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Infection Potential of Hairy Nightshade (*Solanum sarrachoides*) by *Phytophthora infestans* and Late Blight Implications of the Alternate Host

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Abstract

Infection of hairy nightshade (*Solanum sarrachoides* Sendt.) by *Phytophthora infestans* has been reported. However, the epidemiological significance of hairy nightshade to potato late blight is not well known. Disease development and infection rates of *P. infestans* were quantified on hairy nightshade relative to tomato (cv. Brandywine) and potato (cv. Shepody) hosts to evaluate infection potential at 14, 18, 22 and 26°C and 72, 82, 87, and 92% relative humidity (RH). The susceptibility of hairy nightshade to inoculum levels, weed ontogeny, and sporangia production potential were also investigated. Late blight development varied among hairy nightshade, tomato and potato hosts. Pathogen infection rates ranged from 0.0325 to 0.4674 gompits/day (unit for quantifying infection rates), and were significantly ($P < 0.05$) greater on potato and tomato than on hairy nightshade. Late blight severity was variably affected by RH. Disease levels on hairy nightshade varied with inoculum load; and ranged from 9 to 26% and 26 to 37% at low (5×10^3) and high (25×10^3) sporangia concentrations, respectively. Late blight was recorded irrespective of hairy nightshade ontogeny, and was significantly greater on 8–10 than 4–6-week-old plants. These results indicate that pathogen, environmental and host factors affect late blight development on hairy nightshade.

Introduction

Late blight caused by *Phytophthora infestans* (Mont.) de Bary is a major constraint to potato production in many regions of the world. Significant economic losses due to fungicide application costs and late blight infection have been estimated in millions of dollars (Guenthner et al., 2001). The conditions favourable for late blight epidemics on potato and tomato have been well documented in various studies (Minogue and Fry, 1981; Harisson and Rowe, 1989; Campbell and Madden, 1990; Harrison, 1992; Sato, 1994; Becktell et al., 2005). Environmental factors such as temperature, relative humidity (RH) and rainfall have been associated with severe epidemics of late blight (Harrison, 1992). The time required for late blight infection reportedly ranges from 16 h at 7–12°C to only 10 h at 15–27°C when RH exceeds 90% (Wallin, 1962; Krause et al., 1975). Temperature, RH and rainfall or leaf-wetness can also affect different stages of pathogen development (Minogue and Fry, 1981; Harisson and Rowe, 1989).

Alternate hosts may interfere with current late blight management strategies by serving as sources of inoculum, undetected refuges for pathogen dissemination, or possible mechanisms for pathogen survival (Vartanian and Endo, 1985). Reports of alternate hosts of *P. infestans* have been published based on controlled experiments or field observations. Vartanian and Endo (1985) reported *P. infestans* on hairy nightshade (*Solanum sarrachoides* Sendt.) and other solanaceous plant species in California, but little information was provided on infection conditions, disease levels or its significance. The epidemiological implication of hairy nightshade as an alternate host of *P. infestans* has not been well established. The susceptibility of cultivated solanaceous hosts and weed species to artificial inoculation with A1 or A2 mating types of *P. infestans* was examined in greenhouse settings in Prince Edward Island, Canada (Platt, 1999). In that research, variations in late blight infection and symptom expression were observed among climbing nightshade (*Solanum dulcamara* L.), hairy nightshade, potato (*Solanum tuberosum* L.) and tomato (*Lycopersicum esculentum* L.) hosts. No late blight infection of black nightshade

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(*Solanum nigrum* L.), eggplant (*Solanum melongana* L. var. *esculentum* Nees.), green pepper (*Capsicum frutescens* L.) or tobacco (*Nicotiana tabacum* L.) were noted. In other studies, infection and sporulation of *P. infestans* on petunia was documented under greenhouse conditions in New York (Becktell et al., 2005) and the susceptibility of five nightshade species to *P. infestans* was reported in controlled experiments in Idaho (Dandurand et al., 2006).

In South America, pear melon (*Solanum muricatum* L.) and wild *Solanum* species served as alternate hosts for *P. infestans*, thereby posing significant risks for late blight outbreak (Adler et al., 2002; Garry et al., 2005; Chacón et al., 2006). Similarly, natural infection of *Solanum incanum* L. and *Solanum panduraforme* Drege by *P. infestans* was reported in the highland tropics of East Africa (Nattrass and Ryan, 1951), while *P. infestans* infection of gboma eggplant (*S. macrocarpon*), worowo (*Solanecio biafrae* L.), garden huckleberry (*Solanum scabrum*), billy goatweed (*Ageratum conizoides*), Dichrocephala (*Dichrocephala integrifolia*) and haemorrhage plant (*Aspilia africana*) was observed in controlled experiments in Cameroon (Fontem et al., 2004). The natural occurrence of *P. infestans* was reported on woody nightshade (*Solanum dulcamara* L.) in Ireland (Cooke et al., 2002). Extensive late blight infection of thorny nightshade (*Solanum sisymbriifolium*), woody nightshade and black nightshade (*Solanum nigrum* L.) from natural inoculum were reported in potato fields in the Netherlands (Flier et al., 2003).

During the 2004 and 2005 cropping seasons, we observed *P. infestans* infection of hairy nightshade in potato fields in Maine (Olanya and Larkin, 2005; Olanya et al., 2005). The significance of hairy nightshade infection to late blight epidemiology under conditions in Maine has not been documented. The effects of temperature and RH on late blight development on hairy nightshade and its susceptibility to *P. infestans* in relation to inoculum load and hairy nightshade growth stage have not been established. Hairy nightshade is a common weed in the potato agro-ecosystem in Maine and elsewhere which may pose potential weed and disease management problems. Therefore, the objectives of this research were to: (i) assess the effects of temperature and RH on *P. infestans* infection of hairy nightshade relative to potato and tomato hosts, (ii) evaluate the effects of hairy nightshade ontogeny and *P. infestans* inoculum levels on susceptibility of hairy nightshade plants in controlled experiments and (iii) examine the distribution of hairy nightshade weeds in potato fields in relation to cropping variables and document late blight infection of the weed.

Materials and Methods

Experimental materials

The isolates of *Phytophthora infestans* used in this research consisted of 00-109, 02-1, 00-9, and 00-87 which represented genotype designations 100/111/122, 100/122, 111/122 and 100/100, respectively based on allozyme analysis (Groves, 2002). All isolates were collected from potato and tomato plants in Maine and maintained on Rye A agar (Caten and Jinks, 1968) in the culture collection at USDA-ARS, New England Plant, Soil, and Water Laboratory, Orono, Me. The hairy nightshade plants (*Solanum sarrachoides* Sendt.) used in the experiments were grown in the greenhouse. The seeds were obtained from mature plants collected from potato field sites in northern Maine. Hairy nightshade seeds were pretreated in petri-dishes with 100 ppm of giberellic acid solution for 24 h to break dormancy and stimulate germination of hairy nightshade seeds prior to seeding. The hairy nightshade seeds were planted in metro-mix growing medium in the greenhouse (26°C). After 2 weeks, the germinated seedlings were transplanted to 10 cm diameter pots for further growth. Tomato seeds (cultivar Brandywine) were planted in the greenhouse under the same conditions for germination and growth. Potato seed (cultivar Shepody) was laid in a greenhouse bench until buds were formed and then planted in metro-mix medium and watered periodically as needed. The seedlings of hairy nightshade, tomato and potato were used in experiments on infection rates and late blight development.

