slices of cv. Bintje cut from surface-sterilized tubers. When sporulating mycelium was present after an incubation period of 5–7 days at 15°C in the dark, small pieces of mycelium were placed on the lower epidermis of leaflets of cv. Bintje placed with the abaxial side up in 9 cm Petri dishes containing 10 mL 2% water agar. Inoculated leaflets were kept in a climate chamber at 15°C with a 16 h light period provided by type 33 fluorescence tubes (12 W m^{-2}). Leaflets densely covered with sporulating mycelium were obtained after 7 days' incubation. Oospore-derived offspring were cultured and subsequently identified as *P. infestans* based on the colony morphology of cultures grown on Rye A agar (Caten & Jinks, 1968). Mating type was determined by pairing isolates individually with tester strains F80029 (A1 mating type) and F88133 (A2 mating type) on Rye A agar according to standard procedures (Forbes, 1997).

For long term storage sporangial suspensions were prepared in a 15% dimethylsulphoxide solution, transferred into 1·8 mL cryovials, cooled to −40°C at a rate of approximately 0·5°C min^{-1} using a Neslab CC 60 II immersion cooler (Neslab Instruments Inc., Portsmouth, USA) and an alcohol bath, and stored in liquid nitrogen.

Relationship between lesions per leaflet and oospore incidence

The relationship between the ratio of A1 to A2 mating-type isolates in samples from a location and the percentages of leaflets with multiple lesions in which oospores may be formed was examined in isolates from allotment gardens at Ede in 1994. A total of 168 leaflets, each with two or more lesions, was collected by the end of September and examined for the presence of oospores. Oospores were detected in 56 blighted leaflets.

In this case the mating type ratio can be derived from the proportion of leaflets in which oospores are formed. Let p and q represent the frequencies of *P. infestans* strains of A1 and A2 mating types, respectively, ranging

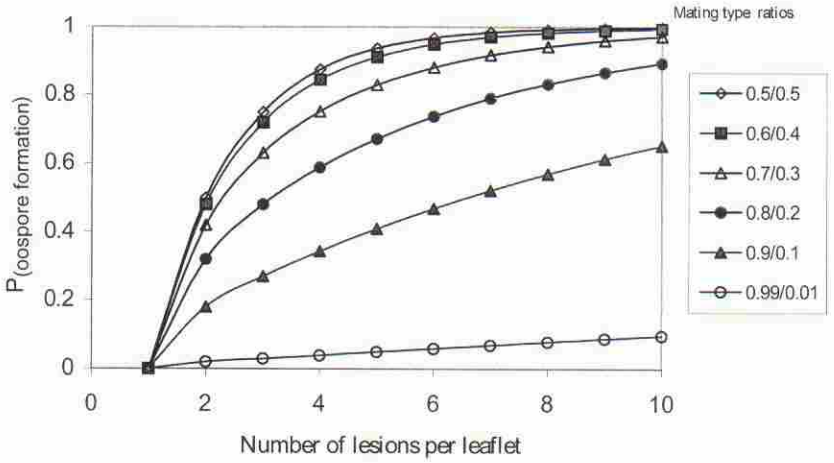

Figure 1 Probability of oospore formation by *Phytophthora infestans* in an infected potato leaflet based on the theoretical relationship between mating type ratio and the number of lesions per blighted leaflet. Data are derived from the equation $P_{(\text{oospore formation})} = 1 - (p^n + q^n)$, where p and q represent the frequencies of *P. infestans* strains of A1 and A2 mating type, respectively, and n represents the number of distinct lesions per leaflet.

from 0·0 to 1·0. In the case of two lesions per leaflet, the probability of oospore formation is estimated as $2pq$ and the probability of absence of oospores as $p^2 + q^2$. The probability of oospore production in leaflets with more than two lesions is $1-(p^n + q^n)$, where n represents the number of distinct lesions per leaflet. The expected fraction of leaflets with oospores was calculated for two to 10 lesions per leaflet for a number of mating type ratios, and the results of this simulation are presented in Fig. 1.

