Plant Pathology (2009) **58**, 87–99 Doi: 10.1111/j.1365-3059.2008.01951.x

Phytophthora infestans in a subtropical region: survival on tomato debris, temporal dynamics of airborne sporangia and alternative hosts

M. A. Lima, L. A. Maffia, R. W. Barreto and E. S. G. Mizubuti*

Departamento de Fitopatologia, Universidade Federal de Viçosa. 365700-000 Viçosa, MG, Brazil

Little is known about inoculum dynamics of late blight caused by *Phytophthora infestans* in tropical/subtropical areas, particularly in Brazil. The objectives of the present study were to assess (i) the survival of the pathogen on stems, leaflets and tomato fruits, either buried or not in soil; (ii) the pathogenicity of *P. infestans* to mostly solanaceous plant species commonly found in Brazil that could act as inoculum reservoir; and (iii) the temporal dynamics of airborne sporangia. *Phytophthora infestans* survived in tomato plant parts for less than 36 days under greenhouse and field conditions. In greenhouse tests, pathogen structures were detected earlier on crop debris kept in dry than in wet soil conditions. Isolates of two clonal lineages of *P. infestans*, US-1 from tomato, and BR-1 from potato, were inoculated on 43 plant species. In addition to potato and tomato, *Petunia* × *hybrida* and *Nicotiana benthamiana* were susceptible to the pathogen. Airborne inoculum was monitored with Rotorod and Burkard spore traps as well as with tomato and potato trap plants. Sporangia were sampled in most weeks throughout 2004 and in the first two weeks of 2005. Under tropical/subtropical conditions, airborne inoculum is abundant and is more important to late blight epidemics than inoculum from crop debris or alternative hosts.

Keywords: epidemiology, inoculum, late blight, potato, tomato

Introduction

Late blight, caused by the oomycete *Phytophthora infestans*, is a destructive disease of tomato (*Solanum lycopersicum* = *Lycopersicon esculentum*) and potato (*Solanum tuberosum*) crops in the tropics. Strategies for late blight management, particularly the use of chemical control, were derived based on inoculum dynamics for epidemic development under temperate conditions. However, dynamics of both primary and secondary inocula of *P. infestans* on tomato and potato remain largely unknown in tropical and subtropical regions. Oospores can act as primary inoculum for late blight epidemics where they are formed (Drenth *et al.*, 1995; Andersson *et al.*, 1998; Fernández-Pavía *et al.*, 2004). In Brazil, oospores have not yet been found associated with late blight epidemics in the field. Although isolates of both A1 and A2 mating types occur, there is no evidence of sexual reproduction in *P. infestans* populations (Reis *et al.*, 2003). Thus, survival of *P. infestans* is most likely to depend on sporangia and mycelium.

*E-mail: mizubuti@ufv.br

Published online 14 October 2008

Survival of hyphae and sporangia associated with crop debris or alternative hosts as well as dynamics of secondary inoculum throughout growing seasons in tropical/subtropical regions are likely to be different from those reported in temperate climate regions. In the tropics, winters are not severe, more than one crop season can be established during the year and alternative hosts are likely to be more abundant. Altogether, the amount of inoculum to start epidemics is expected to be larger in tropical/subtropical regions; however, to date, no proper quantification of this has been conducted.

Persistence and infectivity of asexual structures of *P. infestans* in the soil depend on meteorological variables (Andrivon, 1995), interactions with other organisms (Lacey, 1965) and soil chemical composition (Andrivon, 1994), but data gathered on survival of hyphae, sporangiophores and sporangia of *P. infestans* vary (Andrivon, 1995). Mycelium can survive up to 63 days in sterilized or 35 days in unsterilized wet soil (Zan, 1962). Viability of sporangia is affected by temperature (Melhus, 1915; Sato, 1994), solar radiation (Mizubuti *et al.*, 2000; Porter & Johnson, 2004), moisture (Minogue & Fry, 1981; Harrison & Lowe, 1989; Porter & Johnson, 2004) and soil antagonists (Andrivon, 1995). Half-life of detached sporangia is about 6 h when exposed to temperatures

ranging from 15 to 20°C and relative humidity (RH) from 40 to 88% (Minogue & Fry, 1981). Under natural conditions, sporangia survived 20 days (Porter & Johnson, 2004). However, sporangia are more likely to survive in wet, shaded soil (Porter & Johnson, 2004). In the Mexican Central Plains, *P. infestans* survived up to 30 days in soil with sporangia from a recent infected potato crop (Fernández-Pavía *et al*., 2004). Using a different methodology to quantify survival, the pathogen remained viable for a 32 day-period in unsterilized soil (Lacey, 1965). Pathogen structures associated with crop debris are expected to survive longer than directly in soil. Survival is longer in colonized potato tubers (Kirk, 2003). Survival of *P. infestans* associated with potato debris was estimated to be 15 days in Brazilian conditions (Nazareno *et al*., 2004). To date, there is no information on survival of *P. infestans* associated with tomato debris.

A number of non-cultivated and cultivated plant species, reported as potential hosts to *P. infestans* (Erwin & Ribeiro, 1996), may act as inoculum sources for late blight epidemics. Inoculum produced in potato plants can infect *Solanum dulcamara*, a perennial species commonly found in Northern Ireland (Cooke *et al*., 2002). In Peru, wild *Solanum* species growing close to potato fields were reported to be infected by *P. infestans* (Forbes & Landeo, 2006). Isolates of the A1 and A2 mating types were pathogenic to *S. nigrum* and *S. dulcamara*. These plant species were postulated to act as inoculum reservoir, to allow the development of lesions of both mating types and to provide conditions for sexual reproduction to occur (Flier *et al*., 2003). It is likely that inoculum produced in non-cultivated plants can move to potato or tomato crops. *Phytophthora infestans* can also infect non-solanaceous species, such as *Solanecio biafrae*, *Ageratum conyzoides*, *Aspilia africana* and *Dichrocephala integrifolia*, all belonging to the Asteraceae (Fontem *et al*., 2004). Erwin & Ribeiro (1996) listed 89 plant species as hosts of *P. infestans*, such as eggplant (*Solanum melongena*) and green pepper (*Capsicum annuum*), which are widely grown in Brazil. Susceptibility of these species to *P. infestans* as well as of wild or ornamental plants may be relevant to late blight management because small farmers commonly grow several solanaceous plants in a single area (Becktell *et al*., 2005). Once recognized as an inoculum source, alternative host species should be removed from the vicinity of commercial potato and tomato crops. However, there has been no study to assess the potential of such plants to act as inoculum reservoir in Brazil.

Late blight epidemics in several regions in Brazil are favoured by factors such as monoculture, possibility of year-round successive crop planting, conducive weather conditions and the lack of harsh winters. Usually, the same field is not planted with potato or tomato successively, but new planting areas are commonly established nearby to reduce costs associated with transporting irrigation pipes, stakes and other planting materials. Tomato debris is often removed from the field and potato debris is commonly incorporated into soil. Thus, it is expected that sporangia produced in infected fields are the main inoculum for epidemics. Sporangial dispersal is mainly wind-mediated (Aylor *et al*., 2001). Considering the effects of ultraviolet radiation on sporangia survival, on cloudy, cool and wet days, inoculum can be wind dispersed for long distances with a high chance of causing infection (Mizubuti *et al*., 2000). A peak in sporangia release from canopy occurs between 10·00 and 13·00 hours, when RH is low, which facilitates pathogen dispersal (Aylor *et al*., 2001).

Monitoring wind-borne inoculum contributes to better understanding of plant disease epidemics. Airborne sporangia of *P. infestans* have been quantified throughout growing seasons in India (Phukan & Baruah, 1993) and in North America and Europe (Waggoner, 1952; Hirst & Stedman, 1960). In the temperate regions, winters are severe and asexual structures of *P. infestans* survive poorly (Andersson *et al*., 1998; Kirk, 2003). However, in tropical/subtropical regions, the availability of inoculum to start late blight epidemics is likely to be higher.

Considering the lack of basic knowledge on late blight epidemics under Brazilian conditions, this work aimed to assess: (i) the survival of *P. infestans* associated with stems, leaflets and fruits of tomato, under greenhouse and field conditions; (ii) the pathogenicity of *P. infestans* to plant species commonly found in Brazil; and (iii) the temporal dynamics of pathogen sporangia.

