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Spatiotemporal dynamics of *Phytophthora infestans* airborne inoculum in Belgium

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Abstract For nearly a century, scientists have strived to model the development of *Phytophthora infestans* (*Pi*) to predict late blight infections in potatoes. This has led to the use of decision support systems (DSSs) that rely on forecasting models based on environmental parameters. All these models assume that the primary inoculum is ubiquitous. This study focuses on the spatiotemporal distribution of airborne inoculum of *Pi* to assess the value of incorporating quantitative data of this variable in improving the prediction of primary infection of potato late blight in fields. The daily spatiotemporal distribution of *Pi* airborne inoculum was studied from 2019 to 2022 at Ath, Gembloux, Libramont and Louvain-la-Neuve in Belgium by combining Burkard spore traps with a quantitative PCR assays. The quantities of *Pi* inoculum trapped daily ranged from 0 to 4903 (expressed as sporangia equivalent) depending on the site and the year. The appearance of late blight symptoms in untreated plots located close to spore traps was assessed shortly after the detection of airborne inoculum in all monitored sites. A comparison between airborne inoculum detection and the recommendations provided by three DSSs revealed that fungicide treatments are often recommended at the beginning of the season when airborne inoculum is absent. Two field trials performed in 2021 and 2022 showed that the number of fungicide treatments could be reduced by considering the presence of the inoculum, particularly by delaying the first application. Further knowledge of the relationships between disease pressure, airborne inoculum and meteorological conditions will provide valuable information for improving DSSs and reducing fungicide use.

Keywords Aerobiology · Potato late blight · Decision support system · Airborne inoculum · Burkard spore trap

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Introduction

Potatoes are a major food crop worldwide, with over 359 million tons produced in 2020 over an area of nearly 16.5 million hectares (FAOSTAT, 2022). Northwestern European countries (Germany, France, the Netherlands, the UK and Belgium) are involved in potato production owing to favourable soil and

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climate conditions (Goffart et al., 2022). Due to their high productivity, potato crops are expected to play a key role in European and global food security. However, the potato industry faces major challenges regarding the social and environmental aspects of production, one of the most important of which is reducing the use of synthetic plant protection products (PPP) (Alliot et al., 2022). One option to meet this challenge is to produce more with less through better management and optimization (Andrivon, 2017; Goffart et al., 2022). Much of the PPP used in potatoes protects the crop from potato late light (PLB), a devastating disease caused by *Phytophthora infestans* (*Pi*). This oomycete is responsible for global losses of about 5.2 billion euros each year (Haesaert et al., 2015; Haverkort et al., 2009). *Pi* is a hemibiotrophic pathogen that achieves a complete growth cycle in potato plants. First, it depends on living plant cells in the short biotrophic phase. It then produces multiple rapidly expanding necrotic lesions on leaves and stems, which can destroy plants in a short time. Under favourable conditions (> 90% relative humidity, 15–22 °C), *Pi* is capable of multiple cycles of asexual reproduction with a high sporulation potential, enabling the rapid dissemination of the pathogen by aerial dispersal of asexual propagules called sporangia (Aylor et al., 2001; Fry, 2020). Sporangia are produced in plant tissues under moist conditions and released into the air (Aylor et al., 2001). They can spread at least 90 m above and 500 m downwind (Aylor et al., 2011), possibly up to 700 km (Severns et al., 2019), of diseased plants. When they land on plant tissues, the sporangia either release motile zoospores or encyst. In both cases, a germ tube is produced that penetrates the plant tissues, initiating mycelial growth (Fry, 2008). As a heterothallic species, *Pi* requires contact between isolates of opposite mating types (A1 and A2) to achieve sexual reproduction and produce thick-walled structures called oospores, which can survive in soils when conditions are not appropriate for the development of the oomycete, (e.g. frost or drought) (Drenth et al., 1995; Fernández-Pavía et al., 2004; Fry, 2020). The control of PLB is principally achieved through the repeated use of fungicides, with an average of 12–15 sprays per year in moderately wet climates, such as northwestern Europe, reaching up to 20 sprays during wet growing seasons (Goffart et al., 2022; Haesaert et al., 2015). Even if resistant varieties are available, they are constantly challenged by increasing *Pi* genetic diversity and susceptible cultivars still account for a large proportion of commercially grown potatoes (Fry, 2008; Haesaert et al., 2015). Host resistance must be incorporated into integrated pest management approaches, involving the use of disease prediction models and DSSs to minimize fungicide use and develop sustainable control strategies. Such DSSs have been developed in nearly every potato-growing country. Even though they differ in structure and parameterization, most use mechanistic predictive models capable of predicting PLB risk based on environmental parameters, such as temperature (T), relative humidity (RH) and pluviometry (PLU). However, these systems assume that the primary inoculum is always present and there is doubt whether this assumption is correct (Meno et al., 2023).