These calculations were made under the following assumptions: (i) each individual fungal isolate in the allotment garden has an equal chance of establishing a new lesion (no founder-effects or preferential infection); and (ii) each pair of isolates with opposite mating type is able to form oospores. Even so, the model can at best give only an indication of the chance that oospores will be formed in infected leaflets. No inferences are made about the number of oospores being formed. The validity of the oospore formation model was evaluated by comparing actual oospore production in leaflets with multiple lesions of potato clone Lan 22-21 with the model-predicted probability of oospore formation.

First, the estimated proportion of oospore-containing leaflets was calculated for two, three and four lesions per leaflet for a number of mating-type ratios. These fractions were compared with the observed proportions, and the goodness of fit for mating type ratios was determined using contingency tables in GENSTAT (Payne *et al.*, 1993).

Oospore formation in race-nonspecific susceptible and resistant potato cultivars

The potential oospore production in late blight-affected potato cultivars with different levels of race-nonspecific resistance was assessed in inoculated leaf discs of seven cultivars and one single breeding clone. Leaflets were produced by planting seed tubers in 10 L plastic pots containing steamed potting soil and maintaining in a greenhouse at 20/15°C day/night temperature, with a 16 h light period at 18 W m^{-2} and a relative humidity of 80%.

Two isolates, F80029 (A1) and F88133 (A2) (Drenth *et al.*, 1995), were retrieved from liquid nitrogen storage and recovered on tuber slices of the susceptible cv. Bintje by incubating in the dark at 15°C for 5–7 days. When sporulating mycelium was present, small pieces of mycelium were placed on the lower epidermis of leaflets of the same cultivar placed with the abaxial side up in 9 cm Petri dishes containing 10 mL 2% water agar, and maintained at 15°C with a 16 h light period at 12 W m^{-2}. Sporangial inoculum was prepared by dipping sporulating inoculated leaflets in 10 mL tap water, which was then filtered through a 50 μm filter. Finally, sporangia were collected on a 15 μm filter, resuspended, and the concentration adjusted to 2×10^4 sporangiospores mL^{-1} using a Coulter Counter Z1 (Beckman Coulter BV, Mijdrecht, The Netherlands). The two inocula were then mixed.

Leaf discs (14 mm in diameter) from fully grown leaflets of the fourth or fifth leaf layer from the top of 8- to 10-week-old plants were placed abaxial side up in 9 cm diameter Petri dishes filled with 10 mL 2% water agar. Four replicates of three leaf discs per cultivar were inoculated with 10 μL droplets of the mixed A1/A2 sporangial suspension at the centre of each disc. Dishes with the inoculated leaf discs were placed in plastic trays, enclosed in transparent polythene bags to inhibit desiccation, and incubated for 14 days (15°C) at a light intensity of 12 W m^{-2}, 16 h light per day. The experiment was repeated.

Leaf discs were clarified in ethanol as described previously, and mean numbers of oospores per disc were

assessed using bright-field illumination. An oospore index was defined in terms of oospore formation using a scale from 0 to 13: 0 = 0; 1 = 1–5; 2 = 6–10; 3 = 11–50; 4 = 51–100; 5 = 101–500; 6 = 501–1000; 7 = 1001–2000; 8 = 2001–3000; 9 = 3001–4000; 10 = 4001–5000; 11 = 5001–10 000; 12 = 10 000–15 000; 13 = >15 000 oospores cm^{-2}. ANOVA was performed by Fisher's least significant difference test at the 5% probability level using GENSTAT 5 version 3·2·1 (Payne *et al.*, 1993). The relationship between the level of race-nonspecific host resistance and oospore production, expressed as an index value, was evaluated by means of a polynomial regression model.