Material and methods

Survival of *Phytophthora infestans* in tomato crop debris

Survival of asexual structures in tomato crop debris was assessed in experiments conducted twice in greenhouse and field plots located in the experimental area of the Departamento de Fitopatologia, Universidade Federal de Viçosa (20°44′44″S; 42°50′59″W, 661 m above sea level). In the greenhouse, unsterilized soil was used (red-yellow latosol; 70% clay and 30% sand, no organic matter) in 1·5 L pots. Field plots were set in a red-yellow clay latosol, low level of organic matter (pH = 6·2; K = 114 mg dm^{-3}; P = 24·1 mg dm^{-3} and Ca^{2+} = 2·74 cmol dm^{-3}).

Seedlings of tomato (cv. Santa Clara), susceptible to late blight, were raised under greenhouse conditions and 30-day-old transplants were transferred to the field plots in February and June 2004. In the field, tomato plants were cultivated according to growers' practices, including fungicide sprays up to 35 days after transplanting, when enough plant mass was achieved. From this point on, fungicides to control late blight were no longer applied and natural infection was allowed to occur. Other diseases and pests were controlled by applying specific pesticides.

Diseased plant materials were collected when late blight severity in the plants averaged 25%. Main stems of diseased plants were cut 5 cm above the soil surface and placed in plastic bags moistened inside. To stimulate pathogen sporulation, the bags were kept in a growth chamber, 18°C, 12 h photoperiod. After 48 h, the stems were cut in 8-cm length sections, each with 35 to 50%

disease severity. Diseased leaflets from the upper and middle thirds of the plants, with about 30% severity, were carefully cut from the plants to reduce sporangia detachment. Diseased green fruits with about 40 to 50% severity were also used. Each plant organ was set on a 12 × 13 cm nylon screen envelope (mesh = 1 mm^2). After setting the material on top of a screen section, another screen section (12 × 13 cm) was stapled to envelope the plant materials. An envelope with either three stem sections (15 to 30 g), 10 leaflets, abaxial side up and set to avoid overlapping, or one infected green fruit was considered one sampling unit. Four replicates of each sampling unit were used for each sampling time. The sampling units were set on the surface or buried 10-cm deep in soil, depending on the treatment. Sampling units buried in the field had a nylon string marked at the soil surface to facilitate removal of the envelope. In the greenhouse, pathogen survival in each plant material was studied under two soil conditions: dry or wet. In the dry condition, no water was added to the soil, whereas in the wet condition 240 mL of tap water was uniformly distributed in the pot, every 3 days, to keep soil moisture between field capacity and the saturation point that had been determined in the laboratory. In the greenhouse, plant materials were sampled after 0, 3, 8, 13, 16, 22, 27 and 36 days. In the field runs, survival was assessed at 0, 7, 15, 30, 45, 60, 75 and 90 days after beginning the experiment.

Pathogen survival was assessed following two procedures: by direct observation of sporangiophores with sporangia and by a bioassay (Drenth *et al*., 1995). In both procedures, the plant materials were washed with tap water to remove excess soil while materials were still on the bottom nylon screen. Washing water (about 900 mL/sample) was recovered in a plastic tray (38 × 26 × 7 cm) lined with a triple layer of tissue paper. For direct observation, after washing, each type of plant material was kept in a plastic box (20 × 20 × 20 cm), lined with a wet sponge to form a moist chamber, and incubated in a growth chamber at 18°C, 12 h photoperiod. After 5 days, each sample was examined under a stereoscope and checked for pathogen structures (sporangiophores with or without sporangia). In doubtful cases, microscopic slides were mounted and analyzed under a microscope (100×). The number of fruits, stems and leaflets with structures of *P. infestans* was determined. For the bioassay, 10 healthy tomato leaflets (cv. Santa Clara) were set to float abaxial side down on the washing water, and the plastic trays were covered with plastic bags and incubated at 18°C, 12 h photoperiod (Drenth *et al*., 1995). As a control treatment, healthy leaflets were set in 900 mL tap water in a clean tray. After 2 days, the water was removed, the leaflets were set abaxial side up, the trays were again covered with a plastic bag and taken back to the growth chamber. After 5 days, the leaflets were removed and examined under a stereoscope to check for pathogen structures or examined under a microscope (100×). The number of leaflets with pathogen structures was used to calculate the incidence of diseased leaflets.

In the greenhouse, soil temperature was registered daily at 17·00–18·00 hours. One thermometer was placed in a pot with wet soil and one in a pot with dry soil, bulbs placed 10 cm deep. Relative humidity was recorded with a hygrothermograph. In the field experiments, meteorological variables were registered with a heliograph, two sensors of soil temperature (Temperature Probe Model 108) set at 10 and 20 cm deep, a sensor of soil moisture (Soil Moisture Block Model 227) set at 10 cm deep, air temperature and relative humidity (Temperature and Relative Humidity Probe Model CS500) and rainfall (Tipping Bucket Rain Model TE – 525), coupled to a data logger (CR-10X Campbell Scientific®).

The greenhouse and field experiments were set up in a completely randomized design with four replications. The greenhouse experimental runs were conducted from May to June 2004 and the field runs from September 2004 to January 2005. In the field, soil condition was not considered. Non linear models (exponential decay) were fitted to the data using the SAS System v 8·0. When required, curves were compared by estimating the 95% confidence interval (CI95%) of the difference of the regression parameter estimates, using the approach proposed by Campbell & Madden (1990).

Alternative hosts of *Phytophthora infestans*

Four isolates of *P. infestans* were used, two of US-1 clonal lineage from tomato (one from Minas Gerais (MG) and one from Goiás (GO) states), and two of BR-1 clonal lineage from potato (one from Paraná (PR) and one from Rio Grande do Sul (RS) states).

The tomato isolates were maintained either in leaflets (MG isolate) or in green fruits (GO isolate). The MG isolate was maintained on detached leaflets of cv. Santa Clara tomato plants, 5–6 weeks old, grown in the greenhouse. The leaflets were kept in plastic trays as before, previously disinfested with 70% ethanol, with the abaxial side up, and their petioles were enclosed with wet cotton plugs. Distilled water was sprayed on the leaflets and 40 mL of a suspension with $6{\cdot}5 \times 10^6$ sporangia mL^{-1} from lesions formed in inoculated tomato leaflets was sprayed with a pressurized spray pack (Home Hardware Store Limited). The trays were covered with wet plastic bags and kept at 18°C, 12 h photoperiod. The GO isolate was inoculated in 36 green tomato fruits (12 per tray) that had been previously disinfested with 70% ethanol. In each fruit, a cross-shaped cut (2 × 2 cm length) was made with a sterilized scalpel and the pathogen was inoculated at the intersection of the cuts.

Potato tubers (cv. Bintje) were washed in tap water, left to dry under laboratory conditions, submerged in ethanol, flamed and cut in 0·5–0·8 cm thick slices that were inoculated with potato isolates (RS and PR). For each isolate, 35 plastic boxes previously disinfested with 70% ethanol were prepared, each with four slices. To keep moisture high, a wet cotton plug was set in each box.

Inocula for MG and GO tomato isolates were raised after inoculation of both isolates in tomato fruits. Inocula for RS and PR potato isolates were raised in potato slices.

