Plant Pathology (2003) **52**, 595–603

Epidemiological importance of *Solanum sisymbriifolium*, *S. nigrum* and *S. dulcamara* as alternative hosts for *Phytophthora infestans*

W. G. Flier[a]*†, G. B. M. van den Bosch[a] and L. J. Turkensteen[b]

[a]*Plant Research International, PO Box 16, 6700 AA Wageningen; and* [b]*Kampsweg 31, 9418PD Wijster, the Netherlands*

Lesions of *Phytophthora infestans* were found on woody nightshade (*Solanum dulcamara*), black nightshade (*S. nigrum*) and *S. sisymbriifolium* during a nationwide late blight survey in the Netherlands in 1999 and 2000. Pathogenicity and spore production of *P. infestans* isolates collected from potato (*S. tuberosum*), *S. nigrum*, *S. dulcamara* and *S. sisymbriifolium* were determined on several host plant species, and oospore formation in naturally infected and inoculated foliage of hosts was quantified. The present population of *P. infestans* in the Netherlands is pathogenic on *S. nigrum*, *S. dulcamara* and *S. sisymbriifolium*. Oospores were produced in leaves of *S. nigrum*, *S. dulcamara* and *S. sisymbriifolium* following infection with A1 and A2 isolates. Therefore these plant species should be regarded as alternative hosts for the late blight pathogen. In the case of *S. nigrum* and *S. dulcamara* infection was a relatively rare event, suggesting that diseased plants do not significantly contribute to the overall late blight disease pressure present in potato-production areas. Oospore production in ageing *S. nigrum* and *S. dulcamara* plants in autumn, however, may generate a considerable source of (auto) infections in following years. Considerable numbers of sporangia and oospores were produced on *S. sisymbriifolium* following infection with *P. infestans*. Additional field infection data are needed to evaluate the epidemiological consequences of a commercial introduction of *S. sisymbriifolium* as a potato cyst nematode trap crop.

Keywords: black nightshade, host range, nonhost resistance, potato late blight, woody nightshade

Introduction

The oomycete *Phytophthora infestans*, the cause of late blight in potato and tomato, is considered one of the most important pathogens of potatoes worldwide. The pathogen affects leaves, stems and tubers, leading to serious yield losses and high costs for chemical control. In Europe, potatoes and tomatoes are the principal hosts for *Phytophthora infestans*. An introduction of a diverse population of *P. infestans* into Europe that is likely to have originated in central Mexico (Spielman *et al*., 1991) triggered important changes in the population structure and epidemiology of *P. infestans* in western Europe. Evidence is accumulating that *P. infestans* reproduces sexually in many countries in western Europe (Drenth *et al*., 1994; Sujkowski *et al*., 1994; Andersson *et al*., 1998; Turkensteen *et al*., 2000). Sexual reproduction changes the epidemiology of potato late blight for two reasons. First, infected potatoes can produce oospores, the sexual resting spores that serve as survival structures and primary inoculum. Second, sexual reproduction provides a means of meiotic recombination and increases genetic variation in sexually reproducing populations of *P. infestans*.

Apart from oospores surviving in the soil, *P. infestans* overwinters in potato tubers in storage, in potato cull piles and in tubers left on the field after harvest (van der Zaag, 1956; Hirst & Stedman, 1960). Infected tubers and oospores are thought to be the initial sources of inoculum in the following growing season (Hirst & Stedman, 1960; Andersson *et al*., 1998; Turkensteen *et al*., 2000). The introduction of a highly variable and sexually reproducing *P. infestans* population in the Netherlands raises concerns about the potential of *P. infestans* to infect other solanaceous plant species. A broadening of host range could interfere with current late blight management strategies, as alternative hosts may act as overwintering hosts, and could serve as refuges for the pathogen from which *P. infestans* can infect potato crops throughout the growing season. Few reports exist on colonization of alternative hosts in Europe prior to the displacement of the US-1 clonal lineage by a new *P. infestans* population. The

*To whom correspondence should be addressed.

†E-mail: wilbert.flier@wur.nl

Accepted 6 June 2003

© 2003 BSPP

herbaceous perennial woody nightshade (*Solanum dulcamara*) and annual black nightshade (*S. nigrum*) have both been identified as native host plants (Erwin & Ribeiro, 1996), but confirmed records of field infections are scarce (Hirst & Stedman, 1960; Patino *et al*., 1996) and *S. nigrum* is often regarded as a nonhost for *P. infestans* (Pieterse *et al*., 1994; Platt, 1999; Vleeshouwers *et al*., 2000). Field infection of *S. dulcamara* by *P. infestans* has recently been reported in Ireland (Cooke *et al*., 2002). Adaptation of potato isolates on *S. dulcamara* by means of recurrent inoculations on detached leaves has been described by Turkensteen (1973). In Wales, blighted *S. nigrum* plants were found, and compatible *P. infestans* isolates have been collected (D. S. Shaw, University of Wales, Bangor, UK, personal communication). A nationwide late blight survey aimed at characterizing early outbreaks of late blight in the Netherlands from 1999 to the present has repeatedly confirmed the presence of sporulating lesions of *P. infestans* on *S. dulcamara*, *S. nigrum* and *S. sisymbriifolium* (W. G. Flier, unpublished results). In the Netherlands, both *S. nigrum* and *S. dulcamara* are native plant species. The annual *S. nigrum* has little frost resistance, while the extensive root system and woody stems of *S. dulcamara* classify the latter species as a herbaceous perennial. Originating from the pampas of South America, *S. sisymbriifolium* has been regarded as a nonhost species for *P. infestans* (Sen, 1997; Scholte, 2000) and is currently being evaluated as a potential trap crop for potato cyst nematodes (PCN) (Scholte, 2000; Scholte & Vos, 2000).