The rapid development of molecular tools in the 2000s relieved the technical constraints hindering the use of aerobiological data for day-to-day monitoring of plant-pathogenic airborne inocula (Van der Heyden et al., 2021). This information can be used to inform disease management; however, spatiotemporal concentrations of airborne inocula are highly variable (Duvivier et al., 2013, 2016; Hellin et al., 2018, 2020; Van der Heyden et al., 2012). Aylor et al. (2001) reported that the release of *Pi* sporangia from the potato canopy followed a diurnal pattern, increasing until noon, then decreasing and being nearly absent at night. This scheme was recently confirmed and is influenced by an increase in RH and a decrease in T (Meno et al., 2023). It has also been reported that *Pi* airborne inoculum in New Brunswick (Canada) was heterogeneous and increased during the potato-growing season. The same authors found that epidemics seemed to be influenced by the size of the initial spore load and suggested that monitoring the *Pi* airborne inoculum throughout the potato production season could be useful for adjusting PLB management strategies as a complement to DSSs (Fall et al., 2015a, 2015b). Similarly, Meno et al. (2023) found high daily variability in aerial sporangia concentrations and established correlations between infection pressures computed using the Danish late blight model (BlightManager) and disease severity. Fall et al. (2015a) found that the number of sporangia sufficient to cause one lesion per leaf varied from 10 to 25 per m^3 of air, depending on clonal lineage. Therefore, these studies suggest that it cannot be assumed

that *Pi* inoculum always present everywhere in sufficient quantities to initiate a PLB epidemic.

The aim of the present study was to assess the spatiotemporal distribution of the aerial inoculum of *Pi* in Belgium and quantify this inoculum to improve the prediction of infection periods using DSSs. A network of spore traps associated with weather stations and field trials, including potato varieties highly susceptible to PLB, was deployed in Belgium for four years to test this hypothesis. The opportunity to use such systems to improve the current DSS in Wallonia (Belgium) and to better understand the epidemiology of *Pi* is discussed in this study.

Materials and methods

Assessment of potato late blight severity in fields

The presence of PLB on untreated potatoes was assessed from 2019 to 2022 during the cropping season (Table 1) in two to four experimental fields, with a minimum surface of three ares located beside the Burkard spore traps (Burkard Manufacturing Co. Ltd., Rickmansworth, Hertfordshire, UK). A minimum of 200 plants were inspected for PLB symptoms after potato emergence. The severity of the disease was quantitatively assessed twice a week by visually estimating the percentage of leaf area covered by symptoms using a custom scale modified from (James, 1971) after the first detection of PLB.

Table 1 Potato planting and harvesting dates at the four locations

Location	Year	Planting	Harvest
Ath	2019	19–04	26–09
	2020	22–04	09–09
Gembloux	2019	22–05	16–09
	2020	20–04	07–09
	2021	05–05	22–09
	2022	04–05	13–09
Libramont	2019	02–05	23–10
	2020	27–04	02–09
	2021	30–04	13–09
	2022	17–05	07–09
Louvain-la-Neuve	2019	24–05	15–09
	2020	06–05	16–10
	2021	13–05	25–09
	2022	06–05	30–09

Weather data

Weather conditions were recorded in Ath, Gembloux, Libramont and Louvain-la-Neuve from 2019 to 2022 to model the risk of infection at the plot level over the growing season and study the relationship between meteorological parameters and aerial capture of *Pi*. Meteorological conditions in the vicinity of each experimental field were assessed using the weather station from AGROMET (https://www.agromet.be) (Belgium), the Royal Meteorological Institute of Belgium, or the "Centre pour l'Agronomie et l'Agro-industrie de la Province de Hainaut" (CARAH) networks to evaluate the risk of infection of *Pi* at the trial plot level. Each weather station was placed in an open area with uniform vegetation, away from buildings and tree rows more than 10 times the height of the stations. Hourly data on temperature, relative humidity, irradiance, wind speed and rainfall were collected and used as explanatory variables to interpret the aerobiological data and late blight development.