Table 1 Reaction of plants from 43 species to *Phytophthora infestans* isolates from A1 and A2 compatibility groups evaluated in two field runs

Family	Species	Reaction[a]	Family	Species	Reaction
Solanaceae	*Brugmansia suaveolens*	–	Acanthaceae	*Pachystachys lutea*	–
	Capsicum annuum	–	Asteraceae	*Ageratum conyzoides*[b]	–
	C. frutescens	–		*Emilia sonchifolia*	–
	Cestrum sp.	–		*Erigeron bonariensis*	–
	Datura stramonium	–		*Galinsoga parviflora*	–
	Lycopersicon esculentum	+		*Sonchus oleraceus*	–
	Nicandra physaloides	–	Bignoniaceae	*Pyrostegia venusta*	–
	Nicotiana benthamiana[b]	+	Caprifoliaceae	*Sambucus nigra*	–
	N. rustica	–	Convolvulaceae	*Ipomoea purpurea*	–
	N. tabacum cv. TNN	–		*Cuscuta racemosa*[b]	–
	Petunia × hybrida	+	Geraniaceae	*Pelargonium hortarum*	–
	Physalis sp.	–	Hydrophyllaceae	*Phacelia tanacetifolia*	–
	Solanum americanum	–	Nyctaginaceae	*Mirabilis jalapa*	–
	S. capsicoides	–	Oleaceae	*Jasminum officinale*	–
	S. cernuum	–	Plantaginaceae	*Plantago major*	–
	S. gilo	–	Polemoniaceae	*Phlox drummondii*[b]	–
	S. lycocarpum	–	Polygonaceae	*Polygonum hydropiper*	–
	S. mauritianum	–		*Rumex acetosa*	–
	S. melongena	–	Rubiaceae	*Coffea arabica*	–
	S. paniculatum	–	Verbenaceae	*Lantana camara*	–
	S. viarum	–			
	S. tuberosum	+			
	Vassobia breviflora	–			

[a]+ or – = Late blight symptoms/pathogen structures present or absent, respectively.
[b]Species included in just one run.

Mycelium of potato isolates was transferred to both ends of each potato slice. The trays and boxes were covered with plastic bags and transferred to a growth chamber, 18°C, 12 h photoperiod. After 6 days, the plant material was transferred to 250 mL distilled water in a beaker, which was gently agitated. Sporangial suspension was filtered and final concentration adjusted to $3{\cdot}0 \times 10^3$ sporangia mL^{-1}. Suspensions of the four isolates were mixed in equal proportions to prepare 4·5 L of the final sporangial suspension. This suspension was placed in a refrigerator for 60 min to induce zoospore formation, and 0·45 mL of Tween 20 (0·01%) was added.

Choice of plant species to be tested as hosts followed Wapshere (1974), who evaluated specificity and risk analysis of potential weed biocontrol agents. In total, 43 species were tested, 23 of the Solanaceae family, and the rest from species decreasing in affinity in relation to tomato and potato, and species reported by Erwin & Ribeiro (1996) as hosts of *P. infestans* (Table 1). The plants were grown in plastic pots under greenhouse conditions and all were established from seeds, except plants of *Cuscuta racemosa*, which were grown as parasites of stems of *Hybiscus* sp. kept in pots with water. Plants varied in age because the time to develop a suitable leaf canopy varied between species. One week before inoculation, all plants were confirmed to be healthy and were transferred to field conditions. The field was located in the university campus area and no commercial solanaceous crops were grown within about a 10 km-radius. In the field, the plants were sprayed with water for about 30 min and inoculated by spraying a sporangial suspension to the runoff point. The experiment was conducted in 2004 and repeated in 2005. In 2004, the plants were inoculated at 21·00 hours on 24 November, and evaluated until 15 December. In 2005, the plants were inoculated at 22·00 on 2 January and evaluated until 23 January. At 12 h post inoculation, as well as three times in the next days, the plants were sprinkler-irrigated for 30 min. Plants of tomato cv. Santa Clara and potato cv. Bintje were used as controls.

The plants were examined daily with a magnifying lens (20×). Plants presumed infected by *P. infestans* were examined under a stereoscope and checked for structures of the pathogen. *Phytophthora infestans* was considered established only when sporangia were observed on the diseased tissue. When the diagnosis was doubtful, microscopic slides were prepared and observed under the microscope (100×).

Meteorological variables were registered with a hygrothermograph located 10 m from the experiment and by sensors of temperature and relative humidity (Probe Model CS500), rainfall (Tipping Bucket Rain Model TE – 525) and wind speed (Model-05403 wind monitor) connected to a data logger (CR-10X Campbell Scientific®) located 1 km from the experimental area.

Each experiment was in a completely randomized design, with four replications. A pot with one plant was considered one experimental unit.

Temporal dynamics of airborne sporangia of *Phytophthora infestans*

Airborne sporangia of *P. infestans* were monitored in the experimental field plots with three traps: Rotorod model 20 (Multidata LLC), Burkard volumetric spore trap (Burkard Scientific) and trap plants. To the east, south and west, the experimental area was surrounded by high hills, and a road passed by to the north. The east and west hills were planted with coffee and the south had eucalyptus trees. The nearest commercial tomato field was located approximately 10 km away in a straight line to the south. The prevalent wind direction was from north to south. There were no commercial potato fields in the area.

The Rotorod spore trap was set 4 m high, rotating at 2400 rpm, sampling about 3·12 m^3 air min^{-1}. Acrylic rods (32 × 1·52 mm) covered with silicon grease were set in the trap, between 9·00 and 14·00 hours, when there is a peak in the concentration of airborne *P. infestans* sporangia (Bashi *et al*., 1982). Daily, from 1 March, 2004 to 15 January, 2005, the rods were taken to the laboratory and observed under the microscope (100×). The Burkard spore trap was set at 3 m high. The tape covered with silicone grease was replaced every 7 days. Sampling period ran from 15 January, 2004 to 15 January, 2005. For both traps, concentration of sporangia/m^3 air was estimated following the manufacturer's recommendations.

Groups of 10 tomato plants (cv. Santa Clara), 45-days old, grown under greenhouse conditions, were transferred to the experimental area. Because of the short latent period of late blight, plants were replaced twice a week (after 4 and 3 days) to avoid formation of new lesions from inoculum produced in lesions that could have been established on the first day they were transferred to the field. The same procedure was followed with groups of 10 potato plants (cv. Bintje), 50 days old. The plants also remained in the field for 3 and 4 days, but because of shortage of plants, the procedure was repeated at 15-day intervals. After October 2004, the potato cultivar was changed to Monalisa, also susceptible to late blight. Potato and tomato plants were arranged alternated in a single row, 0·5 m between plants, and set perpendicularly to the prevailing wind direction. Two tomato plants or two potato plants were considered one experimental unit. The plants were sampled from 26 February, 2004 to 13 January, 2005.

Two upper, two middle and two lower tomato leaves, located respectively at 10, 20 and 35 cm from the plant apex, were detached. All leaves of a potato stem randomly chosen were detached. Leaves from healthy potato and tomato plants grown in the greenhouse were used as controls. The leaves were enclosed in a plastic bag that had been wetted with distilled water and kept in a growth chamber at 18°C, 12 h photoperiod (Drenth *et al*., 1995). After 5 days, all leaflets were examined under a stereoscope (20×) or microscope (100×). The incidence of leaflets (diseased leaflets/total leaflets in a sample) with pathogen structures was determined.

Values of temperature, relative humidity, rainfall and wind speed were registered in a data logger as described above. Values of meteorological variables were lagged for up to 7 days from the sampling day and were each correlated to the number of sporangia trapped (Spearman correlation coefficient), using the SAS v 8·0 program.

Results

Survival of *Phytophthora infestans* in tomato crop debris

Data from both runs in the greenhouse were pooled. The pathogen survived for at least 36 days in tomato debris under greenhouse conditions (Fig. 1). Sporangiophores with or without sporangia were directly observed up to the 8th day in stems left on the soil surface and buried in dry soil (Fig. 1a). Under wet soil conditions, sporangia were detected in plant materials only until 3 days (Fig. 1b). Pathogen structures were more abundant in plant parts kept under dry than under wet conditions (data not shown). With the bioassay, the pathogen survived for up to 36 days in stems kept at the dry soil surface and stems and fruits buried under dry conditions (Fig. 1c). The pathogen survived for up to 36 days in leaflets either left on soil surface or buried in wet soil and also in fruits at the wet soil surface (Fig. 1d). In both greenhouse runs, the relative humidity ranged between 75 and 95%. The temperature of dry soil was between 19 and 29°C and of wet soil between 17 and 26°C. Average daily temperature ranged between 15 and 20·8°C.

There was no difference between the rate of survival for materials kept at the soil surface or buried. Comparisons of survival at surface versus buried were made for dry and wet conditions using direct observation or the bioassay. In all comparisons, the CI95% included zero.