Effects of temperature and pathogen isolates

The infection rates of *P. infestans* were assessed on potato (cv. Shepody), hairy nightshade, and tomato (cv. Brandywine) plants in growth chamber experiments at four different temperatures and with four different pathogen isolates, representing diverse pathogen genotypes. Seed pieces of potato, tomato and hairy nightshade plants were planted in sterilized soil/peat mixture under greenhouse conditions. Planting dates were synchronized for the three hosts in order to have relatively uniform seedling plant age at inoculation time. Hairy nightshade, potato and tomato seedlings at 6–8 weeks, were each inoculated simultaneously with isolates 00-109, 02-1, 00-9, and 00-87. Three plants from each host (potato, hairy nightshade and tomato) were inoculated at the same time by atomizing with sporangia suspension (5×10^3 sporangia/ml) onto leaves and placing the plants in growth chambers set at 14, 18, 22 and 26°C. These settings were chosen to represent the ranges in field temperature observed during the potato growing season in Maine. Relative humidity during incubation was maintained at 95%, and growth chambers were illuminated with 12 h of fluorescent light/12 h of darkness. The experimental design was a factorial (four temperatures × three hosts × four pathogen isolates) arranged in a randomized block design with three replications. Each temperature chamber served as a block, and separate randomizations of hosts and pathogen isolates within a chamber were performed.

Incidence and severity of late blight were assessed at 2-day intervals following inoculation. Late blight incidence was visually assessed as number of leaves diseased/total leaves per plant × 100%. Late blight severity was assessed as percent leaf area diseased on

each plant. The incidence of late blight on hairy nightshade stems was also visually assessed by counting number of diseased stems and expressing as percentage of total stems. The experiment was conducted twice.

An additional experiment with the same four temperature × four isolate × three host treatment structure was conducted to assess temperature effects on pathogen lesions using the same isolates. Six- to eight-week-old hairy nightshade, tomato and potato plants were inoculated at the same time by atomizing leaves with sporangia suspension (5×10^3 sporangia/l) with each *P. infestans* isolate consisting of 00-109, 02-1, 00-9, and 00-87. After inoculation, seedlings were incubated in growth chambers at temperatures of 14, 18, 22 and 26°C, each maintained at 95% RH with the same lighting conditions as described above. The number of pathogen lesions was quantified per plant at 2-day intervals. Lesion expansion rates on diseased hairy nightshade plants were obtained by measuring four lesions per plant, on two hairy nightshade plants per pathogen isolate. The experiment was conducted twice.

Effects of RH

Due to the fact that RH conditions for late blight development are well documented for potato and tomato (Minogue and Fry, 1981; Hartill et al., 1990; Gleason et al., 1995), humidity effects were only evaluated on hairy nightshade. Six to eight-week old hairy nightshade plants were inoculated with sporangia suspension at 5×10^3 sporangia/ml using the 00-109, 02-1, 00-9, and 00-87 isolates. The RH treatments consisted of growth chambers maintained at humidity settings of 72 ± 5%, 82 ± 5%, 87 ± 5%, and at 95 ± 5%, with 12 h light and dark periods. The experimental design consisted of a 4 × 4 factorial (RH × pathogen isolate) with three replications. Three plants per pathogen isolate were inoculated and placed in growth chambers at the above RH settings. The temperature in all growth chambers was maintained at 18°C. The same experiment on RH effects was also conducted with the temperature of growth chambers maintained at 22°C. The foliar blight incidence and severity was assessed at 2-day intervals after inoculation. The experiment was repeated twice.

Effects of inoculum levels

The effects of inoculum levels on infection and development of late blight were determined on 6-week-old hairy nightshade plants. Hairy nightshade plants were inoculated with a single isolate of *P. infestans* (00-109, 100/111/122 genotype). Three inoculum levels of 5×10^3, 10×10^3 and 25×10^3 sporangia/ml of sterile distilled water and an un-inoculated control (sterile distilled water) were used. The four treatments were arranged in a completely randomized design (CRD) in each growth chamber and four replications (growth chambers) were used. Two plants per inoculum level per replicate (eight plants/growth chamber) were inoculated by atomizing sporangia suspension onto leaves of hairy nightshade plants. The growth chambers were maintained at 95% RH/22°C. Foliar blight incidence and severity (%) as well as stem blight incidence and severity (%) were assessed on hairy nightshade plants at 2-day intervals subsequent to inoculations. The experiment was repeated twice.

Effect of plant growth stage

To evaluate the susceptibility of hairy nightshade to infection and disease development, hairy nightshade plants at various stages of growth were used. At 4, 6, 8 and 10 weeks old, hairy nightshade plants representing seedling, flowering, berry formation, and maturity stages of growth were inoculated by *P. infestans* (isolate 00-109, 100/111/122 genotype and US 8). The leaves were atomized with 5×10^3 sporangia/ml of distilled water. Plants were incubated in replicated growth chambers at 22°C and 95% RH. The experimental design consisted of plants at four growth stages as the treatment, arranged in a completely randomized design with four replications. The experimental unit was a nightshade plant, and two plants per growth stage per replicate were inoculated with *P. infestans*. Plants were assessed for late blight severity (%), and disease incidence (number of leaves diseased/total leaves × 100%). Assessment for late blight was conducted at 2-day intervals. The hairy nightshade plants were assessed for foliage blight severity and for the number of diseased (symptomatic) leaves. The experiment was conducted two times.

Sporangia production

The potential for sporangia production was monitored on symptomatic and diseased leaves of hairy nightshade, potato and tomato plants. The foliage of three plants of each host was inoculated by atomizing with sporangia (5×10^3/ml of distilled water) of *P. infestans* (isolates 00-109, 02-1, 00-87 and 00-9). The plants were incubated in growth chambers at 22°C under high RH (95%) conditions. The potential for sporangia production was quantified by destructive sampling of 0.5 cm^2 diseased tissue from leaves of each host plant, immersing in 0.5 ml of sterile distilled water, and counting the sporangia microscopically.

Field distribution of hairy nightshade

The occurrence and distribution of hairy nightshade in northern Maine was investigated in a random survey of fields cropped to potato, broccoli or fallow during the cropping seasons in 2005 and 2007. Cultural practices (hairy nightshade control, hilling, crop rotation, fungicide herbicide applications, and tillage) were also examined. Fields were surveyed for hairy nightshade occurrence and density based on accessibility, previous history of late blight, and cultural practices. Within a field, a W-shaped pattern (zig-zag) was used in assessing four to eight sampling sites. Each sample site within a field comprised of two rows, 4.5 × 1.8 m (length × width). The density of hairy nightshade plants in relation to late blight infection was also

noted. The stages of hairy nightshade growth in relation to potato development were recorded. Hairy nightshade density counts were assessed on 7 July, 21 July, 11 August, and 7 September in 2005, and on 18 July, 18 August and 31 August in 2007.