Current late blight management strategies in the Netherlands are based on the eradication of initial sources of inoculum, and slowing down epidemic development through the application of protectant fungicides. The presence of alternative hosts, on which late blight can develop unnoticed, jeopardizes management strategies aimed at reducing primary inoculum during the early stage of crop development. Formation of oospores and possible overwintering on alternative hosts provide new challenges for an integrated late blight management strategy. This study reports a series of experiments to determine the host specificity and spore production of *P. infestans* isolates collected from *S. tuberosum*, *S. nigrum*, *S. dulcamara* and *S. sisymbriifolium* on several host plant species and quantification of oospore formation in naturally infected and inoculated foliage of the same hosts.

Materials and methods

Isolate collection and culture conditions

For the pathogenicity studies described below, a total of 20 *P. infestans* isolates were collected from potato, *S. dulcamara*, *S. nigrum* and *S. sisymbriifolium* (Table 1). Isolates originating from alternative hosts were collected from diseased plants in the Netherlands between 1999 and 2001. Isolations were made by trapping *P. infestans* with potato tuber slices (Forbes *et al*., 1997) followed by culturing on selective rye agar (RA) (Grünwald *et al*., 2001). Reference isolates from potato were selected to represent strains from old and new populations as well as variation in race complexity. All isolates are part of the *P. infestans* collection of Plant Research International, and are maintained in liquid nitrogen storage facilities.

Table 1 Isolates of *Phytophthora infestans* used in compatibility and oospore production experiments

Isolate	Host	Year of collection	Location[a]	Race	Mating type	mtDNA haplotype
IPO00513	*S. sisymbriifolium*	2000	Hoogezand	1.2.3.4.5.6.7.8.10.11	A2	Ia
IPO00518	*S. sisymbriifolium*	2000	Hoogezand	1.2.3.4.5.6.7.8.10.11	A2	Ia
IPO00520	*S. sisymbriifolium*	2000	Hoogezand	1.2.3.4.5.6.7.10.11	A2	Ia
IPO01521	*S. sisymbriifolium*	2001	Wageningen	1.3.7.8.11	A1	Ia
IPO01522	*S. sisymbriifolium*	2001	Wageningen	1.3.7.8.11	A1	Ia
WAU01001	*S. sisymbriifolium*	2001	Wageningen	1.2.3.7.8.11	A1	Ia
Sn001A	*S. nigrum*	1999	Wageningen	1.2.3.4.5.7.8.10.11	A1	Ia
Sn002	*S. nigrum*	1999	Wageningen	nd[b]	A1	Ia
Wild9	*S. nigrum*	1999	Wageningen	nd	A1	Ia
Wild10	*S. nigrum*	1999	Wageningen	nd	A1	Ia
Wild13	*S. nigrum*	1999	Wageningen	nd	A1	Ia
Wild14	*S. nigrum*	1999	Wageningen	nd	A1	Ia
IPO00501	*S. dulcamara*	2000	Wageningen	nd	A2	IIa
IPO00505	*S. dulcamara*	2000	Wageningen	nd	A1	Ia
IPO00507	*S. dulcamara*	2000	Wageningen	nd	A1	Ia
IPO98014	*S. tuberosum*	1998	Valthermond	1.2.3.4.7.11	A1	Ia
IPO428-2	*S. tuberosum*	1992	Ede	1.2.3.4.5.6.7.8.9.10.11	A2	Ia
IPO82001	*S. tuberosum*	1982	Gembloux (B)[c]	1.2.3.4.5.6.7.10.11	A2	Ia
VK 6C	*S. tuberosum*	1958	nd	1.4	A1	Ib
Race 0	*S. tuberosum*	<1970	USA	0	A1	Ib

[a]All locations are in the Netherlands except where noted.
[b]Not determined.
[c]Belgium.

Isolates used in pathogenicity studies were cultured on tuber slices and detached leaves of the susceptible potato cv. Bintje according to Flier & Turkensteen (1999). Sporangial inoculum was prepared by washing leaves showing abundant sporulation in 15–20 mL tap water followed by concentration adjustment using a flow cytometer (Beckman Coulter, Mijdrecht, the Netherlands). Sporangial suspensions were kept at 18°C and used as inoculum within 30 min of preparation.

Characterization of isolates

Isolates were characterized for mating type, specific virulence (race) and mtDNA haplotype. Mating type was determined by *in vitro* crosses with known A1 and A2 tester strains (IPO98014 and IPO82001, respectively) according to Forbes *et al*. (1997). Race determination was performed according to Flier & Turkensteen (1999) using the R1 to R11 differential set of potato clones (Black *et al*., 1953; Malcolmson & Black, 1966).

DNA was extracted from cultures grown on liquid pea broth according to Flier *et al*. (2003). The P1 (1118 bp), P2 (1070 bp), P3 (1308 bp) and P4 (964 bp) regions of the mitochondrial genome were amplified using primers and methods described by Griffith & Shaw (1998). Reactions were performed in a PTC200 thermocycler (MJ Research/Biozym, Landgraaf, the Netherlands). PCR products were digested with restriction enzymes *Cfo*I, *Msp*I and *Eco*RI, resulting in restriction fragment band patterns that can be classified into four different mitochondrial (mtDNA) haplotypes: Ia, Ib, IIa and IIb.

Compatibility and pathogenicity tests

All compatibility and pathogenicity tests were conducted using the methods of Flier & Turkensteen (1999), with minor modifications. Plants were grown in the greenhouse from certified seed (potato cv. Bintje) or from stem cuttings. Detached leaves were inoculated and incubated in a growth chamber which was maintained at 15°C and illuminated with 40 W fluorescence bulbs type 33 (Philips, Eindhoven, the Netherlands) at an intensity of 15 W m^{-2}, 16 h light per day.