Data from both runs at field conditions were also pooled. Under field conditions, *P. infestans* survived for up to 30 days (Fig. 2). No pathogen structures were found by direct observation under the stereoscope after 7 days (Fig. 2a). At 7 days, sporangia were seen on leaflets and stems kept on soil surface and on buried stems and fruits (Fig. 2a). With the bioassay, pathogen structures were seen on leaflets on soil surface for up to 30 days (Fig. 2b). In both field runs, average relative humidity was 80%. Average temperatures were 21 and 22°C during the first and second runs, respectively (Fig. 3a). Precipitation events were more frequent from the end of November to mid-January (Fig. 3a).

As in the greenhouse experiments, there were no differences regarding the rate of survival in plant materials kept at the soil surface or buried.

Alternative hosts of *Phytophthora infestans*

Most solanaceous plants, including those commercially grown, weeds or native to Brazil, were not susceptible to *P. infestans*. Late blight symptoms and sporangiophores/sporangia of *P. infestans* were seen on plants of tomato and potato, as well as on *Petunia* × *hybrida* and *Nicotiana benthamiana*, all solanaceous species (Table 1). On

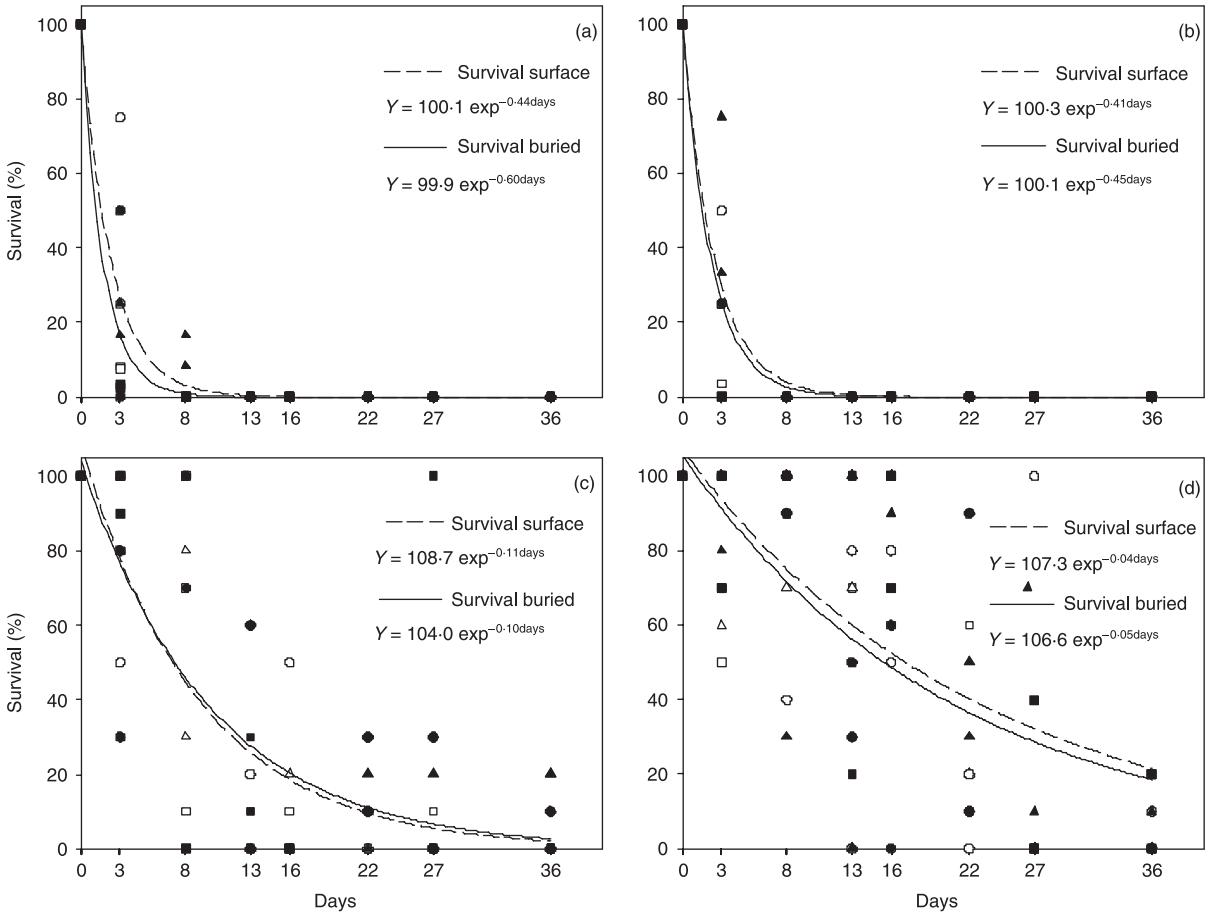

Figure 1 Survival of *Phytophthora infestans* in tomato leaflets (squares), stems (triangles) or fruits (circles) kept on soil surface (open) or buried 10 cm deep (filled) in the soil, under greenhouse conditions. Data from direct observation of structures (a and b) or from the bioassay (c and d), in dry (a and c) or wet (b and d) soil. On all assessment dates, no pathogen structures were seen on control plots of the bioassay. Data are pooled from two experimental runs. The exponential decay model ($Y = a \cdot \exp^{b \cdot X}$, where a and b are parameters that refer to the initial viability and the survival rate, respectively) was fit to the data. Each point is the average of the four replications of each run.
In Figs 1c and 1d some symbols at 36 days are obscured by others.

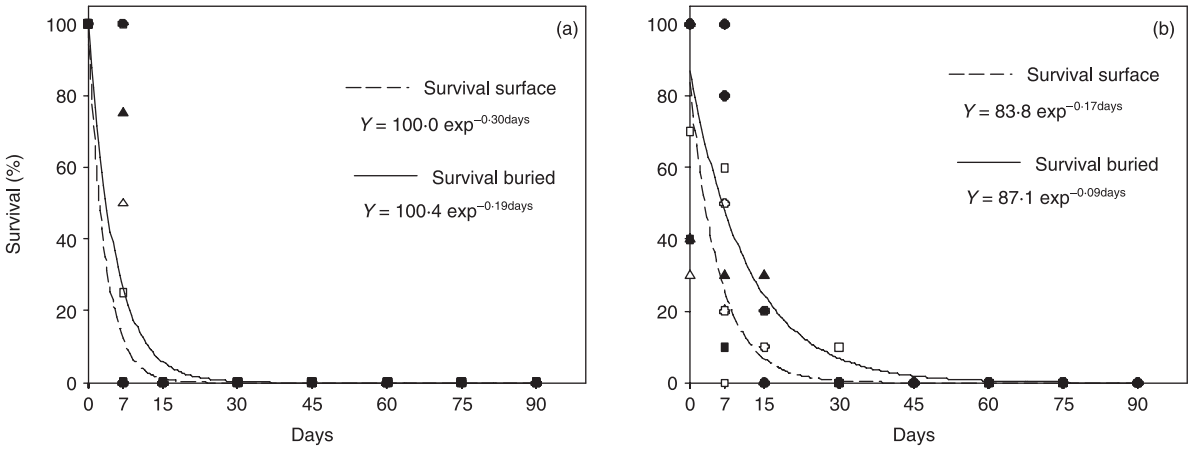

Figure 2 Survival of *Phytophthora infestans* associated with tomato leaflets (squares), stems (triangles) or fruits (circles) kept on the surface (open) or buried (filled), in two experiments conducted under field conditions. Data are from direct observation of pathogen structures (a) or by the bioassay (b). On all assessment dates, no pathogen structures were seen on control plots of the bioassay. The exponential decay model ($Y = a \cdot \exp^{b \cdot X}$, where a and b are parameters that refer to the initial viability and the survival rate, respectively) was fit to the data. Data are pooled from two experimental runs. Each point is the average of the four replications of each run.