Statistical analysis

Data on the effects of temperature on pathogen infection rates were subjected to temporal analysis of disease progress. Data on percent leaf area blighted (late blight severity) was fitted to linear forms of the Gompertz (gompits/day – unit for quantifying infection rates) and Logistic (logits/day – unit for quantifying infection rates) models (Neher et al., 1997) using the Statistical Analysis System, SAS (SAS Institute Inc., Cary, NC, USA, 2005) to describe temporal late blight progress and estimate model parameters (initial disease severity (Y_0), rate of disease progress (r), and final blight severity). The most suitable model for parameter estimation was determined by using standardized residual plots, adjusted coefficients of determination (R^2), and asymptotic standard errors of estimates of initial late blight severity and rates of disease progress (Neher et al., 1997). The significance of the rate parameters (r) among hosts were compared by analysis of variance (ANOVA) and Fisher's LSD statistics. The effects of temperature, pathogen isolates, RH and their interactions on late blight incidence and severity were evaluated by ANOVA. The comparative effects of inoculum levels on susceptibility of hairy nightshade plants were also subjected to GLM analysis of SAS and the significance of average late blight incidence and severity were compared by Fisher's LSD statistics. Lesion numbers on diverse hosts at different temperatures and lesion development on hairy nightshade, plant age effects on late blight severity were compared graphically following mean comparisons by Fisher's LSD statistics. Frequency of occurrence and distribution of nightshade plants in fields were determined by calculating the mean, variance and standard error by Proc Univariate Frequency. Hairy nightshade counts were also analysed using other indices of dispersion such as variance to mean ratios, Morisita's index (Schuh et al., 1986) and binomial distribution (K-values) fitting (Gates and Etheridge, 1970).

Results

Effects of temperature and pathogen isolates

Late blight infection was observed at all incubation temperatures, except at 26°C where no infection was observed on tomato and hairy nightshade plants. The infection rates and other disease estimates were best described by the Gompertz model, therefore; only parameters calculated from this model were presented. The pathogen infection rates (r) differed significantly ($P < 0.05$) between tomato (cv. Brandywine) and potato (cv. Shepody) hosts at 22°C, and between the two hosts with hairy nightshade at 14 and 18°C (Table 1). The infection rates (r) ranged from 0.0325 to 0.4674 gompits/day across incubation temperatures. The pathogen infection rates were greater at 18 and 22°C than at 14 or 26°C. Disease severity was best described with the Gompertz model. At 18°C, infection rates varied among pathogen isolates, ranging from 0.2412 to 0.6416 gompits/day (Table 2). Interactions of temperature x host and isolate x temperature were significant ($P < 0.05$) for late blight incidence and severity (Table 3). When comparisons were made among pathogen hosts, blight incidence and severity were significantly greater on potato and tomato than on hairy nightshade (Fig. 1). Late blight incidence and severity were generally greater on all hosts when inoculated with isolates 00-109 and 02-1 than on hosts inoculated with isolates 00-87 and 00-9, respectively. A quadratic regression showed the trend of foliage blight incidence, severity and stem incidence on hairy

Table 1
Effects of temperature on infection rates of *Phytophthora infestans* on potato, hairy nightshade and tomato hosts in growth chamber experiments[a]

Temperature (°C)	Host	Intercept (Y_0)	Infection rate (r) (gompits/day)	SE of r[b]	[c]R^2 (%)
14	Potato	0.0847	0.4078a	0.0197	0.54
	Hairy nightshade	0.0031	0.1419b	0.0050	0.40
	Tomato	0.0604	0.3582a	0.0604	0.48
18	Potato	0.1155	0.4186a	0.0244	0.65
	Hairy nightshade	0.0085	0.3426b	0.0031	0.63
	Tomato	0.0729	0.4308a	0.0197	0.49
22	Potato	0.1052	0.4674a	0.0246	0.59
	Hairy nightshade	0.0044	0.1654c	0.0009	0.45
	Tomato	0.0685	0.3633b	0.0149	0.54
26	Potato	0.0147	0.0325	0.0147	0.35
	Hairy nightshade	–[d]	–	–	–
	Tomato	–	–	–	–

[a]Parameter estimates are from linear regression of late blight severity on time (days of disease assessment) based on the logistic model. The intercept (Y_0) and rate (r) represent the slope of the equation of predicted line. Different letters indicate significant differences ($P < 0.05$) in infection rate parameter among hosts. Data were computed from three replicates (growth chambers) with three plants per host per pathogen isolate. Four pathogen isolates (00-109, 02-1, 00-87 and 00-9 representing genotypes 100/111/122, 100/122, 100/100 and 111/122 based on allozyme analysis). The units for rate of disease progress are gompits/day. Values followed by the same letter are not significantly different ($P < 0.05$) based on Fisher's LSD statistics; [b]SEM is standard error of the mean; [c]coefficient of determination; [d]disease severity values too low for calculation of infection rates.

Table 2
Parameter estimates for late blight infection of hairy nightshade, caused by *Phytophthora infestans* in growth chamber experiments (18°C)[a]

Isolates[b]	Intercept (Y_0)	Infection rate (r) (gompits/day)	SEM of r[c]	[d]R^2 (%)
00-109	0.0033	0.6416a	0.1091	0.70
00-87	0.0002	0.5263b	0.0616	0.62
02-1	0.0067	0.4887b	0.0469	0.88
00-9	0.0025	0.2412c	0.0440	0.66

[a]Parameter estimates are from linear regression of late blight severity on time (days of disease assessment) based on the logistic model. The intercept (Y_0) and rate (r) represent the slope of the equation of predicted line. Different letters indicate significant differences ($P < 0.05$) in infection rate parameter among pathogen isolates. Data were computed from three replicates (growth chambers) with three plants per isolates. The units for rate of disease progress are gompits/day. Values followed by the same letter are not significantly different ($P < 0.05$) based on Fisher's LSD statistics; [b]Pathogen isolates 00-109, 00-87, 00-9 and 02-1 represent genotype designations 100/111/122, 100/100, 111/122, and 100/122 based on allozyme analysis (glucose-6-phosphate); [c]SEM is standard error of the mean; [d]Coefficient of determination.