Experiment 1: compatibility of P. infestans *to* S. nigrum *genotype Sn001*

The compatibility of *P. infestans* isolates collected in 1999 from diseased *S. nigrum* plants and reference strains from potato were evaluated on *S. nigrum* genotype Sn001 collected from a potato field near Wageningen in 1999.

Plants were transferred to the greenhouse and propagated through stem cuttings. Fully expanded leaves of *S. nigrum* were cut and placed abaxial side up in 9 cm diameter Petri dishes filled with 10 mL 1·5% water agar. Two replicates per plant genotype, each comprising two leaves, were inoculated by spraying a sporangial suspension ($1{\cdot}0 \times 10^4$ sporangia mL^{-1}) with a spray nozzle at a pressure of 0·5 kg m^{-2} until runoff. Nine *P. infestans* isolates were used, three from each alternative host plant species. Inoculated leaves were air-dried 24 h postinoculation after which Petri plates with the inoculated leaves were wrapped in transparent polythene bags and incubated in a climate chamber for 1 week at 15°C with a light intensity of 12 W m^{-2}, 16 h light per day. Data were collected 8 days after inoculation by visual estimation of the lesion area (LA) and the surface area of each leaf using a MINIMOP image analyser (Kontron, Munich, Germany). Sporangia from each individual leaf were collected into a single vial containing 10 mL ISOTON 2 solution (Beckman Coulter) and counted using a flow cytometer. The total number of sporangia produced on each leaf and spore density (SPOR, sporangia mm^{-2} leaf area) were calculated from the average of two counts of 0·5 mL each. The experiment was repeated.

Experiment 2: compatibility of P. infestans *to* S. nigrum *seedlings*

Randomly collected *S. nigrum* seedlings taken from two potato fields were inoculated with isolates originally collected from potato, *S. dulcamara*, *S. nigrum* and *S. sisymbriifolium* in order to evaluate host specificity of *P. infestans* on *S. nigrum*. Four randomly selected *S. nigrum* seedlings were collected from a harvested potato field near the village of Renkum (Province of Gelderland) on 25 November 2000 and from an abandoned potato field near Wijster (Province of Drenthe) on 27 November 2000. Plants were transferred to the greenhouse and propagated through stem cuttings. Bioassays for LA and SPOR were conducted as described for Experiment 1.

Experiment 3: host specificity of P. infestans *isolates collected from various hosts*

Three randomly selected isolates from potato, *S. nigrum*, *S. dulcamara* and *S. sisymbriifolium* were inoculated on potato cv. Bintje, tomato cv. Moneymaker, *S. dulcamara*, *S. sisymbriifolium* and two *S. nigrum* genotypes grown from seed. Bioassays for LA and SPOR were conducted as described for Experiment 1.

Experiment 4: epidemic parameters of late blight on S. sisymbriifolium

Fully grown leaves of potato cv. Bintje and *S. sisymbriifolium* were inoculated with *P. infestans* isolates collected from potato and *S. sisymbriifolium*. Either 10 drops (IF, LP) of 10 μL of a sporangium suspension ($1{\cdot}0 \times 10^4$ spores mL^{-1}) or a single drop (SPOR) of 10 μL of a sporangium suspension ($5{\cdot}0 \times 10^4$ spores mL^{-1}) was placed on the lower side of detached leaves of potato cv. Bintje and *S. sisymbriifolium* that were placed in 9 cm diameter Petri dishes filled with 10 mL 1·5% water agar. Two replicates per plant genotype, each with two leaves, were inoculated. Incubation methods were identical to experiment 1. Infection frequency (IF) based on the fraction of sporulating lesions, and latent period, calculated as time in days from inoculation to sporulation (LP), were calculated from daily observations of infections. Lesion size and SPOR (as described above) were measured after 8 days' incubation. Lesion size (mm^2) was estimated using image analysis as

in experiment 1, but is reported in this experiment in absolute terms and not as a proportion of leaf area.

Oospore formation

Naturally infected leaves of *S. dulcamara*, *S. nigrum* and *S. sisymbriifolium* were collected in 1999, 2000 and 2001, respectively. Fully blighted leaves with multiple lesions were incubated for 6 days at 20°C in Petri dishes (9 cm diameter) containing 1·0% water agar.

Oospore production was measured by extracting and counting oospores from diseased leaves. The area of each individual leaf was measured and then homogenized in 5 mL crushed ice and 5 mL tap water (4°C) with an Ultra Turrax mixer at 24 000 r.p.m. for 90 s. After cooling the suspensions to 4°C, homogenization was repeated without addition of ice or water. The homogenized leaf tissue was macerated on a shaker for 2 h at 20°C by adding Cellulase (C8001, Duchefa, Haarlem, the Netherlands) and Macerase (Duchefa M8002) to the homogenized samples at a final concentration of 0·5 mg mL^{-1} for each enzyme. Samples were sonicated twice for 5 min and left at 20°C overnight on an orbital shaker. Macerated samples were washed sequentially with running tap water on 75 and 20 μm laboratory test sieves (Endecotts Ltd, London, UK), transferred to a 50 mL centrifuge tube and centrifuged for 3 min at 5000 ***g***. Approximately 40–45 mL supernatant was removed and the oospore concentration in the pellet was determined using a Fuchs–Rosenthal haemocytometer.