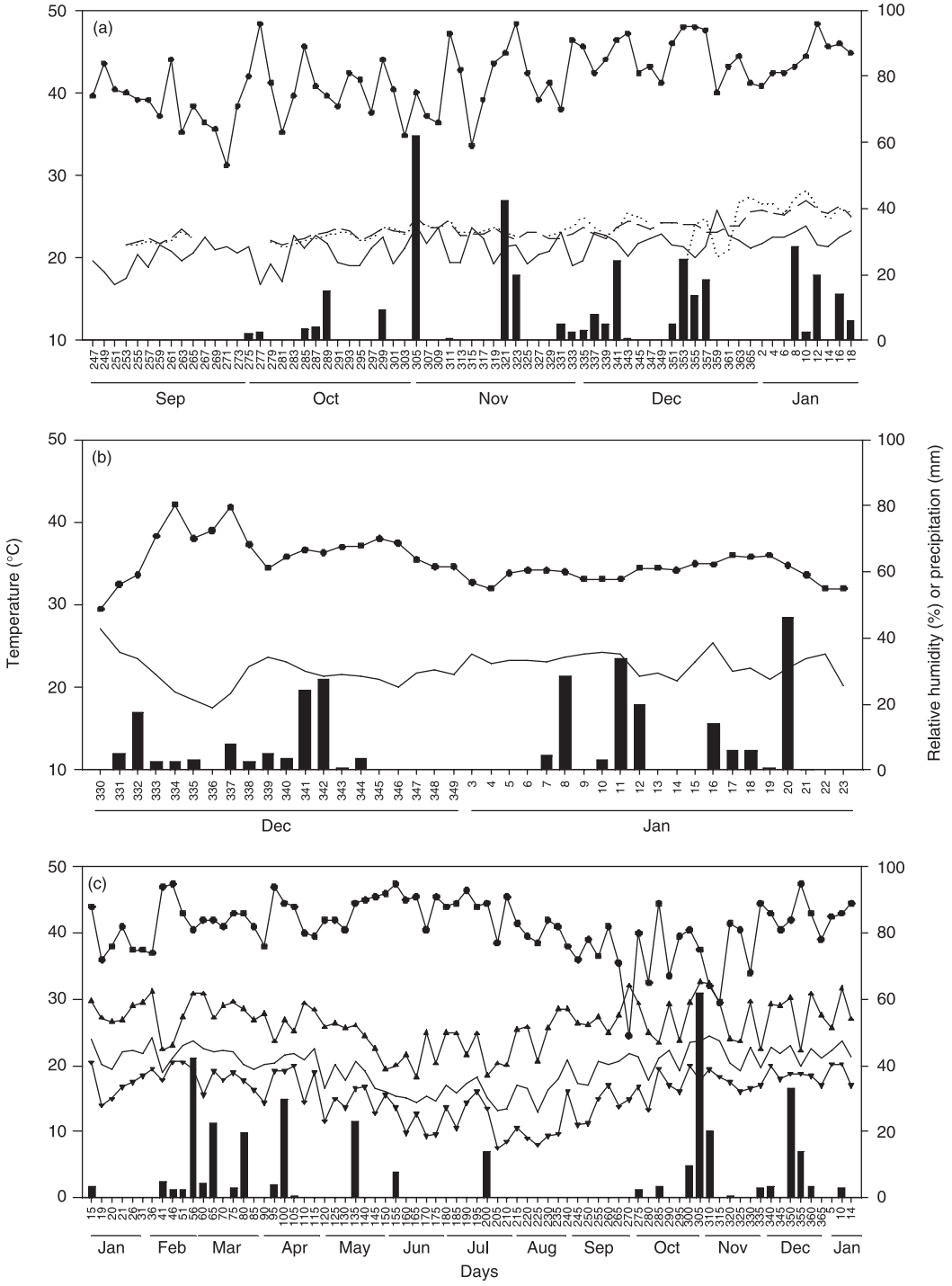

Figure 3 Average values of meteorological variables during the field experiments. Average temperature is represented by a solid line, relative humidity by a solid line with circles and precipitation by vertical bars. a) Survival experiments conducted from September 2004 to January 2005. The soil temperature at 10 cm-deep is represented by a dashed line and at 20 cm-deep by a dotted line. b) Alternative host experiments conducted from December 2004 to January 2005. c) Quantification of airborne inoculum experiments. Maximum temperature is represented by –▲– and minimum temperature by –▼–.

potato leaflets, pathogen structures were noticed 4 days after inoculation in all replicates of both runs. On tomato leaflets, pathogen structures were noticed 6 days after inoculation in all replicates of the first and in two replicates of the second run. Although leaf symptoms were not as evident in *P. hybrida* as in potato and tomato, they appeared 5 and 6 days after inoculation in the first (all replicates) and second (two replicates) runs, respectively.

Nicotiana benthamiana was included only in the first run and disease symptoms and signs were noticed 6 days after inoculation in three replicates. Disease symptoms in both *P. hybrida* and *N. benthamiana* were irregular necrotic, light brown lesions.

In the first run, one week after inoculation, the temperature dropped to 18°C and relative humidity increased up to 80%, conditions favourable to the pathogen. In the second run, temperature remained around 24°C and relative humidity was below 60% (Fig. 3b).

Temporal dynamics of airborne inoculum of *Phytophthora infestans*

Sporangia of *P. infestans* were trapped throughout all the sampling periods, mostly between March and July. The sporangia were sampled in 41 out of 53 weeks and in 42 out of 46 weeks with the Burkard and Rotorod traps, respectively (Fig. 4).

According to the Burkard sampler data, two peaks in the number of trapped sporangia were detected: at 09·00 and 17·00 hours, with an average concentration of 0·19 and 0·11 sporangia/m^3, respectively (Fig. 5). Most sporangia (67%) were trapped in the period between 06·00 and 18·00 hours, concentrating in the morning period (from 06·00 to 12·00 hours) (Fig. 5).

Sporangial dispersal occurred throughout the year with seasonal fluctuation in frequency. The numbers of sporangia trapped by Rotorod and of diseased tomato and potato leaflets had similar trends, with a main peak between March and May. A peak in the bait plants was detected in tomato in May and June and on potato in June (Fig. 4). The highest peak in the Burkard sampler occurred in July. No correlation was found between the number of sporangia trapped by Burkard and incidence on trap plants. There was a positive correlation between the number of sporangia trapped by Burkard and Rotorod ($r = 0{\cdot}16$; $P = 0{\cdot}008$), between the number of diseased tomato and potato leaflets ($r = 0{\cdot}88$; $P < 0{\cdot}0001$) and between the number of sporangia trapped by Rotorod and the number of diseased tomato ($r = 0{\cdot}26$; $P = 0{\cdot}015$) and potato ($r = 0{\cdot}53$; $P = 0{\cdot}020$) leaflets.

To study the correlations between meteorological variables (Fig. 3c) and number of sporangia trapped, the meteorological variables were lagged up to 7 days before sampling the sporangia on all traps. Most correlation values between all meteorological variables and number of sporangia trapped both in the Burkard and Rotorod traps were low and non significant. The correlation coefficient (r) between mechanical traps and meteorological variables ranged from –0·18 to 0·20, showing evidence of no strong relationships. The correlation values between the meteorological variables (except wind speed) and the number of diseased leaflets of tomato and potato were higher. In general, the number of lesions in trap plants was negatively correlated with the minimum and average temperatures. The correlation coefficients between relative humidity and the number of diseased leaflets of tomato and potato plants were positive and most were significant (Table 2). There was no correlation between lesions on potato plants and relative humidity at 3 and 6 days prior to the assessment date (Table 2).

Discussion

Considering the dynamics of inoculum, airborne sporangia seem to be abundant and probably play a major role for late blight epidemics. Survival of the pathogen in tomato debris was short, therefore this source of inoculum is expected to be of lower importance. Even though both potato and tomato crops are always grown in the same regions, they are not continuously grown in the same field at the farm level. However, they are both cultivated throughout the year in several production regions, so infected plants are likely to provide enough sporangia to start or develop late blight epidemics.