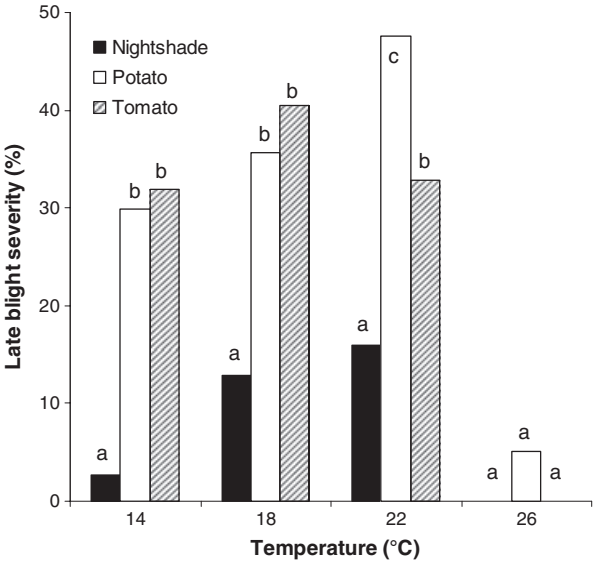

Fig. 1 Effects of temperature and host diversity on late blight severity (%) on plants incubated at 95% RH in growth chamber experiments. Data represents mean of two experiments. The same letters within each temperature group imply no significant differences in disease among hosts, and different letters refer to significant differences ($P < 0.05$) in late blight severity among hosts based on Fisher's LSD statistics

nightshade, in which disease levels were significantly higher at 18 and 22°C than at 14 or 26°C (Fig. 2).

The number of lesions differed, depending on plant host. Across temperatures, fewer lesions were observed on hairy nightshade (0–12) than on potato and tomato (Fig. 3). Very few to no lesions were observed at 26°C. The growth or expansion of lesions on diseased hairy nightshade plants varied among pathogen isolates and ranged from 5 to 36 mm after 7 days of incubation (Fig. 4).

Table 3
Analysis of variance on the effect of temperature and pathogen isolates on development of late blight on hairy nightshade, potato and tomato plants in growth chamber experiments

Source of variation	df	F-value	P > F
Disease severity (%)[a]			
Rep	2	8.73	0.0831
Temp	3	31.35	0.0563
Rep*temp	6	7.74	0.0634
Host	2	88.44	0.001**
Temp*host	6	0.99	0.0003**
Isolate[b]	3	10.25	0.001**
Isolate*temp	9	2.78	0.0079**
Isolate*host	6	19.29	0.038*
Isolate*temp*host	18	1.29	0.2329
Late blight incidence (%)[c]			
Rep	2	8.73	0.1531
Temp	3	14.78	0.0385*
Rep*temp	6	1.31	0.2719
Host	2	43.47	0.001**
Temp*host	6	6.67	0.0520
Isolate[X]	3	21.96	0.0192**
Isolate*temp	9	7.65	0.0098**
Isolate*host	6	17.50	0.055
Isolate*temp*host	18	2.04	0.2610

[a]Refers to % leaf area diseased based on visual assessment of hairy nightshade, potato and tomato plants incubated at 14, 18, 22 and 26°C; [b]Isolates consisted of 00-109, 02-1, 00-87 and 00-9 with 100/111/122, 100/122, 100/100 and 111/122 genotype designations; [c]Number of diseased plants/total plants × 100; *Significant at 0.05 levels; **significant at 0.01 levels.

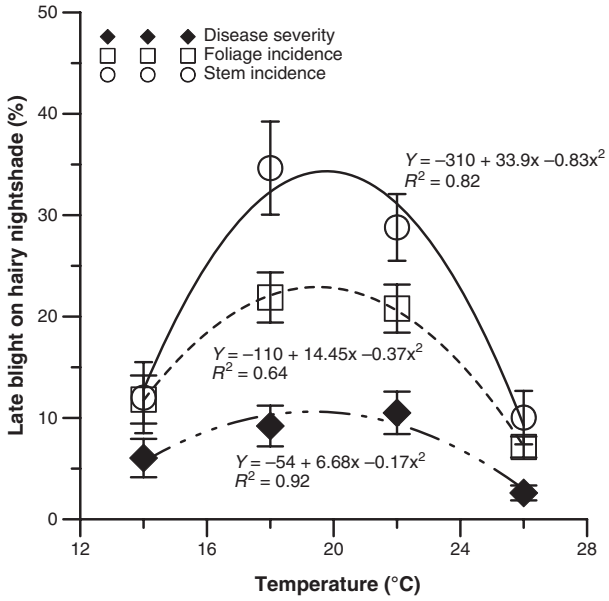

Fig. 2 Effect of temperature on development of late blight on hairy nightshade plants in controlled experiments. Six-week-old plants were inoculated with *P. infestans* (isolate 00-109) and incubated at 95% RH. Disease severity (% leaf area diseased), foliage incidence (leaves diseased/total leaves × 100%), and stem incidence (stems diseased/total stems × 100%) were plotted at temperatures (14–26) of incubation. Data represent means from three experiments and error bars refer to standard errors

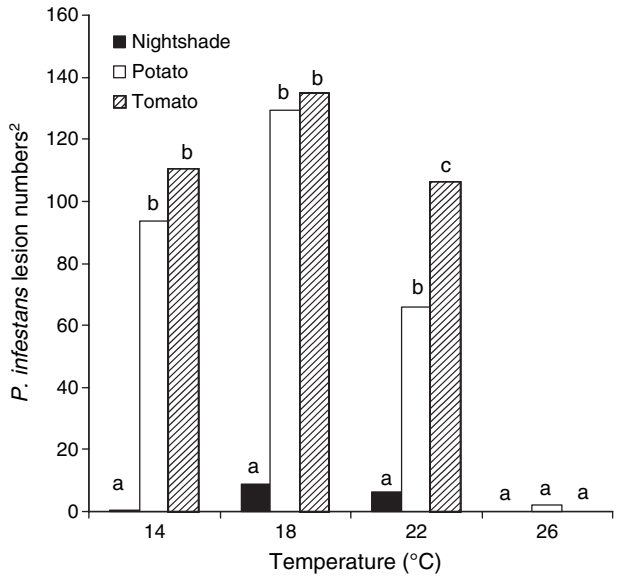

Fig. 3 Average lesion numbers on hairy nightshade, potato and tomato plants inoculated with isolates of *Phytophthora infestans* and incubated at various temperatures at 95% RH in growth chamber experiments. The lesion numbers on potato, tomato and hairy nightshade were quantified from average leaf sizes of 38.6, 18.7 and 12.9 cm^2, respectively. Data represent mean for two experiments and different letters on bar graphs within a temperature indicate significant differences ($P < 0.05$) in disease levels based on Fisher's LSD statistics

Effects of RH

The effects of RH on blight development varied among pathogen isolates in these experiments (Table 4). The interaction of isolates x RH was significant ($P < 0.05$) for disease incidence and severity.