Spore trapping

A network of Burkard 7-day spore-recording traps (Burkard Manufacturing Company Limited, Hertfordshire, UK) was deployed from 2019 to 2022 in Ath, Gembloux, Libramont and Louvain-la-Neuve, with one trap per location, to monitor the spatiotemporal distribution of *Pi*. Spore trap openings were placed 1 m above ground level near untreated plots planted with potato varieties susceptible to PLB (Bintje or Agria) during the growing season. The vacuum flow was regulated at 10 L min^{-1}, corresponding to 14.4 m^3 every 24 h. The traps collected airborne particles on a wax-coated Melinex tape glued to a rotating drum with a 7-day cycle (Burkard Manufacturing Co., Ltd.) (345 mm × 20 mm). After exposure, Melinex tapes were collected weekly and stored at 4 °C until being cut into seven daily segments of 48 × 20 mm. Fragments were subsequently stored in 2-mL microtubes at − 20 °C until DNA extraction. During the winter of 2022, two juxtaposed spore traps were operated over a two-month period in Libramont to study the variability of trapped material in a single location and validate the method.

Spore disruption and DNA extraction

Total DNA was extracted from the material collected in daily segments using the phenol–chloroform method, adapted from the method described by Duvivier et al. (2013). Briefly, samples were homogenized in 200 µL of Triton X-100 (0.1%) using a high-speed benchtop homogenizer (FastPrep, Thermo Savant, US) for 40 s at 6 $m.s^{-1}$. After 5 min cooling on ice, 400 µL of extraction buffer was added and tubes were shaken again for 40 s. The extraction buffer comprised one equivalent of beta-mercaptoethanol mixed with 100 equiv lysis buffer. The samples were then incubated at 65 °C for 1 h and the DNA was extracted with 600 µL phenol:chloroform (1:1). Each sample was precipitated with isopropanol, NaOAc and 1 µL of glycerol, then washed with ethanol. The DNA pellet was dissolved in 50 µL of molecular biology grade water and samples were conserved at − 20 °C.

Quantification of *Pi* by quantitative PCR

Pi DNA was quantified using quantitative PCR with primers and a Taqman probe targeting *Pi* ITS 1 region (167 bp), as described by Lees et al. (2012), using Takyon No ROX Probe 2X MasterMix Blue dTTP. A calibration curve was established from three independent experiments, each encompassing five tenfold serial dilutions of sporangia suspensions to allow the conversion of Cq values into the number of sporangia. Suspensions of sporangia were obtained from 4-week-old cultures of *Pi* on rye-agar, counted using a Fuchs–Rosenthal counting chamber (depth 0.1 mm, 0.0025 mm^2, Superior Marienfeld, Germany), added to wax-coated Melinex tape and extracted under the same conditions as the field samples. Each sample was run as two technical replicates during development and routine use**.**

Characterization of *Pi* genotypes in DNA extracts from airborne inoculum

Selected DNA extracts from trapped airborne inocula were characterized using sequencing and simple sequence repeat (SSR) genotyping. Twelve DNA samples extracted from winter captures representing the four locations in 2019, 2020 and 2021 were amplified using three primer pairs: primers used for qPCR quantification targeting *Pi* internal transcribed spacer rDNA (ITS1) region (Lees et al., 2012), primers targeting the heat shock protein 90 (HSP90) gene of *Pi* (Blair et al., 2008) and the cytochrome c oxidase subunit 1 (COI) gene of *Pi* (Robideau et al., 2011). All PCRs were done using GoTaq G2 DNA polymerase (Promega) following the manufacturer's instructions. The amplification reaction comprised a denaturation step at 95 °C for 3 min; 35 cycles of 30 s at 95 °C, 30 s at the locus-specific annealing temperature and the locus-specific time at 72 °C; and a final elongation step of 7 min at 72 °C. PCR products were purified using the SmartPure PCR Kit and DNA Purification Kit (Eurogentec, Seraing, Belgium) according to the manufacturer's instructions and sequenced using Microsynth SeqLab (Goettingen, Germany). The sequences were manually cleaned on the Benchling platform (https://benchling.com/), aligned with reference sequences using MAFFT and compared to NCBI nr using BLASTn.