Oospore production in *S. dulcamara*, *S. nigrum* and *S. sisymbriifolium* was also studied in inoculation experiments. Leaves from fully grown greenhouse plants were placed abaxial side up in 16 cm diameter Petri dishes filled with 20 mL 2% water agar. Two replicates of two leaves per host species were inoculated by spraying a mixed A1/A2 sporangial suspension ($1{\cdot}0 \times 10^4$ sporangia mL^{-1}) with a spraying nozzle at a pressure of 0·5 kg m^{-2} until runoff. The parental strains used were IPO98014 (A1) and IPO428-2 (A2) (Table 1). Plates containing inoculated leaves were placed in plastic trays, enclosed in transparent polythene bags to inhibit desiccation, and incubated for 14 days (11°C) at a light intensity of 12 W m^{-2}, 16 h light per day. The experiment was repeated. Afterwards, oospores were extracted as described previously.

Statistical analyses

Data were analysed using the GENSTAT statistical package release 6·0 (Payne *et al*., 1993). Both measured and calculated epidemic parameters were graphically checked for normality and equal variance distributions. The SPOR parameter was log_{10} transformed, and mean values and LSDs were back-transformed to their original dimensions following statistical analysis. ANOVA was used to compare means.

Results

Isolate characterization

Isolates collected from various hosts during 1999 and 2000 were confirmed to be *P. infestans* by morphological and molecular characteristics. All isolates collected from alternative hosts were of either the Ia or IIa mtDNA haplotype (Table 1). Both A1 and A2 mating types were represented in the isolate collection. All isolates were able to infect potato. Both simple and fairly complex physiological races were found among the isolates. Race 1.2.3.4.5.6.7.8.10.11 was the most complex race present, while race 1.3.7.8.11 was the least complex phenotype (Table 1).

Pathogenicity of *P. infestans* on *S. nigrum*

Solanum nigrum genotype Sn001 collected in 1999 was a susceptible host after inoculation with *P. infestans* isolates collected from several accessions of *S. nigrum* and cultivated potato (Table 2). Average lesion area ranged from

Table 2 Relative lesion area and sporulation density of *Phytophthora infestans* isolates collected from infected *Solanum nigrum* plants and potato inoculated on *S. nigrum* genotype Sn001 and potato cv. Bintje

		S. tuberosum cv. Bintje		*S. nigrum* Sn001	
Isolate	Original host	LA (%)[a]	SPOR (cm^{-1})[a]	LA (%)	SPOR (cm^{-1})
IPOSn001a	*S. nigrum*	100	64305	40	2256
IPOSn002	*S. nigrum*	78	40675	44	1321
IPOSn10	*S. nigrum*	98	37519	45	1809
IPOSn13	*S. nigrum*	100	46206	33	2816
IPOSn9	*S. nigrum*	95	40940	80	2811
IPOSn14	*S. nigrum*	100	47526	58	3063
IPO428-2	*S. tuberosum*	100	25682	58	6407
IPO82001	*S. tuberosum*	93	57186	28	3038
IPO98014	*S. tuberosum*	96	53007	25	2114
Race 0	*S. tuberosum*	90	56336	30	2312
VK 6C	*S. tuberosum*	100	47275	28	1443

[a]LA = proportion of leaf area infected; SPOR = sporulation density.
Fisher's least significant difference ($P = 0{\cdot}05$): LA = 29; SPOR = 2245.

41·9 to 95·5% on *S. nigrum* Sn001 and cv. Bintje, respectively. Mean sporulation densities varied between 2704 sporangia cm^{-2} for *S. nigrum* Sn001 and 47 046 sporangia cm^{-2} for cv. Bintje. No significant differences in pathogenicity were found for isolates collected from *S. nigrum* and potato.

All but two *S. nigrum* genotypes, originating as seedlings, were infected by one or more *P. infestans* isolates in detached leaf inoculation experiments (Table 3). Isolates originating from potato, *S. nigrum*, *S. dulcamara* and *S. sisymbriifolium* were equally able to infect *S. nigrum* plants collected from farmers' fields. No sporulation was observed on *S. nigrum* Renkum 2 and Renkum 4. In compatible interactions, mean LA varied from 16·8% for *S. nigrum* Renkum 5 to 75·5% for *S. nigrum* Wijster 5, and average SPOR ranged from 787 to 3587 sporangia cm^{-2} for *S. nigrum* Renkum 5 and Wijster 5, respectively. The main effects of isolate and host, and the isolate–host interaction term, significantly contributed to the estimated LA and SPOR values ($P < 0{\cdot}001$).

Host specificity

Isolates originally collected from potato, *S. nigrum*, *S. dulcamara* and *S. sisymbriifolium* were able to infect all these hosts as well as tomato cv. Moneymaker (Table 4). Potato and tomato were the most susceptible host plant species with average LA ranging from 79·0 to 95·7% and average SPOR varying between 5760 and $4{\cdot}1 \times 10^4$ sporangia cm^{-2} for tomato and potato, respectively. As in previous experiments, no infections were observed on *S. nigrum* Renkum 2 (Tables 3 and 4). Average values for LA and SPOR were low in *S. nigrum* genotype Wijster 3 and *S. sisymbriifolium* compared to *S. dulcamara* (Table 4). Significant isolate, host, and isolate × host effects were detected for both parameters evaluated ($P < 0{\cdot}001$).

Epidemic parameters of *S. sisymbriifolium*

Three out of five isolates originally collected from *S. sisymbriifolium* and reference potato strains IPO428-2 and IPO82001 were able to infect and sporulate on detached leaves of *S. sisymbriifolium* (Table 5). Isolates IPO00521 and IPO522 only caused small hypersensitive reactions on the inoculated leaves, and no sporulation was observed until 14 days after inoculation when the experiment was terminated. Consequently, these isolates were considered as incompatible with the *S. sisymbriifolium* genotype tested. The epidemic parameters IF, LP, LA and SPOR varied considerably among isolates and inoculated host plants. The reaction of *S. sisymbriifolium* to infection with a compatible *P. infestans* isolate was marked by LP values comparable with potato cv. Bintje (ranging from 4 to 6 days) and low SPOR values (ranging from 1400 to 1700 sporangia cm^{-2}) compared to Bintje.