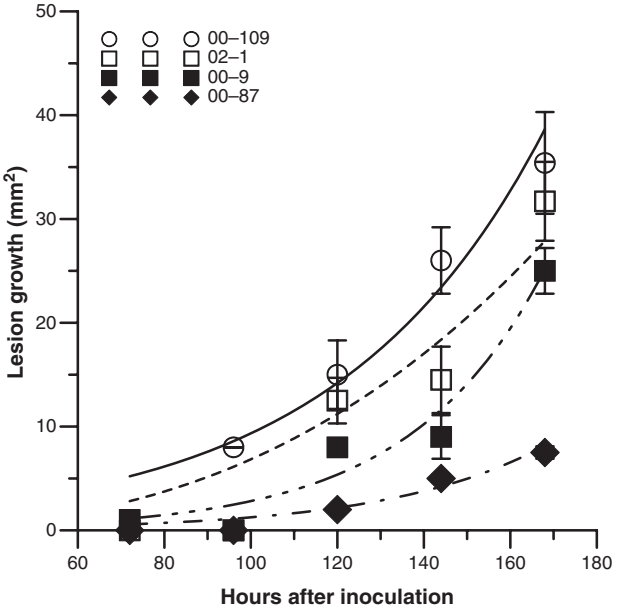

Fig. 4 Lesion expansion on leaves of diseased hairy nightshade (*Solanum sarrachoides* Sendt.) plants inoculated with diverse isolates of *Phytophthora infestans* and incubated at 22°C/95% RH. The error bars refer to standard errors and data represents mean of two experiments

Table 4
Analysis of variance on the effect of relative humidity (%) and pathogen isolates on development of late blight on hairy nightshade plants in growth chamber experiments

Source of variation	df	F-value	P > F
Disease severity (%)[a]			
Rep	2	0.06	0.9669
Isolate[b]	3	6.29	0.0003**
Rep*isolate	6	1.35	0.2323
Relative humidity (RH)[c]	3	3.12	0.0258*
Isolate*RH	9	1.18	0.0351*
Late blight incidence (%)[d]			
Rep	2	0.24	0.8592
Isolate	3	11.50	0.0001**
Rep*isolate	6	2.05	0.0746
RH	3	3.55	0.0145*
Isolate*RH	9	2.14	0.0250*

[a]Percent of leaf area diseased based on assessment of hairy nightshade plants incubated at 22°C; [b]Isolates 00-109, 02-1, 00-87 and 00-9; [c]The relative humidity values were 72, 82, 87 and 92% in growth chambers where nightshade plants were incubated following artificial inoculations; [d]Number of diseased leaves/total leaves on a plant × 100; *Significant at 0.05 levels; ** significant at 0.01 levels.

Among pathogen isolates, late blight incidence and severity on hairy nightshade varied and disease incidence levels ranged from 10.7 to 40.8% at 18°C, and 0.4 to 47.9% at 22°C, respectively (Table 5). Late blight severity (%) ranged from 5.8 to 17.4% and from 0.25 to 17.5% at 18 and 22°C, respectively. Once infection was established, there was consistent late blight development on hairy nightshade plants regardless of the RH conditions maintained in the growth chambers; however, significant effects of RH among pathogen isolates were observed (Table 5).

Effect of inoculum levels

Infection of hairy nightshade was observed at all inoculum levels applied, when plants were incubated at 22°C and 95% RH (Table 6). Inoculum load affected foliar and stem blight severity, as well as disease incidence. No disease was detected on the un-inoculated control. Foliar blight severity did not differ significantly between inoculum levels of 5000 and 10 000 sporangia/ml, but disease level increased slightly at higher inoculum level (25 000 sporangia/ml of distilled water). Foliar and stem blight incidence were similar regardless of inoculum level applied (Table 6).

Effect of plant growth stage

Late blight infection was observed at different stages of hairy nightshade growth (Fig. 4). Blight severity ranged from 6.3 to 19.5% across hairy nightshade growth stages of 4–10 weeks, while foliage infection frequency ranged from 10.9 to 40% (Fig. 5). Significant difference in foliar blight severity was not observed between plants at 4–6 weeks old, or between 8 and 10 week-old plants. The infection frequency of nightshade (foliage blight incidence %) ranged from 10.9 to 36.4% across hairy nightshade plants at 4–10 weeks old and there were significant differences

Table 5
The effects of relative humidity (%) and pathogen isolates on average late blight severity on hairy nightshade in growth chamber inoculations

	Relative humidity (%)[a]							
	72	82	87	92	72	82	87	92
Isolates[b]	DS (%)[c]	DS (%)	DS (%)	DS (%)	DI (%)[d]	DI (%)	DI (%)	DI (%)
Temperature (18°C)								
00-109	12.7	17.4	13.2	15.9	14.0	40.8	33.4	33.9
02-1	10.9	6.8	14.9	12.5	10.7	18.2	35.8	34.2
00-87	8.1	6.2	6.3	6.6	16.8	15.3	25.6	17.2
00-9	7.8	5.8	12.2	10.3	15.2	12	11.9	18.7
LSD $_{(0.05)}$	4.3	4.5	8.2	7.8	6.6	11.2	14.0	13.7
Temperature (22°C)								
00-109	7.3	7.0	17.5	16.1	23.73	15.1	47.9	46.4
02-1	1.4	6.3	9.2	7.7	2.69	12.5	23.9	25.1
00-87	0.69	1.1	4.9	5.3	1.8	2.4	11.0	15.0
00-9	0.25	0.9	0.5	0.4	0.51	1.5	0.4	1.8
LSD $_{(0.05)}$	1.6	3.2	3.2	2.7	3.6	4.5	5.9	4.2

[a]Relative humidity of growth chambers in which inoculated hairy nightshade plants were placed for late blight development; [b]*Phytophthora infestans* isolates 02-1, 00-19, 00-9 and 00-87 are of 100/122, 100/111/122, 111/122 and 100/100 genotype designations based on allozyme analysis with glucose 6-phosphate; [c]Percentage leaf area diseased based on visual assessment of hairy nightshade foliage. Data represent mean of three replicates; [d]Number of diseased leaves/total leaves on a plant × 100. Means are based on Fisher's LSD statistics.

Table 6
Effects of inoculum levels on late blight development on hairy nightshade inoculated in growth chamber experiments at 22°C

Inoculum levels (sporangia/ml)[a]	Foliar blight severity (%)[b]	Foliar blight incidence (%)[c]	Stem blight severity (%)[d]	Stem blight incidence (%)[e]
25 000	15.3	51.5	10.7	45
10 000	10.8	42.3	9.4	41
5000	10.3	40.2	7.3	30.7
LSD $_{(0.05)}$	3.4	8.6	5.8	12.5

[a]Inoculum levels represent low, moderate and high concentrations of sporangia. Data means from two experiments; [b]Average leaf area diseased (%) incited by *P. infestans* isolate 00-109 (100/111/122 genotype) based on visual symptoms assessed on two plants per inoculum level per replicate; [c]Mean number of leaves diseased/total leaves on a plant expressed as a percentage; [d]Mean disease severity (%) on stems, with symptomatic lesions of *P. infestans*; [e]Mean number of diseased stems/total stems on a plant expressed as a percentage. Mean values are based on Fisher's LSD statistics.

($P < 0.05$) in disease levels at different plant-age groups. A significant increase in late blight severity and infection frequency was detected on older plants.