Oospore survival under field conditions

Oospores, formed in potato leaves after inoculation with a mixture of isolates F80029 and F88133, were mixed with river sand or light clay (825 oospores cm^{-3} soil) and placed in 800 mL clay pots as described by Drenth *et al.* (1995). Pots were buried in a field on 9 November 1992. From 16 November 1992 until 10 June 1998, soil infectivity was assessed by means of a spore-baiting bioassay (Drenth *et al.*, 1995). Pot contents (approximately 800 cm^3) were placed in a freezer (−20°C) for 24 h in order to eliminate contamination by sporangia or mycelium of *P. infestans*. After thawing the soil was transferred to a plastic container with a transparent lid and mixed with 2 L tap water. After 2 days' incubation at 15°C under a light intensity of 12 W m^{-2}, 16 h/day, 15–20 greenhouse-grown leaflets of cv. Bintje were floated abaxial side up on the water and incubated for 2 weeks. Developing lesions were counted and isolations were made from these by placing pieces of infected leaf tissue under potato tuber slices that were then incubated at 17–19°C for 5 days until sporulation occurred. An inoculation needle was used to transfer sporangia from the tuber slice to Rye A agar (Caten & Jinks, 1968), supplemented with ampicillin (200 mg l^{-1}), Benlate (50% WP, 100 mg l^{-1}), PCNB (75% WP, 67 mg l^{-1}), polymixin B (50 mg l^{-1}) and rifampicin (20 mg l^{-1}), and then incubated at 20°C for 1–2 weeks. Subsequently small pieces of selective medium containing actively growing *P. infestans* hyphae were transferred to Rye A agar plates. Isolates were maintained on Rye A agar at 20°C and subsequently stored in liquid nitrogen.

Infectivity of oospore-contaminated soils

The minimum response time for oospores to germinate in flooded soil, release zoospores and produce sporulating lesions on floating leaflets was established, and the longevity of soil infectivity after flooding was also determined. For this purpose, 10 clay pots of sandy soil containing oospores (as described above) were taken at random on 28 February 1995. These soil samples had been exposed to local weather conditions since November 1992. Ten simultaneous spore-baiting bioassays were initiated to test the infection potential of a 200 g sample of sandy soil. For this purpose, 20 leaflets of cv. Bintje were placed on the water surface at the start of each bioassay, and replaced by fresh ones twice a day during the first 10 days of the experiment. After 10 days the leaflets were replaced every 5 days until day 25. Each of the exposed leaflets was placed in a separate Petri dish and incubated at 15°C with 16 h light (12 W m^{-2}) per day. After 5–7 days the leaflets were examined for the presence of lesions.

In order to determine the germination potential of oospores in a sandy soil, a sequential soil infectivity test was performed by alternately flooding and drying the soil samples. A sample of approximately 500 g sandy soil from the survival experiment was used. The experiment started on 28 October 1994 (day 0) with bioassay 1. On day 3 of bioassay 1, 20 leaflets of cv. Bintje were placed on the water surface, and were subsequently replaced by new ones every 3 days. The exposed leaflets were incubated and examined as described above. On 16 November bioassay 1 was terminated and the water was poured off. The soil was air-dried at room temperature (15–20°C) and then divided into three equal portions. One portion (A) was frozen at −20°C for 24 h in order to eliminate viable sporangia and zoospores that might still have been present. The other soil samples (B and C) were stored at 5°C and subsequently frozen (−20°C) before the following sequential bioassay was initiated. Bioassay 2 started on 25 November when 1·5 L water was added to soil sample A. Bioassay 2 was terminated on 8 December when new lesions ceased to appear. The water was poured off and the soil of sample A was air-dried and frozen for 24 h on 21 December. The second sequential bioassay (3) with soil sample A started on 22 December. In total the sequential testing procedure was carried out eight times in 7 months. Soil sample A was tested in all sequential bioassays, sample B in assays 1, 4, 5, 6, 7 and 8, and sample C in assays 1, 7 and 8.