The survival time in tomato debris estimated in the present study is in accordance with data previously reported for studies of survival of sporangia in soil (Lacey, 1965; Fernández-Pavía *et al*., 2004; Porter & Johnson, 2004). However, the survival time in potato debris was estimated to be 15 days (Nazareno *et al*., 2004). This apparent discrepancy between survival associated with potato and tomato debris under Brazilian conditions could have been caused by factors such as the amount of tissue diseased, pathogen genotypes and soil conditions (physical, chemical and biological properties) among others. However, the methodology used to quantify survival was probably the most influential cause of the discrepancy. In the potato experiment, survival was quantified by direct observation, which underestimated survival capacity when compared to the bioassay. The short time of survival of *P. infestans* in tomato debris could have been caused by the average soil temperature and the cycles of humid/dry soil conditions due to precipitation throughout the experimental runs. Survival of asexual structures of *P. infestans* is favoured by cool temperature and constant dry conditions (Harrison, 1992). Another important issue to be considered is the decomposition of crop debris mediated by the interaction of weather and soil microbes, which is expected to occur at a more constant rate in the tropical areas due to the less variable temperature regime and intense microbial activity during the year.

Brazilian isolates of *P. infestans* infected plants of tomato, potato, *P. hybrida* and *N. benthamiana*. Late blight symptoms in *P. hybrida* have already been observed under field (Hirst & Moore, 1957; Deahl *et al*., 2006) and greenhouse (Vartanian & Endo, 1985; Becktell *et al*., 2005) conditions, but have not yet been reported for Brazilian conditions and isolates. *Nicotiana benthamiana* is not commonly reported as a host of *P. infestans* because special conditions seem to be required for infection to develop. Under controlled conditions, symptoms occurred on detached leaflets of *N. benthamiana*, after silencing the *P. infestans inf1* elicitin gene (Kamoun *et al*., 1998). *Phytophthora infestans* isolates with the INF1 protein infected *N. benthamiana* plants growing under both field and greenhouse conditions (Becktell *et al*.,

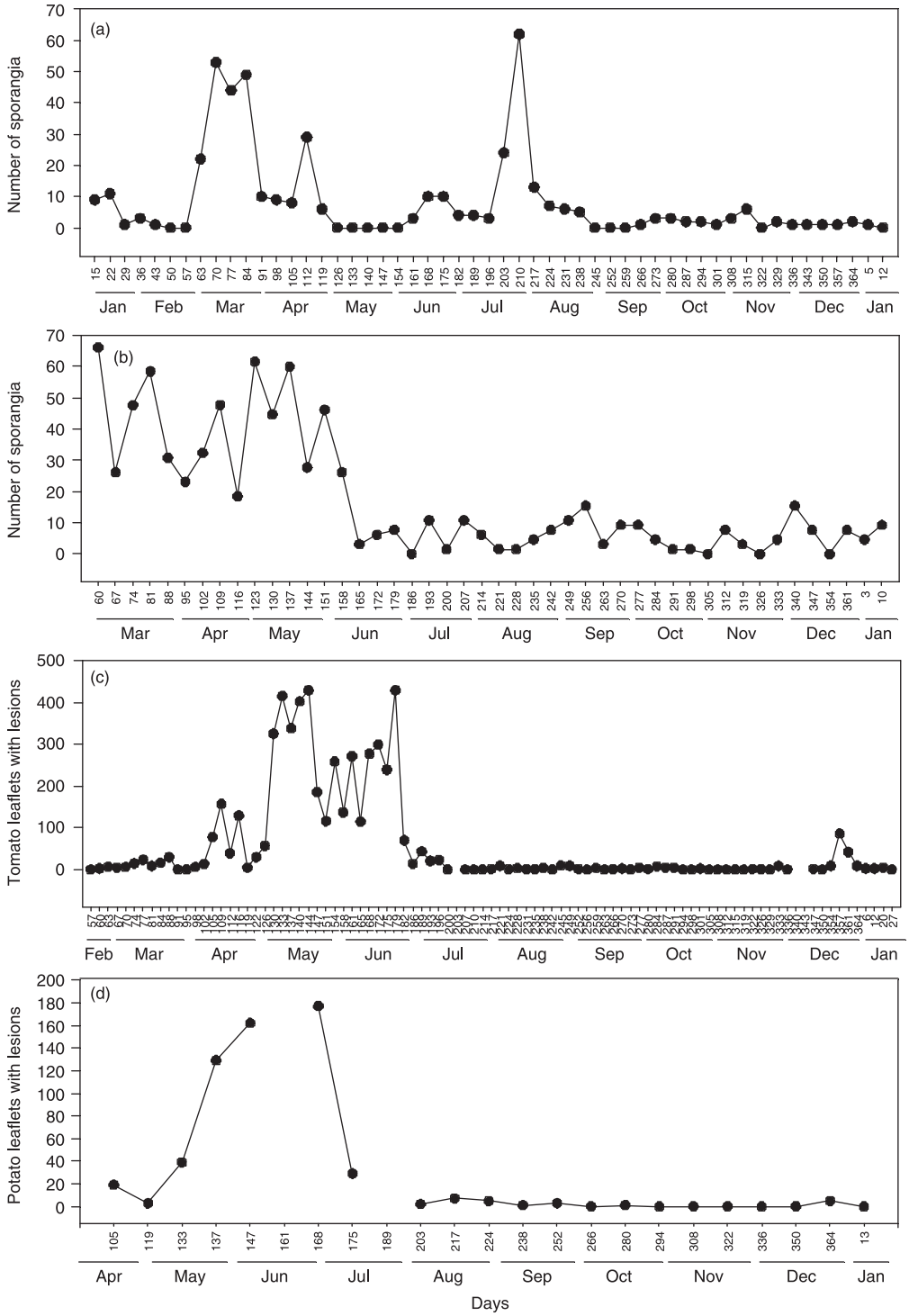

Figure 4 Number of *Phytophthora infestans* sporangia sampled by Burkard (a) and Rotorod traps (b) and cumulative number of diseased leaflets of tomato (c) and potato plants (d) used as biological traps. Missing points in panel D were because trap plants died from insect attack.

2006). Therefore, two factors seem to affect susceptibility of *N. benthamiana* to *P. infestans*: pathogen genotype and leaf physiological stage (Colon *et al*., 1993; Becktell *et al*., 2006). The inoculum used here was a mixture of isolates of A1 and A2 groups, from distinct geographical Brazilian regions. It is likely that the high aggressiveness of one or more isolates used may have facilitated infection of *N. benthamiana*. Plant physiological stresses may also contribute to sporulation of *P. infestans* (Hirst & Stedman, 1960; Bhattacharyya & Sharma, 1976; Colon *et al*.,

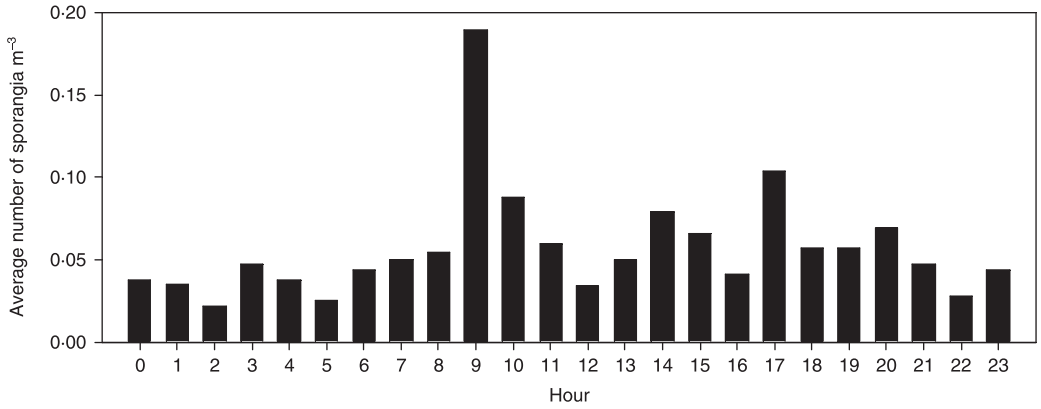

Figure 5 Average number of *Phytophthora infestans* sporangia per cubic metre of air sampled by the Burkard trap during a day.