Sporangia production

Sporangia production on hairy nightshade, potato and tomato leaves was observed when plants were inoculated and incubated at 22°C at 95% RH. More sporangia were produced on diseased potato and tomato than on hairy nightshade plants (Fig. 6). No sporangia were produced when inoculated plants were incubated at 14 or 26°C with excess RH of 95% (data not shown).

Field distribution of hairy nightshade

Hairy nightshade plants were observed at different stages of growth from seedling to berry formation. Variation in average hairy nightshade infestation of fields or density was detected across sampling times from July to September (Table 7). The percentage of fields infested with hairy nightshade varied among sampling times and between years. Hairy nightshade plants were observed in 38–50% of the fields surveyed in 2005 and in 67–80% of fields surveyed in 2007. Hairy nightshade density varied widely among fields

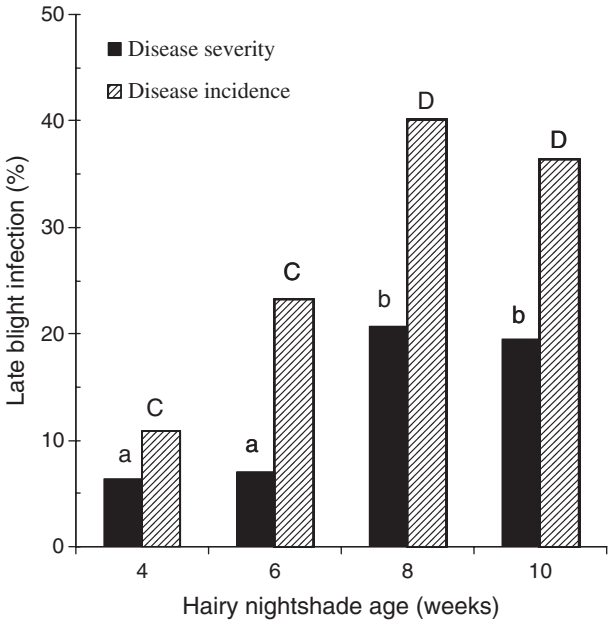

Fig. 5 Effects of hairy nightshade plant age at the time of inoculation on development of *P. infestans* in controlled experiments. Plants were inoculated with *P. infestans* (isolate 00-109) and incubated at 18°C/95% R.H. Data represents average of two experiments consisting of three replicates. Disease severity refer to percent leaf area diseased, and disease incidence refer to number of leaves diseased/total leaves on plant × 100%. Different lower case and upper case letters among plant age refer to significant differences in disease severity and infection frequency, respectively; while the same letters imply no significant differences ($P < 0.05$) in disease levels among plant ages based on Fisher's LSD statistics

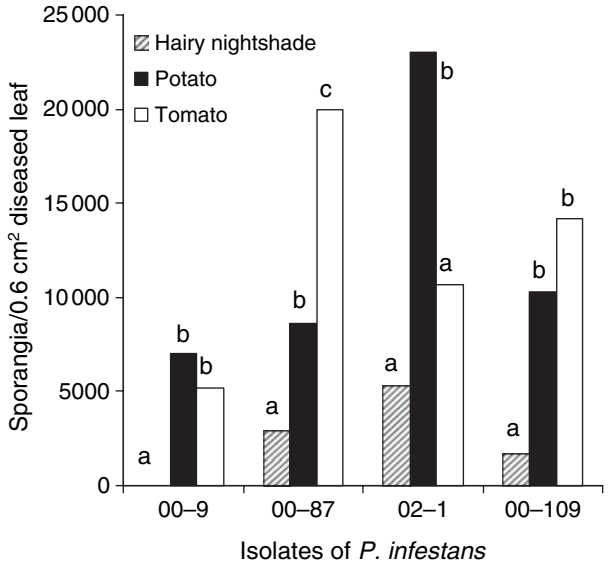

Fig. 6 Comparative production of sporangia on symptomatic leaves of hairy nightshade, potato and tomato hosts artificially inoculated with *P. infestans* isolates and incubated at 22°C/95% RH for 7 days in controlled experiments. Three symptomatic leaves from each diseased host and three replicates were detached and quantified for sporangia numbers microscopically following immersion in 500 μl of distilled water. The same letters among host plants within an isolate refer to non-significant differences in sporangia numbers. Different letters refer to significant differences ($P < 0.05$) in sporangia production among diseased hosts within an isolate based on Fisher's LSD statistics

and sampling periods (Table 7). Based on the variance-to-mean ratios, and Taylor's index of dispersal, an aggregated spatial distribution of hairy nightshade was generally observed within fields (Table 7). The indices of dispersion indicated spatial aggregation of hairy nightshade plants within fields in many instances. Across sampling times, the spatial pattern of weed plants within fields was also aggregated (Table 7). The occurrence and density of hairy nightshade plants within fields varied with cropping pattern or history. During the 2005 survey, hairy nightshade field density was low in broccoli and small grain crops (0–100 plants/m^2 on July 7, and 51–86 plants/m^2 on July 21), but much higher in potato (21 015 plants/m^2 on July 7 and 40 231 plants/m^2 on July 21). Overall, fields previously cropped to broccoli and currently planted with potato had higher density of hairy nightshade. No late blight was detected on hairy nightshade during our sampling surveys, but low levels of late blight were reported elsewhere in the state on hairy nightshade by September of 2005.

Discussion

Isolates of *P. infestans* from potato and hairy nightshade caused typical symptoms of late blight under field and growth chamber experiments (Olanya et al., 2005). Infection of foliage and stems occurred under natural and artificial inoculations, implying that hairy nightshade can be readily infected by *P. infestans* and may therefore serve as a potential host for this pathogen. In contrast to findings of Dandurand et al. (2006) in which excised leaves of nightshade plants and berries were wounded prior to inoculation, our experimental results showed that wounding prior to inoculation may not be necessary for hairy nightshade infection by *P. infestans*.

We observed differences in infection rates among hairy nightshade, potato and tomato hosts. The low infection rates observed on hairy nightshade suggest that overall infection levels and disease progress would be expected to be lowest on hairy nightshade compared to tomato and potato hosts. This also suggests that hairy nightshade may potentially be of significance as a secondary source of inoculum for *P. infestans*. In our experiments, average disease severity on hairy nightshade did not exceed 50% in growth chamber inoculations. In field observations, less than 10% disease levels were observed on hairy nightshade infected naturally by *P. infestans*.

This is the first research to document a comparative analysis of the infection rates of *P. infestans* on hairy nightshade relative to tomato and potato hosts under controlled conditions. Even though no significant infection of hairy nightshade by *P. infestans* was observed in field surveys, results from greenhouse experiments indicate that *P. infestans* infection of hairy nightshade in the field could occur in the presence of inoculum and favourable conditions. Previous studies have documented significant late blight epidemics as a consequence of infection of alternate hosts such as woody nightshade (*Solanum dulcamara*), black nightshade (*Solanum nigrum*), and thorny nightshade (*Solanum sisymbriifolium*) in Holland (Zwankhuizen et al., 2000; Flier et al., 2003).