Oospore formation in host plants

Oospores of *P. infestans* formed readily in leaves of potato, *S. dulcamara*, *S. nigrum* and *S. sisymbriifolium* inoculated

Table 3 Relative lesion area and sporulation density on eight genotypes of *Solanum nigrum* and potato cv. Bintje eight days following inoculation with 12 *Phytophthora infestans* isolates

		S. tuberosum		*S. nigrum* Renkum								*S. nigrum* Wijster							
		cv. Bintje		Renkum 2		Renkum 3		Renkum 4		Renkum 5		Wijster 2		Wijster 4		Wijster 5		Wijster 6	
Isolate	Original host	LA[a]	SPOR[a]	LA	SPOR	LA	SPOR	LA	SPOR	LA	SPOR	LA	SPOR	LA	SPOR	LA	SPOR	LA	SPOR
IPO00501	*S. dulcamara*	100	32758	0	0	61	1515	0	0	26	269	71	2041	35	1804	60	3243	61	2086
IPO00505	*S. dulcamara*	100	47363	0	0	43	4481	0	0	19	1472	97	6453	31	2382	99	4240	65	2164
IPO00507	*S. dulcamara*	75	43783	0	0	46	2999	0	0	0	0	74	4465	45	4795	66	5540	24	585
IPOSn001A	*S. nigrum*	100	41403	0	0	38	885	0	0	18	1383	87	3750	44	4061	97	5404	50	2431
IPOSn13	*S. nigrum*	100	26124	0	0	8	155	0	0	2	435	57	2347	63	1841	71	2092	53	2018
IPOSn9	*S. nigrum*	88	23504	0	0	69	2953	0	0	19	299	58	3778	26	1186	74	3300	63	1738
IPO00513	*S. sisymbriifolium*	72	10933	0	0	26	1199	3	0	0	0	56	2634	2	0	66	2136	28	863
IPO00520	*S. sisymbriifolium*	100	20422	0	0	8	645	0	0	33	1573	89	3349	9	0	61	1951	19	907
IPO01521	*S. sisymbriifolium*	100	43546	0	0	50	1997	0	0	29	726	79	3219	59	2660	81	4558	78	2147
IPO428-2	*S. tuberosum*	100	33797	0	0	30	643	0	0	38	1990	69	4144	41	1728	79	3471	51	1787
IPO98014	*S. tuberosum*	100	46181	0	0	4	0	0	0	3	302	81	2639	13	1991	86	4185	21	755
Race 0	*S. tuberosum*	100	37171	0	0	30	730	0	0	14	1001	72	2650	23	697	64	2921	40	1158

[a]LA = proportion of leaf area infected; SPOR = sporulation density.
Fisher's least significant difference ($P = 0{\cdot}05$): LA = 26; SPOR = 1050.

Table 4 Relative lesion area and sporulation density on leaves of five host plant species eight days following inoculation with 12 *Phytophthora infestans* isolates

		S. tuberosum cv. Bintje		*S. dulcamara*		*S. nigrum* (Renkum 2)		*S. nigrum* (Wijster)		*S. sisymbriifolium*		*S. lycopersicum* cv. Moneymaker	
Isolate	Original host	LA (%)[a]	SPOR[a]	LA (%)	SPOR	LA (%)	SPOR	LA (%)	SPOR	LA (%)	SPOR	LA (%)	SPOR
IPO00501	*S. dulcamara*	83	28232	56	4245	0	0	0	0	15	1029	63	1809
IPO00505	*S. dulcamara*	100	64390	50	3513	0	0	5	614	40	7203	69	6365
IPO00507	*S. dulcamara*	100	81123	49	6638	0	0	0	0	11	1434	50	2400
IPOSn001a	*S. nigrum*	100	63750	78	8018	0	0	0	0	3	649	93	16113
IPOSn13	*S. nigrum*	88	17210	13	1781	0	0	25	1289	0	0	68	2500
IPOSn9	*S. nigrum*	100	33972	55	2567	0	0	0	0	4	0	91	3929
IPO00513	*S. sisymbriifolium*	80	10814	23	681	0	0	13	348	72	3763	96	9788
IPO00520	*S. sisymbriifolium*	100	13628	23	781	0	0	0	0	83	3445	89	4976
IPO01521	*S. sisymbriifolium*	100	41279	65	4067	0	0	15	718	8	0	72	2525
IPO428-2	*S. tuberosum*	100	41781	74	1685	0	0	15	443	36	3222	97	5770
IPO98014	*S. tuberosum*	100	52604	75	5615	0	0	5	1375	24	2666	92	10179
Race 0	*S. tuberosum*	99	40905	46	822	0	0	0	0	2	0	70	2766

[a]LA = proportion of leaf area infected; SPOR = sporulation density.
Fisher's least significant difference ($P = 0{\cdot}05$): LA = 24; SPOR = 980.