Sixteen DNA samples from spore traps, ranging from 10.6 to 1456.6 sporangia equivalent per day, were genotyped by the Multi-infections and Multiresistances (MIR) team at the National Research Institute for Agriculture, Food and the Environment (INRAE) (France) using a 17-plex of (SSR) markers, as described in Beninal et al. (2022). Samples were selected to represent two years (2020 and 2021) at two locations (Gembloux and Libramont), during (six samples) and outside (ten samples) the potato growing season.

Analysis of predicted infection risks by the Guntz–Divoux (GD) model and calculation of false alarm of infection on the basis of airborne inoculum data

The Guntz–Divoux (GD) model (Arora et al., 2014; Goeminne et al., 1997) was designed to indicate when the meteorological conditions were favourable for PLB development and is used by producers. The model was used to calculate the risk of infection (*ir*) according to the following quadratic equation:

$$ir = -2.21 + 0.155wp - 0.211mt + 0.013wp.mt + 0.00093wp^2 + 0.0094mt^2$$

where *wp* is the duration (h) of the wet period and *mt* is the mean temperature over this period. When *ir* was

greater than one, the incubation period began and the model predicted the appearance of symptoms after the incubation period. This model was used in the four experimental fields each year. The risk of infection was considered a false alarm when no symptoms appeared in untreated plots after the infection risk. Airborne inoculum data were included in the model by analysing their presence or absence on the day before an alarm was triggered. The number of false alarms that integrated the absence of airborne inoculum during the preinfection period was calculated.

Modeling of the survival probability of *Pi* airborne inoculum

The survival probability of *Pi* airborne inoculum was estimated using the model developed by Skelsey et al. (2018) and based on cumulative solar radiation (MJ/m^2) during each day. A mean-centred transformation was performed on the solar radiation data to match the range of values Skelsey et al. (2018) used to develop the formula (min = 1.1 $MJ.m^{-2}$, max = 6.7 $MJ.m^{-2}$ and mean = 3.80 $MJ.m^{-2}$) to use the model.

$$Survival\ probability\ of\ sporangia = \frac{1}{1 + e^{-(2.37-0.45X)}}$$

with X as the cumulated solar radiation ($MJ.m^{-2}$).

The inoculum was considered viable when the survival probability was equal to or greater than 0.6. This cutoff was chosen through statistical analysis, as it offers the best predictive capabilities, according to Skelsey et al. (2018).

Inclusion of airborne inoculum data to Vigimap DSS in two field trials

Three fungicide programmes were compared: (1) treatments based on Vigimap (the DSS currently used in the Walloon region of Belgium and operated by CARAH asbl) warnings only ("Vigimap"), (2) treatments based on Vigimap combined with airborne inoculum values in order to delay the first treatment ("Spore trap") and (3) untreated controls ("Control"). These treatments were conducted on three potato varieties with increasing sensitivities to *Pi*: Louisa, Agria and Bintje. A randomized block design field experiment with four replicates was set up and each plot comprised four rows of ten plants per variety. Melinex tapes of the spore traps were collected twice a week during the assay to quantify the airborne inoculum in real-time. If the number of captured sporangia per day was higher than ten and the meteorological conditions were favourable to PLB infection according to Vigimap, treatment in the "Spore trap" programme was justified.

Results

Weather data in relation to PLB disease severity in untreated potato plots

A summary of the weather data is shown in Fig. 1A. Weather conditions varied significantly across the four years and locations included in the study. Colder weather was recorded in Libramont than in other places, with more frost during winter and lower mean temperatures during the cropping season. Overall, Ath experienced higher relative humidity and rainfall than Louvain-la-Neuve and Gembloux despite having similar temperatures. The 2020 and 2022 growing (May–September) were unusually hot and dry, which is unfavourable for PLB epidemics. In contrast, the summer of 2021 was cool and rainy, which was particularly favourable for the development of *Pi*. Weather conditions were more contrasted during the 2019 cropping season, but overall, they were not favourable for PLB.