Results

Oospore formation under natural conditions

In 1992 all four sites showed blight-affected tomatoes and potatoes when samples were collected. Oospores of *P. infestans* were detected at three of the four sites in the central Netherlands, in 12 out of 35 affected green tomato fruits (Table 1). At the Wageningen site oospores were found in eight of 20 leaflets of tomato line W.Va. 63, and in four of 60 leaflets of cv. Bintje showing two or more lesions per leaflet. However, oospores were not observed in 310 infected stems of cv. Bintje with coalescent lesions sampled at Wageningen, nor in potato and tomato leaflets with multiple lesions collected at Renkum and Zeewolde.

In the following 2 years in the starch potato region in the Province of Drenthe, seven out of 98 and five out of 100 potato leaflets with two blight lesions, respectively,

Table 1 Incidence of oospores of *Phytophthora infestans* in blighted green tomato fruits, September 1992

Source	Cultivar	Number of tomato fruits	
		With blight	With oospores
Wageningen, Gelderland	W.Va. 63	12	8
Renkum, Gelderland	"	7	2
Zeewolde, Flevoland	"	7	0
Allotment gardens, Ede	Moneymaker and others	9	2

yielded oospores. Oospores were extracted from each oospore-containing leaflet, and oospore-derived progeny were established. A total of 13 *P. infestans* cultures were established from three leaflets (two in 1993 and one in 1994). The A1 : A2 ratios of isolates originating from each leaflet were 5 : 1 and 0 : 3 (1993 sample) and 1 : 3 (1994 sample), leading to a pooled A1 : A2 ratio of 6 : 7.

In September 1994, oospores were found in leaflets with multiple lesions collected from potato clone Lan 22-21 in an allotment garden complex at Ede (Fig. 2). A total of 168 leaflets with two or more lesions were examined for the presence of oospores. Oospores were found to be present in 56 leaflets (Table 2). The percentage of oospore-containing leaflets ranged from 26% for leaflets with two lesions to 47% for those with four lesions. However, no significant differences in the likelihood of oospore formation were observed when the probability of oospore formation for leaflets with either two, three, four or more than four lesions per leaflet was compared using contingency testing ($\chi^2_{[3]} = 3{\cdot}55$; $P = 0{\cdot}314$; Table 2).

Calculations based on the postulated relationship between lesions per leaflet and oospore incidence, using the oospore incidence data from potato clone Lan 22-21 at Ede in 1994, predicted a mating type ratio at that location of 1 : 5, based on the goodness-of-fit test statistic ($\chi^2_{[2]} = 0{\cdot}20$; $P = 0{\cdot}906$). The actual (observed) mating type ratio in the same allotment garden complex in July 1994 was 1 : 3, based on mating type determination of 78 isolates.

Oospores were detected in 61% of the multiple lesion-carrying leaflets collected from volunteer potato plants in six commercial fields in 1998 ($n = 462$) (Table 3). The percentage of leaflets with oospores present ranged from 33·3% at Velingerveen to 87·0% at Rolde. The proportion of leaflets containing oospores was higher in samples collected in September than in those collected in July/August.

Oospore formation in susceptible and partially resistant potato varieties

Oospores were detected in leaf tissue of all potato cultivars tested (Table 4). The highest numbers of oospores were observed in cvs Bintje and Pimpernel, and the lowest in Bildtstar, Nicola and Spunta. No significant difference in oospore production between the two experiments ($P = 0{\cdot}281$) was observed, and no significant cultivar–experiment interaction occurred ($P = 0{\cdot}501$). Differences in oospore production between cultivars proved to be highly significant ($P < 0{\cdot}001$). For example, cv. Pimpernel consistently produced large numbers of oospores (average oospore