Table 5 A comparison of four epidemic parameters of potato cv. Bintje and *S. sisymbriifolium* following inoculation of detached leaves with seven *Phytophthora infestans* isolates and incubation at 15°C

		S. tuberosum cv. Bintje				*S. sisymbriifolium* Wag-1			
Isolate	Original host	IF (%)[a]	LP (days)[a]	Lesion size (mm^2)	SPOR (cm^{-2})[a]	IF (%)	LP (days)	Lesion size (mm^2)	SPOR (cm^{-2})
WAU01001	*S. sisymbriifolium*	98	4	20	20400	55	4	17	1600
IPO00513	*S. sisymbriifolium*	49	10	3	2300	81	6	13	1400
IPO00518	*S. sisymbriifolium*	74	13	6	10500	39	5	19	1700
IPO01521	*S. sisymbriifolium*	98	5	2	25600	0	>14	3	0
IPO01522	*S. sisymbriifolium*	99	5	17	24800	0	>14	8	0
IPO428-2	*S. tuberosum*	100	4	30	26400	0	>14	9	0
IPO82001	*S. tuberosum*	75	5	15	14000	0	>14	3	0

[a]IF = infection frequency; LP = latent period; SPOR = sporulation density.
Fisher's least significant difference ($P = 0{\cdot}05$): IF = 28; LP = 8; lesion size = 9; SPOR = 257.

with A1 and A2 isolates from potato (Fig. 1). Oospores were also observed in diseased leaves of both *S. nigrum* (46 oospores cm^{-2}) and *S. dulcamara* (178 oospores cm^{-2}) collected from roadsides and fields in 2000 and 2001. In *S. sisymbriifolium*, abundant oospore formation was found in diseased leaves taken from a blighted experimental crop in 2000 (297 oospores cm^{-2}) and after inoculation of detached leaves with A1 and A2 strains (147 oospores cm^{-2}). Oospore densities were higher in naturally infected leaves compared to inoculated tissues for all host plant species tested, with average densities of 179 and 102 oospores cm^{-2}, respectively.

Discussion

The increased problems with late blight control in northwest Europe coincided with the displacement of the asexually reproducing population made up of the US-1 clonal lineage by a variable population of *P. infestans* (Spielman *et al*., 1991). In the Netherlands, increased aggressiveness of local pathogen populations and earlier onset of late blight epidemics have led to a greater disease pressure in potato crops. The establishment of genetic variation in the pathogen population through sexual reproduction in *P. infestans* may also lead to a broadening of the host range in both cultivated and native host plants. This is the first report of *P. infestans* causing late blight symptoms on *S. nigrum*, *S. dulcamara* and *S. sisymbriifolium* in the Netherlands.

Isolates collected from *S. nigrum*, *S. dulcamara* and *S. sisymbriifolium* caused typical late blight symptoms on leaves of five host plant species included in this study. Infection levels and sporulation densities varied with isolate, plant genotype and specific combinations of isolate × plant genotype. In general, relative lesion areas of inoculated detached leaves were greater for potato cv. Bintje and tomato cv. Moneymaker than for the other three solanaceous hosts. Sporulation density was higher on potato, tomato and *S. dulcamara* compared to *S. nigrum* and *S. sisymbriifolium*. Oospores were extracted from

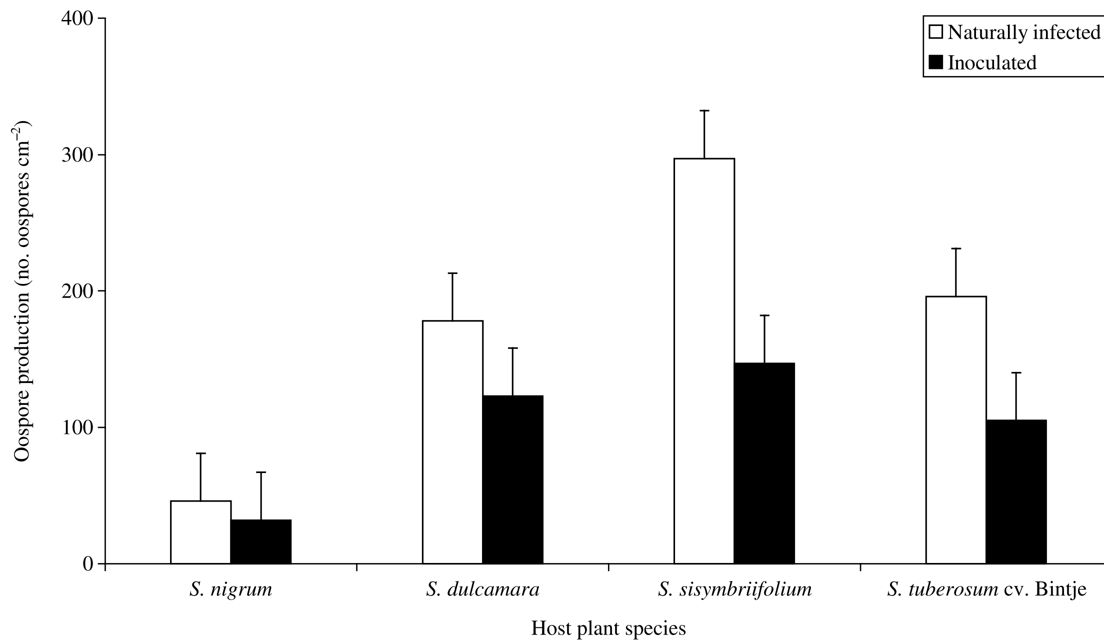

Figure 1 Oospore production in leaf tissue of four host plant species following either inoculation with compatible A1 (IPO98014) and A2 (IPO428-2) mating type strains, or natural infection in the field.

blighted *S. nigrum* leaves collected from farmers' fields and *S. dulcamara* leaves collected from plants growing along a roadside. Oospores were also found in diseased leaves of *S. sisymbriifolium* and potato cv. Bintje. Inoculation of host plants with A1 and A2 mating type strains of *P. infestans* yielded fewer oospores compared with leaves taken from naturally infected plants. Overall, oospore production per unit leaf surface was highest in *S. sisymbriifolium* and lowest in *S. nigrum*.