Table 2 Correlation coefficients (level of significance) between meteorological variables and number of diseased leaflets in the plant traps

Meteorological variable	Lag period[a] (days)	Plant trap[b]	
		Tomato ($n = 90$)	Potato ($n = 20$)
Average temperature (°C)	0	–0·28 (0·009)	–0·60 (0·005)
	1	–0·21 (0·043)	–0·43 (0·061)
	2	–0·25 (0·018)	–0·50 (0·024)
	3	–0·21 (0·048)	–0·45 (0·048)
	4	–0·25 (0·019)	–0·64 (0·003)
	5	–0·24 (0·021)	–0·60 (0·006)
	6	–0·22 (0·033)	–0·46 (0·039)
	7	–0·23 (0·031)	–0·44 (0·051)
Maximum temperature (°C)	0	–0·31 (0·003)	–0·55 (0·012)
	1	–0·21 (0·049)	–0·37 (0·110)
	2	–0·31 (0·003)	–0·36 (0·117)
	3	–0·23 (0·027)	–0·37 (0·112)
	4	–0·30 (0·004)	–0·54 (0·014)
	5	–0·33 (0·002)	–0·58 (0·007)
	6	–0·28 (0·007)	–0·41 (0·074)
	7	–0·28 (0·009)	–0·54 (0·013)
Relative humidity (%)	0	0·47 (< 0·0001)	0·50 (0·026)
	1	0·53 (< 0·0001)	0·62 (0·004)
	2	0·51 (< 0·0001)	0·48 (0·032)
	3	0·47 (< 0·0001)	0·36 (0·115)
	4	0·50 (< 0·0001)	0·78 (< 0·0001)
	5	0·54 (< 0·0001)	0·62 (0·003)
	6	0·46 (< 0·0001)	0·39 (0·086)
	7	0·50 (< 0·0001)	0·50 (0·024)

[a]Lag = days previous to the sampling day.
[b]n = number of observations.

1993). Hirst & Stedman (1960) found pathogen structures only on yellowing and senescing parts of *S. dulcamara* and *S. nigrum* plants. Although no clear symptoms of stress were detected at the time of inoculation of *N. benthamiana* plants, it is likely that the plants were stressed when transferred from the greenhouse to the field. This hypothesis must be checked by inoculating plants under different physiological conditions and at varying phenological stages.

Several plant species are reported as hosts of *P. infestans*, but many reports are either based on studies conducted using detached leaves or on observations of symptoms in the field, without microscopic analysis of pathogen structures. Late blight-like symptoms were described in *Bupleurum maddeni*, *Erigeron multicaulis*, *Geranium nepalense*, *Ipomoea purpurea*, *I. hederacea*, *Sonchus oleraceus*, *Aster thomsoni*, *Galinsoga parviflora* and *Rumex acetosa*, but pathogen structures were not found on the plants (Bhattacharyya & Sharma, 1976). Experiments with detached leaves may generate confusing diagnoses regarding susceptibility of the target plant. Detached leaflets undergo physiological alterations that reduce the chance of defence responses to invading organisms and the host range can be overestimated.

The host range of *P. infestans* should be determined under field conditions. In an experiment conducted under controlled conditions that included plants of 39 species of Solanaceae and nine of other families, plants of 11 solanaceous species (*Datura meteloides*, *D. stramonium*, *Lycium chinense*, *Nicandra physaloides*, *Nicotiana acuminata*, *N. clevelandii*, *P. hybrida*, *Schizanthus pinnatus*, *Solanum aviculare*, *S. sarrachoides* and *S. sisymbriifolium*) were susceptible to the pathogen. However, when the experiment was conducted under field conditions, only tomato, potato and *S. sarrachoides* plants were infected (Vartanian & Endo, 1985). Analyses under field conditions are preferred because the interactions of biotic and abiotic factors are considered (Flier *et al.*, 2003). It is also known that isolates of both clonal lineages of *P. infestans* have host specificity in Brazil: US-1 is associated with tomato and BR-1 with potato (Suassuna *et al.*, 2004). Thus, inoculum of different origins may vary in aggressiveness in alternative host plants.

Airborne inoculum of *P. infestans* was available almost all year round. Most sporangia were trapped in the autumn-winter period due to more favourable environmental conditions to late blight epidemics. The high numbers of sporangia trapped between March and July 2004 may be due to a highly blighted tomato crop near the experimental area (about 50 m). At this time, late blight occurred in commercial crops located in fields

surrounding the experimental area, thus favouring both production and dispersal of sporangia. Tomato was continuously grown throughout the year, and potato was planted in November 2004, when the sporangia were sampled. Neither crop was artificially inoculated. The plantings, located in a 40 to 100 m-radius from the traps, probably acted as inoculum source. Sporangia of *P. infestans* may be dispersed up to 20 km away in less than 3 h when wind speed is 1 to 2 m s^{-1} and the interaction of wind speed and sporangia concentration may explain the increase in the amount of airborne inoculum (Aylor *et al.*, 2001).

The yearly pattern of sporangia dispersal has important implications for late blight management regarding the use of decision support systems (DSS). In temperate zones, most DSS assume that inoculum is present in the area and control recommendations are driven by the accumulation of favourable weather conditions. One difficulty in adopting the DSS is to determine where, when and how important the sources of inoculum are to late blight epidemics. In tropical areas, this does not seem to be an issue, since inoculum is present throughout the year. Thus, recommendations based on units of cumulative favourable weather conditions seem reasonable. However, the threshold that triggers events of interest can be adjusted according to the higher risk periods conditioned by higher amounts of airborne sporangia. This hypothesis needs to be tested under field conditions.

There were quantitative and qualitative differences between trapping efficiency of Burkard and Rotorod traps. Throughout the year, considering counts of at least 40 sporangia trapped, eight and three peaks were detected with the Rotorod and Burkard, respectively. This difference is probably due to each trap's mechanism and time of trapping. Sporangia are trapped by impact in the Rotorod and by suction in the Burkard. The higher number of sporangia sampled in the Burkard is due to its continuous operating mode, whereas the Rotorod sampled 5 h a day. The suction capacity of the Burkard trap used in this study was set to 65% of the maximum due to motor limitations. Nevertheless, even sucking 0·39 m^3 h^{-1} of air, sporangia were efficiently trapped throughout the period.

The daily pattern of sporangia was similar to that observed in other studies. During a 24 h period, two peaks of trapped sporangia were recorded in the Burkard: at 09·00 hours, with the largest concentration, and at 17·00 hours. Phukan & Baruah (1993) also observed two peaks: at 06·00 and 17·00 hours. A long duration of leaf wetness, mostly due to dew formation, can hold sporangia formed at night (Bashi *et al.*, 1982) and may be associated with the highest inoculum concentration in the first hours after sunrise (Rotem *et al.*, 1970; Phukan & Baruah, 1993). The sporangia concentration is reduced when the temperature increases and moisture decreases, conditions that are unfavourable to sporangia production. This condition is reversed at sunset when the amount of airborne sporangia gradually increases (Phukan & Baruah, 1993).

Given the high sensitivity of sporangia to UV radiation, airborne sporangia trapped at night are more relevant to epidemics (Mizubuti *et al.*, 2000). The high amounts of sporangia in the morning are likely to be inactivated by UV radiation during sunny days. However, the second peak detected in late afternoon and those present during the night may contribute more effectively to epidemic development. Besides not being damaged by UV radiation, these sporangia are likely to encounter cooler temperatures and higher relative humidity at night, conditions that favour germination and the establishment of infection (Mizubuti & Fry, 1998). The way the trap plants were used in this study did not allow this hypothesis to be tested. Comparative studies between plants exposed during the day versus night would allow better inferences.

Tomato and potato plants were efficient as traps of sporangia of *P. infestans*. From July 2004 to January 2005, the low frequency of diseased leaflets on these biological traps was correlated to the low sporangia concentration sampled in the mechanical traps. The use of trap plants may allow conclusions to be drawn about inoculum viability and even availability, considering the correlation with the Burkard and Rotorod traps. Plants as traps do not provide reliable quantitative information about sporangia, but may be useful in decision support systems related to disease forecast due to the low cost and the ease of adoption by growers.