Hairy nightshade infection varied with pathogen isolates or genotypes used in inoculations. The isolate 00-109 consistently produced higher disease levels and lesion expansion rates compared to other isolates. This suggests that the pathogen isolate of genotype designation 100/111/122 is more virulent or pathogenic on hairy nightshade than the isolates 02-1, 00-87 and 00-9, representing other *P. infestans* genotypes. The variability among pathogen isolates with regard to *P. infestans* is not specific to hairy nightshade host. Previous research documented pathogen variation on a potato host (Olanya and Larkin, 2005). Late blight infection of hairy nightshade has been reported previously (Vartanian and Endo, 1985; Platt, 1999; Dandurand et al., 2006); however, no assessment or quantification of pathogen isolate effects have been documented previously.

Late blight development on hairy nightshade depended on temperature. Foliar blight severity, foliar blight incidence, and stem blight incidence had quadratic response to temperature, with greater late blight at 18 and 22°C than at 14 or 26°C. During the potato production season in Maine, the average ambient temperature conditions in the field often range from 10 to 26°C (Olanya et al., 2007). Because hairy nightshade is a weed in the potato agroecosystem with similar

Table 7
Occurrence and distribution of hairy nightshade (*Solanum sarrachoides*) in potato fields during 2005 and 2007 cropping seasons in northern Maine

Year	Sample Data	% fields with[a] hairy nightshade	Samples in field[b]	Mean[c] (plants/m^2)	Variance/ mean[d]	Variance	*K*-value[e]
2005							
	July 7	50 (4/8)	6	26	113	4	8
	July 7	7	2	18	8	0.3	
	July 7	6	8	332	42	0.3	
	July 7	3	12660	395388	3	123	4
	July 21	37.5 (3/8)	6	4	15	4	1
	July 21	6	11	604	55	0.2	
	July 21	6	28	1195	43	0.7	
	Aug 11	44.4 (4/9)	5	412	18949	133	1
	Aug 11	5	43.6	4349	100	0.4	
	Aug 11	6	4	40	11	0.3	
	Aug 11	4	49,319	628053	12.7	2.4	
	Sept 7	40 (2/5)	6	11	475	44	0.3
	Sept 7	6	24	1724	71	0.4	
2007							
	July 18	80 (4/5)	7	2	14	9	0.2
	July 18	7	87	23100	266	0.3	
	July 18	6	0.3	0.7	2	0.3	
	July 18	6	27	2934	109	0.3	
	Aug 18	66.7 (4/6)	5	150	58719	391	0.4
	Aug 18	4	47	7312	156	0.3	
	Aug 18	5	1	5	5	0.25	
	Aug 18	5	141	23818	168	0.8	
	Aug 18	5	11	264	25	0.4	
	Aug 31	80 (4/5)	5	10	105	11	1
	Aug 31	5	13	135	10	1.4	
	Aug 31	5	42	1145	27	2	
	Aug 31	5	13	198	15	1	

[a]Percentage of fields surveyed with hairy nightshade. Numbers in parenthesis refer to fields with hairy nightshade/total number of fields surveyed during each sampling period; [b]Number of samples from contiguous two-row plots (8.3 m^2) assessed for hairy nightshade plants within potato fields during cropping season. The counts were based on W-shaped sampling pattern within a field and where there was no hairy nightshade, the fields were not included in the analysis. A total of four positive fields with 6, 7, 6 and 3 sample units within fields were done on 7 July 2005. On July 18, 4 fields with positive hairy nightshade counts had 7, 7, 6 and 6 sampling units within fields, respectively; [c]Density of hairy nightshade plants/8.3 m^2 were obtained from counts sampled from several sampling sites within field; [d]Based on Morisita's index of aggregation and variance to mean ratios, values >1 indicates clumped distribution of weed plants in a field, values <1 indicate random distribution; [e]Index of aggregation based on binomial probability distribution of counts. Values of $K < 3$ indicate clumped distribution and >3 refers to slight aggregation of weed plants within a potato field.

physiological growth as potato, infection of hairy nightshade in the field can be expected under this range of temperatures, if inoculum is present. To our knowledge, no previous research has demonstrated the effect of temperature on late blight development on hairy nightshade. Our data shows that the optimum temperature range (18–22°C) for infection of hairy nightshade is the same as that previously reported for potato and tomato (Minogue and Fry, 1981; Hartill et al., 1990; Sato, 1994; Mizubuti and Fry, 1998; Becktell et al., 2005).

Variation in RH effects on foliar incidence and late blight severity was observed in our experiments. This suggests that once infection occurs, blight development on hairy nightshade can be expected at RH ranges of 72–92%. This implies that moisture requirements for late blight development on hairy nightshade are not highly specific within this range of humidity. However, we did not investigate RH requirements for pathogen infection itself. It is also plausible that humidity and temperature may interact to influence infection and disease development on hairy nightshade. There is little published research on the effects of RH or their interactions with temperature on late blight development on hairy nightshade; however, numerous reports are available on this topic for potato or tomato hosts (Wallin, 1962; Krause et al., 1975; Minogue and Fry, 1981; Harisson and Rowe, 1989; Harrison, 1992; Gleason et al., 1995).

Late blight infection of hairy nightshade foliage and stems occurred at various inoculum levels. This suggests that inoculum load is not crucial for pathogen infection of hairy nightshade but may impact disease development on plants. This is similar to potato in which even a low amount of inoculum can result in the initiation of late blight infection, however; optimum inoculum loads have been associated with greater spore dispersal and severe epidemics (Rotem et al., 1971). In our experiments, variable sporangia production was recorded on diseased leaves of hairy nightshade, tomato and potato when plants were inoculated with various pathogen isolates and incubated at 22°C/95% RH. Although inoculum levels may have little consequence on infection potential of hairy nightshade, the potential for sporangia production may be crucial in evaluating

disease risk posed by hairy nightshade in potato fields. Even a relatively small amount of sporangia inoculum can initiate late blight epidemics if hairy nightshade control options are not exercised. It would seem prudent late blight management strategy to recognize that infection of an alternate host may pose a disease risk, irrespective of whether it is a primary or secondary source of inoculum for *P. infestans*.

Infection of hairy nightshade was detected from 2 to 10 weeks of hairy nightshade growth in growth chamber experiments. This implies that various stages of hairy nightshade plant growth (seedling to physiological maturity) can theoretically be infected by *P. infestans*. In this study, we observed various stages of hairy nightshade growth during the field survey on hairy nightshade distribution in potato fields. Precautions should therefore be taken in controlling hairy nightshade to minimize its infection potential and possibility of serving as an inoculum source.

The occurrence of hairy nightshade was detected in 56 and 75% of potato fields surveyed in northern Maine during the 2005 and 2007 field surveys. During the field surveys, we observed potato rotated predominantly with broccoli and small grains, but recorded hairy nightshade in greater numbers in fields previously or currently cropped to broccoli and potato. This suggests that cropping pattern may affect hairy nightshade occurrence and distribution. Within fields, the spatial pattern of hairy nightshade plants was aggregated. The spatial distribution of hairy nightshade plants across sampling time was also aggregated, implying that hairy nightshade density may be increased by weed seed production at specific sites within fields. Despite the above findings, no late blight infections of hairy nightshade were reported in our field surveys in 2005 and 2007, even though a low level of blight was observed in potato.