Based on the presence of field infections and results obtained in detached leaf inoculation studies, the present status of *S. nigrum* as a nonhost plant for *P. infestans* needs to be reconsidered. According to Pieterse *et al.* (1994) and Vleeshouwers *et al.* (2000), *S. nigrum* is to be regarded as a nonhost of *P. infestans*, while others have speculated on the stability and durability of the hypersensitive resistance response characteristic for *P. infestans* × *S. nigrum* interactions (Colon *et al.*, 1993; Kamoun *et al.*, 1999). Observations of blighted *S. nigrum* and *S. dulcamara* plants in the field remain very rare. The first European report on the incidence of *P. infestans* on *S. nigrum* and *S. dulcamara* dates back to the 1950s (Hirst & Stedman, 1960). More recently, *P. infestans* has been collected from *S. nigrum* in the Andean highlands of Colombia (Patino *et al.*, 1996, 1998) and from North Wales (D. S. Shaw, personal communication). In a recent study on host specificity of *P. infestans* in Canada (Platt, 1999), isolates classified as US-8 and US-11 as well as US-1 infected leaf and stem tissues of *S. dulcamara* in a bioassay. No disease symptoms other than a hypersensitive response were observed when *S. nigrum* was inoculated with North American *P. infestans* isolates, and it was therefore suggested that *S. nigrum* should be regarded as a nonhost. The results presented here clearly indicate the presence of a basic compatibility between *S. nigrum* and the *P. infestans* population present in the Netherlands. The observations on leaf blight in *S. dulcamara* support the findings of Cooke *et al.* (2002), reporting the natural occurrence of *P. infestans* on *S. dulcamara* in Ireland. This study confirms that the experimentally grown PCN trap crop *S. sisymbriifolium* should be regarded as an alternative host for *P. infestans*, despite earlier reports suggesting a nonhost status for *S. sisymbriifolium* (Sen, 1997). Isolates have been collected from *S. sisymbriifolium* growing in two experimental fields in 2000 and 2001.

The results shown here demonstrate the presence of differential interaction in the *S. nigrum* and *P. infestans* pathosystem. The observed specificity between host and pathogen may be explained by the presence of R-genes in *S. nigrum* that are matched by race specificity in *P. infestans*. One of the tested *S. nigrum* genotypes (Sn001) appears to carry no R-genes effective against the *P. infestans* strains tested. Support for the *S. nigrum* R-gene hypothesis is provided by Horsman *et al.* (1999), who reported on backcrosses between somatic hybrids of *S. nigrum* and potato to both parental species. In backcrosses with potato, they observed that the BC1 genotype was as resistant as the *S. nigrum* fusion partner, but found that two out of eight BC2 genotypes showed sporulating lesions. Inoculation studies also suggest the presence of R-genes in *S. sisymbriifolium*. Two isolates collected from an experimental PCN trap crop near Wageningen in 2001 were not able to infect a genotype of *S. sisymbriifolium* collected from an infected field near Hoogezand in 2000.

Testing the possibility of host range extension with randomly selected isolates collected from principal hosts may

not be the most efficient method for establishing host/nonhost status for potential new hosts. The presence of R-genes in a native host plant with low abundance and a highly dispersed distribution pattern, or in a newly introduced host for which compatibility is found at very low frequencies, could easily disguise the compatibility of a host. It is therefore recommended that, when possible, a number of genotypes of a potential host should be exposed to a variable pathogen population, preferably under field conditions.

Based on infection levels, asexual sporulation and oospore formation in diseased leaves, it is concluded that basic compatibility exists between the present *P. infestans* population in the Netherlands and the host plant species *S. nigrum*, *S. dulcamara* and *S. sisymbriifolium*, and that these plant species may host late blight. In the case of *S. nigrum* and *S. dulcamara*, infection appears to be a relatively rare event, and it is not very likely that diseased *S. nigrum* and *S. dulcamara* plants significantly contribute to the overall disease pressure present in potato production areas. The observations are therefore in concordance with Cooke *et al.* (2002). A commercial introduction of *S. sisymbriifolium* as a PCN trap crop could potentially have a significant impact on late blight epidemics through the production of inoculum during the growing season and the formation of functional oospores in infected leaf tissue that can serve as initial inoculum for following potato crops. In order to induce sufficient hatching of larvae of PCN, *S. sisymbriifolium* does not need to be present during the whole growing season. Therefore the impact of *S. sisymbriifolium* on late blight epidemics may depend on its planting time and on the duration of the period in which the crop is allowed to grow. However, introduction of *S. sisymbriifolium* as a commercial trap crop may require a chemical control strategy to prevent late blight development on this crop.

Acknowledgements

This research was conducted as part of the research programme (DWK-397) 'Plant Pathogen Management', funded by the Dutch Ministry of Agriculture, Fisheries and Nature Management. The Hilbrands Laboratory kindly supplied *S. sisymbriifolium* plants. We thank Bart Timmermans and Jan Vos (Department of Agronomy, Wageningen University) for providing isolate WAU01001 and stimulating discussions, and Petra van Bekkum and Marieke Kanne-Förch for excellent technical support.

References

Andersson B, Sandström M, Strömberg A, 1998. Indications of soil borne inoculum of *Phytophthora infestans*. *Potato Research* **41**, 305–10.

Black W, Mastenbroek C, Mills WR, Peterson LC, 1953. A proposal for an international nomenclature of races of *Phytophthora infestans* and of genes controlling immunity in *Solanum demissum* derivates. *Euphytica* **2**, 173–240.

Colon LT, Eijlander R, Budding DJ, van IJzendoorn MT, Pieters MMJ, Hoogendoorn J, 1993. Resistance to potato late blight (*Phytophthora infestans* (Mont.) de Bary) in *Solanum nigrum*, *S. villosum* and their sexual hybrids with *S. tuberosum* and *S. demissum*. *Euphytica* **66**, 55–64.