Inoculum is present during all growth stages of both tomato and potato crops. Inoculum may be formed either externally or internally to the crop. Successive plantings of both solanaceous crops in an area may be affected earlier, considering the availability of airborne sporangia, as well as sporangia produced in crop debris, although debris is less important as a primary inoculum source. In small areas, tomato may be planted in the same or contiguous areas. Although not quantified, inoculum produced in the debris probably does not contribute efficiently to the start of late blight epidemics. Considering the high amount of airborne sporangia formed in successive plantings of tomato and potato and the presence of potential host plants in a region, the availability of inoculum is expected to be continuous, constantly threatening the new tomato and potato plantings. Two strategies are proposed to reduce the efficiency of these plantings as inoculum sources: promoting a coordinated crop rotation in the producing regions and starting to spray protective fungicides right after transplanting tomato seedlings or after emergence of potato plantlets.

In conclusion, *P. infestans* survives for a short time in tomato debris, and this inoculum source probably does not contribute effectively to late blight epidemics under Brazilian conditions. Apart from tomato and potato, two other solanaceous plants were found to be hosts of *P. infestans* in the field: *P. hybrida* and *N. benthamiana*. Although both species may contribute as inoculum sources they are not commonly found in potato and tomato growing areas. The most important inocula for late blight epidemics are probably airborne sporangia of *P. infestans*, which are available throughout the year. The availability is higher in some months, because either weather favours disease development or there are more

tomato plantings. Even in months unfavourable to disease, sporangia were trapped by the mechanical and trap plants, although at a low frequency. Understanding inoculum dynamics can help establish times of the year that are more favourable to late blight and enable planters to make more rational decisions regarding disease control.

Acknowledgements

We are grateful to FAPEMIG and CNPq for providing financial support for this research. L. A. Maffia, R. W. Barreto and E. S. G. Mizubuti acknowledge the CNPq research fellowships. We also thank André R. Duarte and Ducélio H. Lana for the many hours spent at the microscope counting sporangia.

References

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

Andrivon D, 1994. Fate of *Phytophthora infestans* in a suppressive soil in relation to pH. *Soil Biology & Biochemistry* **26**, 953–6.

Andrivon D, 1995. Biology, ecology, and epidemiology of the potato late blight pathogen *Phytophthora infestans* in soil. *Phytopathology* **85**, 1053–6.

Aylor DE, Fry WE, Mayton H, Andrade-Piedra JL, 2001. Quantifying the rate of release and escape of *Phytophthora infestans* sporangia from a potato canopy. *Phytopathology* **91**, 1189–96.

Bashi E, Ben-Joseph Y, Rotem J, 1982. Inoculum potential of *Phytophthora infestans* and the development of potato late blight. *Phytopathology* **72**, 1043–7.

Becktell MC, Daughtrey ML, Fry WE, 2005. Epidemiology and management of petunia and tomato late blight in the greenhouse. *Plant Disease* **89**, 1000–8.

Becktell MC, Smart CD, Haney CH, Fry WE, 2006. Host-pathogen interactions between *Phytophthora infestans* and the solanaceous hosts *Calibrachoa* × *hybridus*, *Petunia* × *hybrida*, and *Nicotiana benthamiana*. *Plant Disease* **90**, 24–32.

Bhattacharyya SRSK, Sharma SR, 1976. New host of *Phytophthora infestans* in nature. *Indian Phytopathology* **29**, 342–3.

Campbell CL, Madden LV, 1990. *Introduction to Plant Disease Epidemiology*. New York, USA: John Wiley & Sons.

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.

Deahl KL, Jones RW, Perez FM, Shaw DS, Cooke LR, 2006. Characterization of isolates of *Phytophthora infestans* from four solanaceous hosts growing in association with late blight-infected commercial potato crops. *HortScience* **41**, 1635–9.

Drenth A, Janssen EM, Govers F, 1995. Formation and survival of oospores of *Phytophthora infestans* under natural conditions. *Plant Pathology* **44**, 86–94.

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

Fernández-Pavía SP, Grünwald NJ, Díaz-Valasis M, Cadena-Hinojosa M, Fry WE, 2004. Soilborne oospores of *Phytophthora infestans* in Central Mexico survive winter fallow and infect potato plants in the field. *Plant Disease* **88**, 29–33.

Flier WG, van den Bosch GBM, Turkensteen LJ, 2003. Epidemiological importance of *Solanum sisymbriifolium*, *S. nigrum* and *S. dulcamara* as alternative hosts for *Phytophthora infestans*. *Plant Pathology* **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. *Journal of Phytopathology* **152**, 331–6.

Forbes GA, Landeo JA, 2006. Late blight. In: Gopal J, Khurana SMP, eds. *Handbook of Potato Production, Improvement, and Postharvest Management*. Binghamton, NY, USA: Haworth Press Inc., 279–320.

Harrison JG, 1992. Effects of the aerial environment on late blight of potato foliage – a review. *Plant Pathology* **41**, 384–416.

Harrison JG, Lowe R, 1989. Effects of humidity and air speed on sporulation of *Phytophthora infestans* on potato leaves. *Plant Pathology* **38**, 585–91.

Hirst JM, Moore WC, 1957. *Phytophthora infestans* on *Petunia* and *Datura*. *Plant Pathology* **6**, 76.

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

Kamoun S, van der Lee T, van den Berg-Velthuis G, Groot KE, Govers F, 1998. Loss of production of the elicitor protein INF1 in the clonal lineage US-1 of *Phytophthora infestans*. *Phytopathology* **88**, 1315–23.

Kirk WW, 2003. Tolerance of mycelium of different genotypes of *Phytophthora infestans* to freezing temperatures for extended periods. *Phytopathology* **93**, 1400–6.

Lacey J, 1965. The infectivity of soils containing *Phytophthora infestans*. *Annals of Applied Biology* **56**, 363–80.

Melhus IE, 1915. Germination and infection with the fungus of the late blight of potato (*Phytophthora infestans*). *Agricultural Experiment Station of the University of Wisconsin Research Bulletin* **37**, 1–64.

Minogue KP, Fry WE, 1981. Effect of temperature, relative humidity, and rehydration rate on germination of dried sporangia of *Phytophthora infestans*. *Phytopathology* **71**, 1181–4.

Mizubuti ESG, Fry WE, 1998. Temperature effects on developmental stages of isolates from three clonal lineages of *Phytophthora infestans*. *Phytopathology* **88**, 837–43.

Mizubuti ESG, Aylor DE, Fry WE, 2000. Survival of *Phytophthora infestans* sporangia exposed to solar radiation. *Phytopathology* **90**, 78–84.

Nazareno NRX, Boschetto N, Pinto JA, 2004. Sobrevivência em campo de *Phytophthora infestans* em hastes de batata no Paraná, Brasil. *Fitopatologia Brasileira* **29**, 573.

Phukan SN, Baruah CK, 1993. Studies on the factors influencing diurnal periodicity in dispersal, deposition and infectivity of sporangia of the late blight fungus *Phytophthora infestans*. *Advances in Plant Sciences* **6**, 158–60.

Porter LD, Johnson DA, 2004. Survival of *Phytophthora infestans* in surface water. *Phytopathology* **94**, 380–7.
Reis A, Smart CD, Fry WE, Maffia LA, Mizubuti ESG, 2003. Characterization of *Phytophthora infestans* isolates from Southern and Southeastern Brazil from 1998 to 2000. *Plant Disease* **87**, 896–900.
Rotem J, Palti J, Lomas J, 1970. Effects of sprinkler irrigation at various times of the day on development of potato late blight. *Phytopathology* **60**, 839–43.
Sato N, 1994. Effect of sporulating temperature on the limit temperature in indirect germination of the sporangia of *Phytophthora infestans*. *Annals of the Phytopathological Society of Japan* **60**, 60–5.
Suassuna ND, Maffia LA, Mizubuti ESG, 2004. Aggressiveness and host specificity of Brazilian isolates of *Phytophthora infestans*. *Plant Pathology* **53**, 405–13.
Vartanian VG, Endo RE, 1985. Overwintering hosts, compatibility types, and races of *Phytophthora infestans* on tomato in Southern California. *Plant Disease* **69**, 516–9.
Waggoner PE, 1952. Distribution of potato late blight around inoculum sources. *Phytopathology* **42**, 323–8.
Wapshere A, 1974. A strategy for evaluating the safety of organisms for biological weed control. *Annals of Applied Biology* **77**, 201–11.
Zan K, 1962. Activity of *Phytophthora infestans* in soil in relation to tuber infection. *Transactions of the British Mycological Society* **45**, 205–21.