The occurrence and severity of PLB epidemics in potato varieties susceptible to *Pi* showed significant variation between years and locations. No epidemic development of *Pi* was observed in 2020 or 2022 in the untreated plots assessed, regardless of the location, whereas exceptionally high disease pressure was witnessed in 2021, with total foliage destruction at the beginning of July (Fig. 1b). In 2019, the first symptoms occurred later in the season (end of July–August) in Gembloux and Libramont than usual (June–July), but the severities were higher (80–100%).

Overview of airborne inoculum capture patterns in four locations and four years

The airborne inoculum of *Pi* captured by the Burkard spore traps was quantified using qPCR. The amplification curves of serial dilutions of plasmid constructs

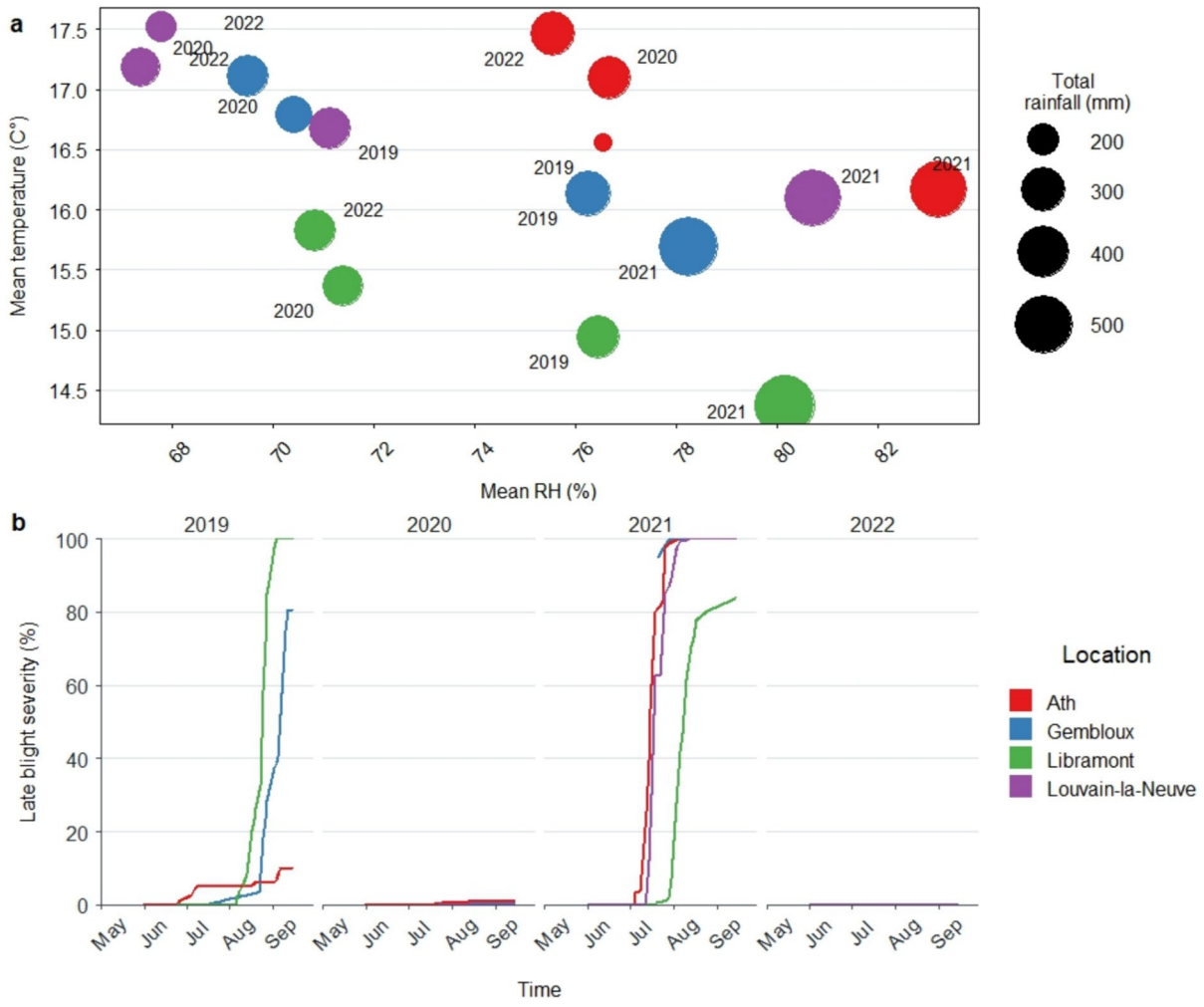

Fig. 1 (**a**) Mean temperature (°C), mean relative humidity (%) and total precipitation (mm) for all four locations (Ath, Gembloux, Libramont and Louvain-la-Neuve) during the cropping season (May–September) from 2019 to 2022. (**b**) PLB severity on susceptible cultivars (Bintje or Agria) was monitored in the four locations from 2019 to 2022

containing *Pi* target template showed 90–110% efficiency and an R^2 value of over 0.98, confirming the efficiency of the qPCR assay under the conditions applied in this study. A calibration curve was constructed, allowing conversion of the quantification cycle (Cq) from qPCR in terms of sporangia equivalents. Taking the three independent assays together (serial sporangia dilutions, DNA extraction and qPCR), a high correlation between spore concentrations and Cq values was obtained, enabling the establishment of a reliable calibration curve ($R^2 = 0.98$). The equation $y = -0.2439x + 8.1746$, with y the number of sporangia and x the Cq, was used to interpret the Cq values from the qPCR analysis in terms of the sporangia-equivalent per cubic metre of sampled air. The mean Cq values for DNA from 1000 to one sporangium ranged from 21.22 to 33.52.

The amounts of *Pi* airborne inoculum captured by two spore traps placed at 15 m intervals in Libramont during a period extending from the end of January 2022 to the end of April 2022 were compared to demonstrate the uniformity of captures between distinct devices, showing similar profiles (data not shown).

The spatiotemporal profiles of *Pi* airborne inoculum captured at four locations from 2019 to 2022 are shown in Fig. 2. In 2019 and 2020, variable