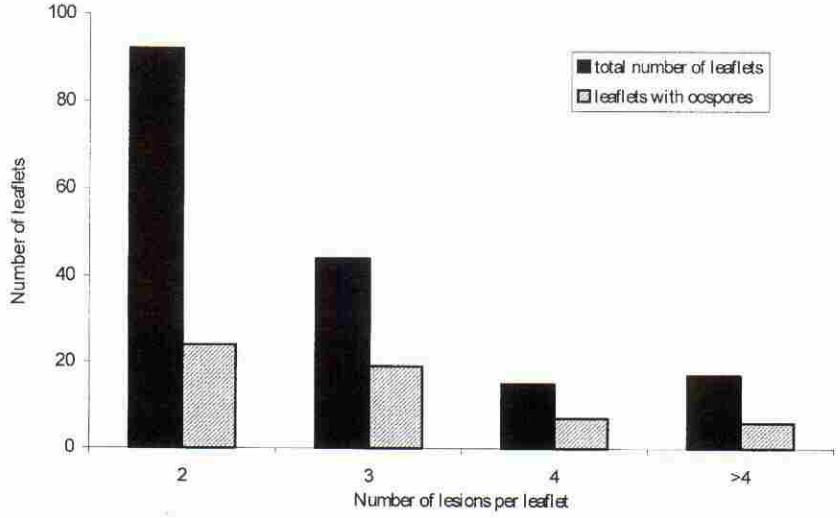

Figure 2 Incidence of oospores of *Phytophthora infestans* in naturally infected leaflets of clone Lan 22-21 with two or more lesions growing in an allotment garden at Ede in 1994.

Table 2 Contingency test of observed and expected numbers of leaflets containing oospores of *Phytophthora infestans* in the race-nonspecific breeding clone Lan 22-21 growing in an allotment garden at Ede in 1994

Lesions per leaflet	Total number of leaflets	Leaflets with oospores		Test statistic
		Observed (O)	Expected (E)	$(O-E)^2/E$
2	92	24	30·7	1·45
3	44	19	14·7	1·28
4	15	7	5·0	0·80
>4	17	6	5·7	0·02
Total	168	56	56	3·55[a]

[a] $\chi^2_{[3]} = 3{\cdot}55$; $P = 0{\cdot}314$ (ns).

index 9·45), while cv. Bildtstar produced few (average oospore index 0·75). Except for cv. Bintje, there appears to be a nonlinear relationship between foliar resistance, as presented by a 1–9 resistance rating (1 = very susceptible, 9 = highly resistant according to the Dutch national list of recommended potato cultivars; Ebskamp *et al.*, 1998), and oospore production. A third-order polynomial model gave the best fit, with significant associations between oospore production and the regression coefficients ($P < 0{\cdot}004$). The fitted curve has the following equation:

$$\text{oospore index} = 29{\cdot}3 - 15{\cdot}9 \times \text{resistance rating} + 2{\cdot}7 \times \text{resistance rating}^2 - 0{\cdot}1 \times \text{resistance rating}^3$$

Only 59% of the variability of oospore production could be attributed to the foliar resistance rating ($R^2_{\text{adjusted}} = 0{\cdot}593$). The data for Bintje clearly represent outlying values for oospore production (Table 4).

Oospore survival under field conditions

Oospores remained infectious in clay and sandy soils for up to 34 and 48 months after the start of the experiment, respectively (Fig. 3). The infection pattern, as assessed by means of the spore-baiting assay, was irregular and did not indicate a seasonal pattern of inducible oospore germination. Incidence of infection was higher in sandy than in clay soil. The infectivity of oospore-contaminated clay soil decreased faster than that of sandy soil, with the highest infectivity after 7 and 28 months' exposure to the prevailing field conditions, respectively.

Infectivity of oospore-contaminated soils

The first blight lesions were detected by the floating leaflet bioassay at 84–96 h after flooding of the oospore-contaminated soil (Fig. 4). The total number of lesions reached a maximum of 21 after 240 h.

The results obtained by sequential testing of an oospore-contaminated sandy soil using the floating leaflet bioassay are presented in Table 5. Soil sample A was tested eight times within a period of 7 months. Infection became visible in bioassays 1, 2 and 4, but not in assays 3 and 5–8. High numbers of infections were found during the second bioassay of soil samples B and C. When tested for the third time, both these soil samples showed a dramatic drop in soil infectivity. None of the three soil portions was exhausted after a single spore-baiting experiment. Soil sample A remained infectious after three periods with frosts between 28 October 1994 and 17 January 1995.