Cooke LR, Carlisle DJ, Wilson DG, Deahl KL, 2002. Natural occurrence of *Phytophthora infestans* on woody nightshade (*Solanum dulcamara*) in Ireland. *Plant Pathology* **51**, 392.

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.

Erwin DC, Ribeiro OK, 1996. *Phytophthora Diseases Worldwide*. St Paul, MN, USA: APS Press.

Flier WG, Turkensteen LJ, 1999. Foliar aggressiveness of *Phytophthora infestans* in three potato growing regions in the Netherlands. *European Journal of Plant Pathology* **105**, 381–8.

Flier WG, Grünwald NJ, Kroon LPNM, Sturbaum AK, van den Bosch GBM, Garay-Serrano E, Lozoya-Saldaña H, Fry WE, Turkensteen LJ, 2003. The population structure of *Phytophthora infestans* from the Toluca Valley of central Mexico suggests genetic differentiation between populations from cultivated potato and wild *Solanum* species. *Phytopathology* **93**, 382–90.

Forbes GA, Escobar XC, Ayala CC, Revelo J, Ordonez ME, Fry BA, Doucett K, Fry WE, 1997. Population genetic structure of *Phytophthora infestans* in Ecuador. *Phytopathology* **87**, 375–80.

Griffith GW, Shaw DS, 1998. Polymorphisms in *Phytophthora infestans*: four mitochondrial haplotypes are detected following PCR amplification of DNA from pure cultures or from host lesions. *Applied and Environmental Microbiology* **64**, 4007–14.

Grünwald NJ, Flier WG, Sturbaum AK, Garay-Serrano E, Van den Bosch GBM, Smart CD, Matuszak J, Turkensteen LJ, Fry WE, 2001. Genetic population structure of *Phytophthora infestans* within the Toluca valley of central Mexico. *Phytopathology* **91**, 882–90.

Hirst JM, Stedman OJ, 1960. The epidemiology of *Phytophthora infestans*. II. The source of infection. *Annals of Applied Biology* **48**, 489–517.

Horsman K, Fratini R, Huigen DJ, Jacobsen E, 1999. Successful first and second backcrosses of *S. nigrum* (+) *S. tuberosum* somatic hybrids with both *Solanum* parents. *Sexual Plant Reproduction* **12**, 144–51.

Kamoun S, Huitema E, Vleeshouwers VGGA, 1999. Resistance to oomycetes: a general role for the hypersensitive response? *Trends in Plant Science* **4**, 196–200.

Malcolmson JF, Black W, 1966. New R genes in *Solanum demissum* Lindl. and their complementary races of *Phytophthora infestans* (Mont.) de Bary. *Euphytica* **15**, 199–203.

Patino LF, Morales JG, Buritica CP, Jaramillo VS, 1996. Infectivity of *Phytophthora infestans* isolates from high altitudes in plants in different ecological zones. *ASCOLFI Informa* **22**, 86–90.

Patino LF, Morales JG, Buritica CP, Zapata JL, Jaramillo VS, 1998. Ecological behaviour and potential interaction between *Phytophthora infestans* and Solanaceae. *Fitopatologia Colombiana* **22**, 59–64.

Payne RW, Lane PW, Digby PGN, Harding SA, Leech PK, Morgan GW, Todd AD, Thompson R, Tunnicliffe-Wilson G, Welham SJ, White RP, 1993. *GENSTAT 5, Release 3, Reference Manual*. Oxford, UK: Clarendon Press.

Pieterse CMJ, Derksen AMCE, Folders J, Govers F, 1994. Expression of the *Phytophthora infestans ipi*B and *ipi*O genes *in planta* and *in vitro*. *Molecular and General Genetics* **244**, 269–77.

Platt HW, 1999. Response of solanaceous cultivated plants and weed species to inoculation with A1 or A2 mating type strains of *Phytophthora infestans*. *Canadian Journal of Plant Pathology* **21**, 301–7.

Scholte K, 2000. Screening of non-tuber bearing Solanaceae for resistance to and induction of juvenile hatch of potato cyst nematodes and their potential for trap cropping. *Annals of Applied Biology* **136**, 239–46.

Scholte K, Vos J, 2000. Effects of potential trap crops and planting date on soil infestation with potato cyst nematodes and root-knot nematodes. *Annals of Applied Biology* **137**, 153–64.

Sen S, 1997. Host range of *Phytophthora infestans*, the incitant of leaf blight of Kangaroo apple. *Journal of Mycology and Plant Pathology* **27**, 244.

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

Sujkowski LS, Goodwin SB, Dyer AT, Fry WE, 1994. Increased genotypic diversity via migration and possible occurrence of sexual reproduction of *Phytophthora infestans* in Poland. *Phytopathology* **84**, 201–7.

Turkensteen LJ, 1973. *Partial Resistance of Tomatoes Against.* Phytophthora infestans, *the Late Blight Fungus*. Wageningen, the Netherlands: Wageningen Agricultural University, PhD thesis.

Turkensteen LJ, Flier WG, Wanningen R, Mulder A, 2000. Production, survival and infectivity of oospores of *Phytophthora infestans*. *Plant Pathology* **49**, 688–96.

Vleeshouwers VGGA, van Dooijeweerd W, Govers F, Kamoun S, Colon LT, 2000. The hypersensitive response is associated with host and nonhost resistance to *Phytophthora infestans*. *Planta* **210**, 853–64.

van der Zaag DE, 1956. Overwintering en epidemiologie van *Phytophthora infestans*, tevens enige nieuwe bestrijdingsmogelijkheden. *Tijdschrift Plantenziekten* **62**, 89–156.