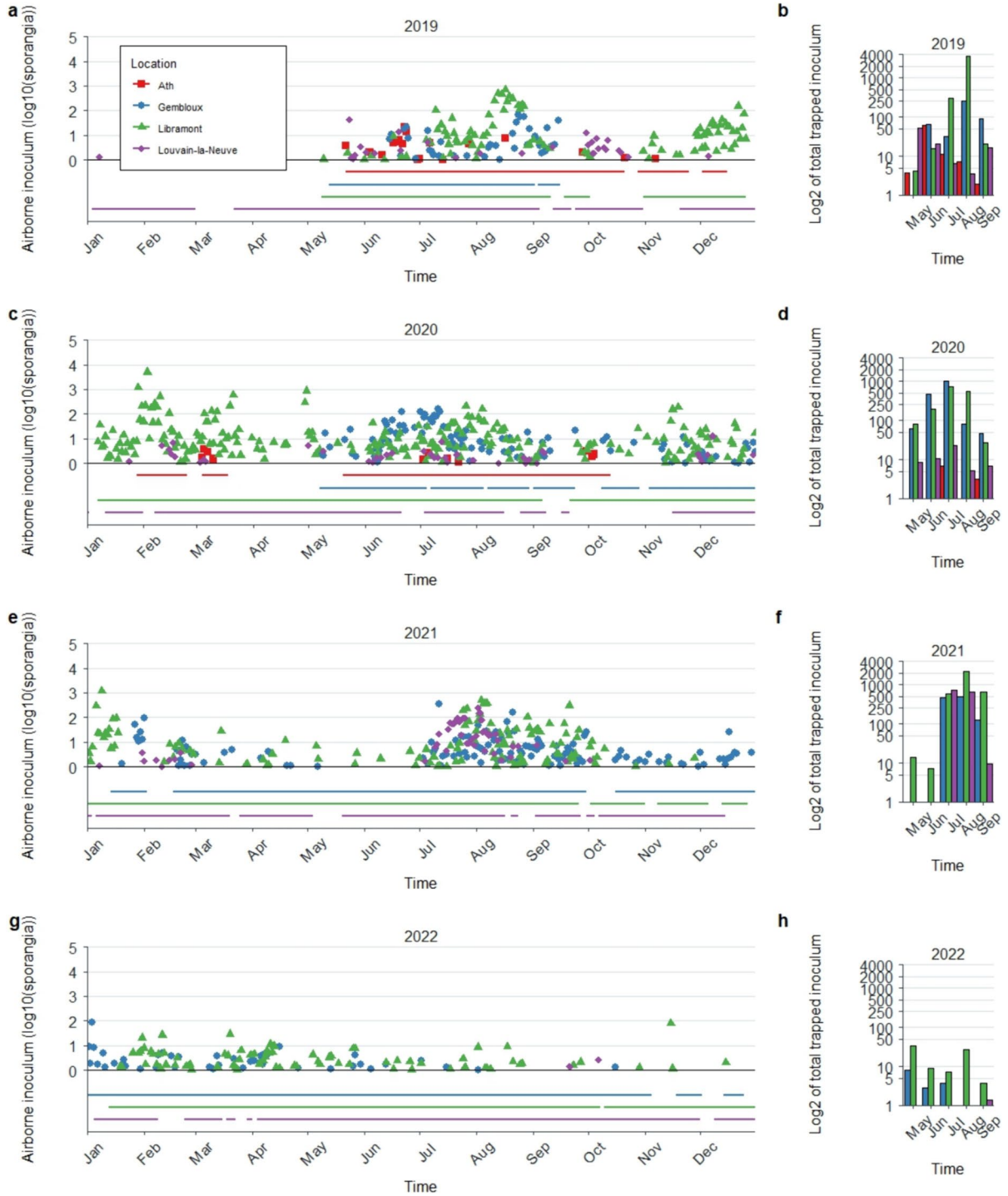

Fig. 2 Daily quantities of *Pi* airborne inoculum trapped by four spore traps in potato fields in Ath, Gembloux, Libramont and Louvain-la-Neuve in 2019 (**a**), 2020 (**c**), 2021 (**e**) and 2022 (**g**). Coloured lines below zero represent trapping periods for each location. Sums of inoculum quantities trapped per month over the cropping season (May–September) in 2019 (**b**), 2020 (**d**), 2021 (**f**) and 2022 (**h**) for the four locations. Airborne inoculum data from 2021 and 2022 are not available for Ath due to technical issues

amounts of *Pi* aerial inoculum were collected over five months of the growing season May–September at the four locations, except in May 2019 and May, June and August 2020 at Gembloux. The monthly trapped quantities of airborne inoculum over the two seasons were higher in Libramont with 3976 and 1587 sporangia over the 2019 and 2020 seasons, respectively, and Gembloux (451 and 1689 sporangia) than in Louvain-la-Neuve (99 and 54 sporangia) and Ath (85 and 10 sporangia). No inoculum was detected in Gembloux and Louvain-la Neuve in May and June 2021, respectively. In 2022, the aerial inoculum was collected during the five months of the growing season in Libramont, from May to July in Gembloux and only in September in Louvain-la-Neuve. No data were available for Ath in 2021 and 2022 due to technical issues. The monthly trapped quantities varied from 0 to 3635, 0 to 1036, 0 to 2198 and 0 to 33 equivalent sporangia during the cropping seasons of 2019–2022 respectively (Fig. 2 b, d, f and g). Airborne *Pi* inoculum was also detected during periods other than the potato growing season at all four locations for several successive months or sporadically, depending on the year and the field. High captures (in quantities comparable to or higher than those collected during the season) were observed during the winter of 2020 and 2021, particularly in Libramont.