Discussion

Viable oospores of *P. infestans* are formed in field crops in the Netherlands. Both potato and tomato leaves serve as good hosts for the formation of oospores. No oospores were found in potato stems.

Oospores were readily found when A1 : A2 mating type ratios were in the ratio 1 : 3 under field conditions. In commercial potato crops, A1 mating type percentages ranged from 30 to 99% according to year and region (Drenth *et al.*, 1994; L. J. Turkensteen, unpublished results). In a recent study, Cohen *et al.* (1997) showed that oospore formation is not significantly inhibited by skewed mating type ratios. In their study, mixed A1/A2 sporangial inocula were used to infect leaflets of potato and tomato under controlled conditions.

Based on the hypothesis that more lesions on a leaflet will increase the probability of both A1 and A2 strains being present, thus facilitating oospore formation, we might expect to find a positive correlation between the

Table 3 The presence of oospores of *Phytophthora infestans* in leaflets with multiple lesions sampled from volunteer potato plants in the starch potato region of the Netherlands in 1998

Locality	Sampling date	Crop	Potato cultivar (volunteers)	Total number of leaflets	% Leaflets with oospores
Muntendam A	29 July	Winter wheat	Karnico	101	35·6
Muntendam B	4 August	"	Karnico	54	35·2
Velingerveen	4 August	"	Kartel	15	33·3
Hooghalen	10 September	Flax	Elkana	109	68·8
Kooyenburg	10 September	Sugar beet	Florijn	106	76·4
Rolde	10 September	"	Florijn	77	87·0

Table 4 Oospore formation by *Phytophthora infestans* isolates F80029 (A1) and F88133 (A2) in inoculated leaf discs of potato cultivars with different levels of race-nonspecific resistance

Cultivar	Foliar resistance rating[a]	Oospore index[c] expt 1	expt 2
Bintje	3	12·1 a	11·3 a
Bildtstar	3·5	0·4 d	1·1 d
Desiree	5	2·4 d	3·2 d
Spunta	5	0·5 d	0·6 d
Nicola	5·5	1·1 d	0·3 d
Surprise	7	5·3 c	5·8 c
Pimpernel	8	10·2 a	8·7 b
Lan 22-21	12[b]	7·8 b	4·0 c

[a]Foliar resistance, 1–9 scale ranging from highly susceptible (1) to highly resistant (9), according to the Dutch National List of Recommended Potato Cultivars 1998.

[b]Resistance rating based on experimental data (L.J. Turkensteen, unpublished results).

[c]Values represent the mean oospore index per leaf disc, averaged over four replicates of three discs each. Oospore indices assigned the same lower case letter are not significantly different within experiments according to Fisher's LSD test ($P = 0{\cdot}05$).

number of lesions and the incidence of oospore-containing leaflets. The proportion of blighted leaflets of clone Lan 22-21 containing oospores actually increased from 26% to 47% for leaflets with two and four lesions, respectively, but the contingency test statistic provided no evidence for the presence of a trend, based on the four lesion incidence classes. The relationship presented here is based on assumptions that are not usually met under field conditions. It is not realistic to assume that each individual fungal strain in the local 'isolate pool' has an equal chance of establishing a new lesion on a random host plant, as this ignores the spatial distribution of both isolates and hosts. Strong founder effects will greatly increase the possibility of a common ancestry for lesions on a single leaflet. The formula presented might prove more useful as guide for breeding efforts to minimize the probability of oospore formation in infected potato crops. Reducing the number of lesions formed, by selecting for an infection resistance component of race-nonspecific resistance, will reduce the chance of oospore formation even under situations in which A1 : A2 ratios are close to 1 : 1.