We conclude that hairy nightshade may serve as a host for *P. infestans* with significant implications based on (i) the distribution of hairy nightshade in the potato agroecosystem, (ii) observed hairy nightshade infection by *P. infestans* in controlled experiments across plant growth stages and inoculum production potential and (iii) the similarity in temperature and humidity requirements for late blight development on hairy nightshade compared to potato and tomato. Even though potato and tomato hosts have greater susceptibility than hairy nightshade, blight management strategies should include control practices that reduce the likelihood of late blight infection of hairy nightshade.

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References

Adler NE, Chacón G, Flier WG, Forbes GA. (2002) The Andean fruit crop, pear melon (*Solanum muricatum*) is a common host for A1 and A2 strains of *Phytophthora infestans* in Ecuador. *Plant Pathol* **51**:802–812.

Becktell MC, Daughtrey ML, Fry WE. (2005) Temperature and leafwetness requirements for pathogen establishment, incubation period and sporulation of *Phytophthora infestans* on Petunia × hybrida. *Plant Dis* **89**:975–979.

Campbell CL, Madden LV. *Introduction to Plant Disease Epidemiology*. New York, NY, USA, John Wiley, 1990, p. 530.

Caten CE, Jinks JL. (1968) Spontaneous variability of single isolates of *Phytophthora infestans*. I. Cultural variation. *Can J Bot* **46**:329–348.

Chacón MG, Adler NE, Jarrin F, Flier WG, Gessler C, Forbes GA. (2006) Genetic structure of the population of *Phytophthora infestans* attacking *Solanum ochranthum* in the highlands of Ecuador. *Eur J Plant Pathol* **115**:235–245.

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

Dandurand LM, Knudsen GR, Eberlein CV. (2006) Susceptibility of five nightshade (*Solanum*) species to *Phytophthora infestans*. *Am J Potato Res* **83**:205–210.

Flier WG, van den Bosch GMB, Turkensteen LJ. (2003) Epidemiological importance of *Solanum sisymbriifolium, S. nigrum and S. dulcamara* as alternate hosts for *Phytophthora infestans*. *Plant Pathol* **52**:595–603.

Fontem DA, Olanya OM, Njualem BF. (2004) Reaction of certain solanaceous and asteraceous plant species to inoculation with *Phytophthora infestans* in Cameroon. *J Phytopathol* **152**:331–336.

Garry G, Forbes GA, Salas A, Santa Cruz M, Perez WG, Nelson RJ. (2005) Genetic diversity and host differentiation among isolates of *Phytophthora infestans* from cultivated potato and wild solanaceous hosts in Peru. *Plant Pathol* **54**:740–748.

Gates CE, Etheridge FG. (1970) A generalized set of discrete frequency distributions with FORTRAN program. *Math Geol* **4**:1–24.

Gleason ML, MacNab AA, Pitblado RE, Ricker MD, East DA, Latin RX. (1995) Disease-warning systems for forecasting tomatoes in eastern North America: are we there yet? *Plant Dis* **79**:113–121.

Groves CL. (2002) Characterization of *Phytophthora infestans* from Maine during 1999 and 2000. *Am J Potato Res* **79**:325–333.

Guenthner JF, Michael KC, Nolte P. (2001) The economic impact of potato late blight on US growers. *Potato Res* **44**:121–125.

Harisson JG, Rowe R. (1989) Effects of humidity and air speed on sporulation of *Phytophthora infestans* on potato leaves. *Plant Pathol* **38**:585–591.

Harrison JG. (1992) effects of the aerial environment on late blight of potato foliage – a review. *Plant Pathol* **41**:384–416.

Hartill WFT, Young K, Allan DJ, Henshall WR. (1990) Effects of temperature and leaf wetness on the potato late blight. *NZ J Crop Hort Sci* **18**:181–184.

Krause RA, Massie LB, Hyre RA. (1975) BLITECAST: a computerized forecast of potato late blight. *Plant Dis Rep* **59**:95–98.

Minogue KP, Fry WE. (1981) Effects of temperature, relative humidity and rehydration rate on germination of dried sporangia of *Phytophthora infestans*. *Phytopathology* **71**:1181–1184.

Mizubuti ESG, Fry WE. (1998) Temperature effects on the developmental stages of isolates from three clonal lineages of *Phytophthora infestans*. *Phytopathology* **88**:837–843.

Nattrass RM, Ryan R. (1951) New hosts of *Phytophthora infestans* in Kenya. *Nature* **68**:85–86.

Neher DA, Reynolds KL, Campbell CL. Analysis of disease progress curves using linear models. In: Francl L, Neher DA (eds), *Exercises in Plant Disease Epidemiology*, St Paul, MN, USA, APS Press, 1997, pp. 29–33.

Olanya OM, Larkin RP. (2005) Temperature and relative humidity effects on late blight incited by *Phytophthora infestans* isolates from diverse genotypes and hosts (Abstract). *Phytopathology* **95**:S77.

Olanya OM, Lambert DH, Plant AB. (2005) Occurrence and cross-infection of *Phytophthora infestans* on hairy nightshade (*Solanum sarrachoides*) and potato (*Solanum tuberosum*) in Maine. *Can J Plant Pathol* **27**:458–460.

Olanya OM, Starr GC, Honeycutt CW, Griffin TS, Lambert DH. (2007) Microclimate and potential for late blight development in irrigated potato. *Crop Prot* **26**:1412–1421.

Platt HW. (1999) Response of solanaceous cultivated plants and weed species to inoculation with A1 or A2 mating type strains of *Phytophthora infestans*. *Can J Plant Pathol* **21**:301–307.

Rotem J, Cohen Y, Putter J. (1971) Relativity of limiting and optimum inoculum loads, wetting durations, and temperatures for infection by *Phytophthora infestans*. *Phytopathology* **61**:275–278.

Sato N. (1994) Effect of sporulating temperature on the limit temperature in indirect germination of the sporangia of *Phytophthora infestans*. *Ann Phytopathol Soc Japan* **60**:60–65.

Schuh W, Frederiksen RA, Jeger MJ. (1986) Analysis of spatial patterns in sorghum downy mildew with Morisita's index of dispersion. *Phytopathology* **76**:446–450.

Vartanian VG, Endo RM. (1985) Overwintering hosts, compatability types and races of *Phytophthora infestans* on tomato in southern California. *Plant Dis* **69**:516–519.

Wallin JR. (1962) Summary of recent progress in predicting late blight epidemics in the United States and Canada. *Am Potato J* **39**:306–312.

Zwankhuizen MJ, Govers F, Zadoks JC. (2000) Inoculum sources and genotypic diversity of *Phytophthora infestans* in southern Flevoland, the Netherlands. *Potato Res* **106**:667–680.