The level of race-nonspecific resistance present in the cultivars tested could explain a considerable part (59%) of the observed variation in oospore production. Elguezabal (1993) provided more evidence for the presence of a positive association between race-nonspecific resistance and oospore production. In a study of Mexican potato cultivars and breeding lines, resistant cultivars such as Norteña and Tollocan showed a remarkable and significantly higher level of oospore production than the susceptible cv. Alpha. In a recent study, Hermansen *et al.* (2000) reported on the presence of oospores in leaves of potato cultivars with intermediate levels of late blight resistance from three locations in the southern part of Norway. These observations are supported by another recent report on oospore production in partially resistant potato cultivars (Hanson & Shattock, 1998). When whole

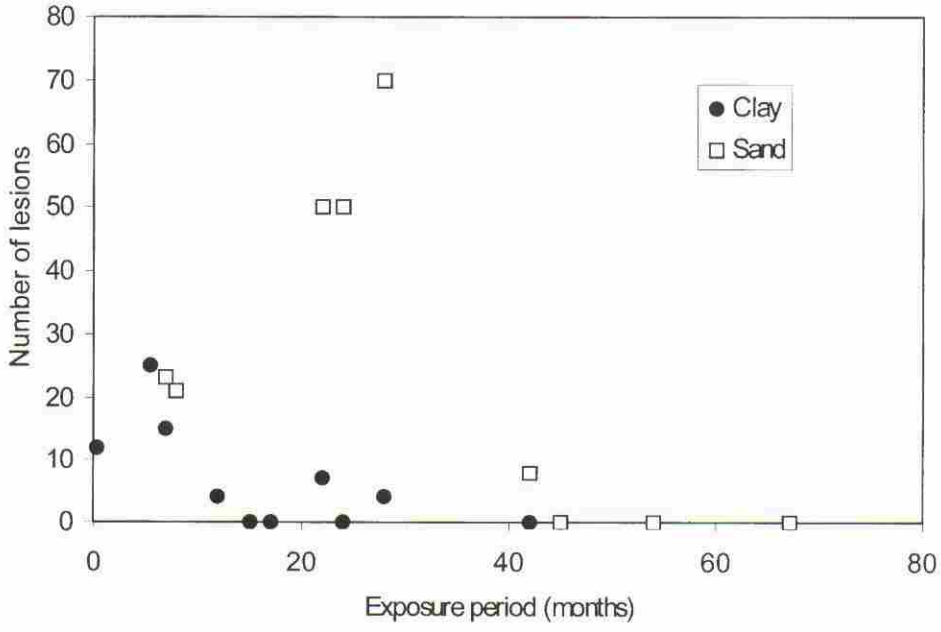

Figure 3 Infectivity of clay and sand soils contaminated with oospores of *Phytophthora infestans* over 6 years. Each data point represents the number of single lesions observed in a single spore-baiting floating leaflet bioassay. Exposure period to local Dutch climatic conditions after start of survival experiment in November 1992.

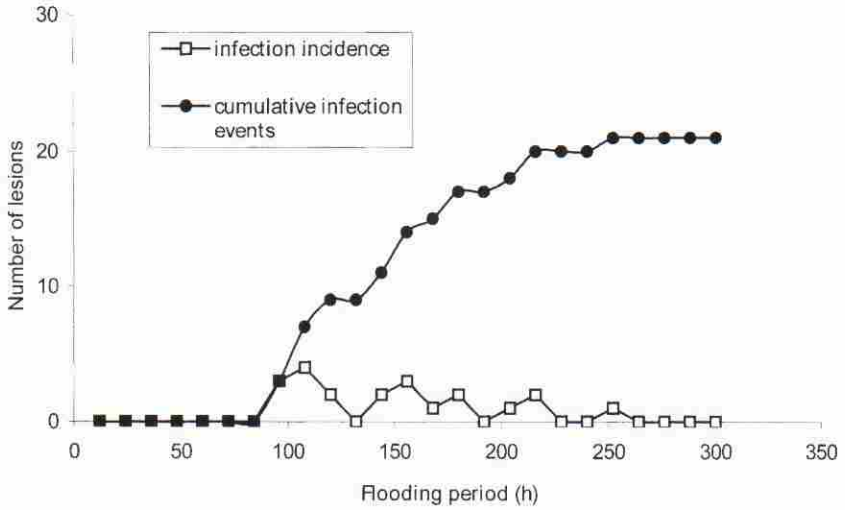

Figure 4 Cumulative mean number of infections by *Phytophthora infestans* on leaflets of cv. Bintje floating on a flooded oospore-contaminated sandy soil. Number of lesions in floating bioassay: each value represents the average of 10 bioassays.

plants and leaf discs of 10 cultivars were inoculated, numbers of oospores were highest in cultivars with medium levels of race-nonspecific resistance. No oospores were observed in the highly susceptible cvs Home Guard and Bintje after inoculation of field and plastic tunnel-grown whole plants.

As postulated by Hanson & Shattock (1998), factors such as methodology and choice of parental isolates and cultivars might strongly affect oospore production. This is supported by the present observations that cvs Bintje and Bildtstar, with comparable levels of race-nonspecific resistance, showed large differences in oospore formation in inoculated leaf discs.

The differences found in oospore production in response to levels of host resistance (Drenth *et al.*, 1995; Hanson & Shattock, 1998; this study) suggest that oospore formation is also affected by factors other than the level of partial resistance, to which 59% of the variability has been attributed. One such factor may be sterol content of the host. Oospore formation is promoted by sterols *in vitro* (Elliot, 1983), and considerable differences in sterol production between potato cultivars have been reported (Langcake, 1974). This factor might explain the very high numbers of oospores found in cvs Bintje and Pimpernel, which have low and high race-nonspecific resistance, respectively. The practical significance of Bintje as a potentially dangerous cultivar in terms of oospore production cannot be neglected. Bintje is still widely grown in the Netherlands (90 000 ha, approximately 50% of the area under potato cultivation). Based on the oospore formation experiment and the 1998 survey in Drenthe, it is concluded that the replacement of this susceptible cultivar by more resistant ones will not automatically lead to a reduction in oospore formation in field crops. It is recommended that future potato-breeding programmes should include an assessment of oospore production in progenitors and advanced breeding materials.

Table 5 Soil infectivity data obtained by a sequential testing procedure of a sandy soil artificially contaminated with oospores of *Phytophthora infestans* by means of a spore-baiting, floating leaflet bioassay

	Lesion incidence in bioassay		
Bioassay date	Soil sample A	Soil sample B	Soil sample C
1. 28 Oct 1994	15[a]	15*	15[a]
2. 25 Nov 1994	8		
3. 22 Dec 1994	0		
4. 17 Jan 1995	2	38	
5. 7 Feb 1995	0	3	
6. 7 March 1995	0	0	
7. 7 April 1995	0	0	41
8. 15 May 1995	0	0	10

[a]All three soil samples were tested together in bioassay 1. In total, 45 lesions were detected in this first bioassay.

Oospores from cross F80029 × F88133 exposed to local weather conditions survived for up to 48 months. Oospore survival time may vary; Pittis & Shattock (1994) reported much shorter survival periods, but longer periods could also be possible as longevity might depend on the parents of the cross and the soil conditions. For example, in the present work, after four periods of 25 days of flooding soil samples, no more oospores appeared to be viable. In a laboratory trial, all oospores germinated within 2 months when stored at 15°C in water in a beaker glass (unpublished results). It appears that the water content of soils may have a major impact on longevity.

No seasonal germination pattern was observed. Although germination peaks were recorded, the general conclusion may be that oospore germination in soil is a

rather erratic process. At present, all the prerequisites are present for oospores to play an active role in the epidemiology of *P. infestans* in the Netherlands. Both mating types are commonly found in field crops and, as a consequence, oospore formation in field crops has become a reality. As oospores of *P. infestans* can survive for up to 4 years, they may form a source of initial inoculum for most common crop rotation schemes.

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