These data revealed that airborne quantities of *Pi* varied greatly from place to place, year to year and across years. The highest peaks in the *Pi* airborne inoculum during the year were related to severe PLB epidemics; however, the airborne inoculum of this microorganism was not always present. In particular, the airborne inoculum captures profiles across the entire year in a wavy fashion, peaking during the crop season and winter period. In 2022 (Fig. 2), which was a particularly hot and dry year, the profiles were flatter with a total of 592 sporangia trapped over the four locations and a maximum 159 sporangia trapped in January, showing a slow decline of 13.3 trapped sporangia per month on average as the year progressed. Forty-two sporangia were trapped in May, whereas 27 sporangia were recorded in August 2022 over the four locations. The quantity of trapped airborne inoculum decreased in September with only 15 sporangia trapped over the four locations (Fig. 2 h). Large quantities of sporangia were detected in August in Libramont compared to the other sites, especially in 2019, with an average of 120 sporangia trapped daily. Almost no airborne inoculum was detected in 2022 at the four sites, indicating a low PLB pressure.

Focus on spore capture profiles during the cropping season

The presence of *Pi* in the air was first detected in May in 10 of the 14 cases studied (Table 2). The first detection in Louvain-la-Neuve in 2021 and 2022 occurred later in the season, on July 4 and September 21, respectively. Similarly, the first detection in Gembloux in 2019 was on June 15 and on July 3 in Ath in 2020. The quantity of trapped inoculum varied greatly depending on the location and year. Libramont was the location where the largest quantities of inoculum were typically detected, except for 2020, when the mean daily detection was 12.2 sporangia per day in Gembloux and 11.4 sporangia per day in Libramont. There was weak late blight pressure during the 2022 growing season, with 28 days of positive *Pi* detection in Libramont, 9 days in Gembloux and only 1 day in Louvain-la-Neuve. The airborne inoculum was always detected 1–4 days before the appearance of the first symptoms of late blight in the field and the cumulative quantity of airborne inoculum was greater than 10 sporangia over the 10 days prior to the infection onset, except in Ath in 2020, where only 3.3 sporangia equivalent were detected.

Spore capture profiles during winter

In 2019, one spore trap was operated throughout the year, including during winter. Because some captures were achieved in January and February in Louvain-la-Neuve (Fig. 2), two other Burkard spore traps were used during the 2019–20 winter in Ath and Libramont and during 2021 and 2022 in Gembloux and Libramont. In each case, spore capture was recorded outside the growing season, particularly during winter. In addition, similar quantities of airborne inoculum were obtained from two juxtaposed spore traps operated at Libramont over a 77-day period between January 27 and March 16, 2022, confirming the reliability of the high capture recorder at this location during winter (data not shown). Twelve DNA samples were amplified using PCR assays and sequenced to confirm the specific detection of *Pi* during winter captures. The 11 ITS1 sequences shared 100% homology

Table 2 Summary of the *Pi* airborne inoculum monitoring and potato late blight occurrence during the cropping season (May–September) from 2019 to 2022 in the four studied locations

Location	Year	Date of first detection	Late blight onset	Last detection before LB onset (sporangia)	Sum of sporangia 10 days prior LB onset	Mean sporangia/day	Max daily quantity of sporangia	Days of positive detection
Ath[a]	2019	22–05	24–06	23–06 (22)	44.4	< 1.0	22.0	37/132
	2020	03–07	20–07	16–07 (2)	3.3	< 1.0	3.1	24/134
Gembloux	2019	15–06	15–07	13–07 (5)	27.6	3.6	64.4	65/126
	2020	07–05	23–07	21–07 (14)	100.0	12.2	152.9	87/138
	2021	07–05	20–07	16–07 (3)	364.0	7.1	352.0	70/153
	2022	14–05				< 1.0	4.0	9/153
Libramont	2019	10–05	06–08	05–08 (9)	52.0	28.6	706.4	68/139
	2020	01–05	06–07	05–07 (31)	103.4	11.4	203.0	107/139
	2021	06–05	20–07	18–07 (7)	13.4	23.2	480.8	80/149
	2022	04–05				< 1.0	8.9	28/153
Louvain-la-Neuve	2019	24–05				< 1.0	44.1	22/146
	2020	01–05				< 1.0	7.3	59/118
	2021	04–07	13–07	12–07 (22)	39.2	11.0	254.9	64/126
	2022	21–09				< 1.0	1.4	1/153

[a]Airborne inoculum data from 2021 and 2022 are not available for Ath due to technical issues

with *Pi*. Therefore, two additional genes (COI1 and HSP90) were targeted. Due to the presence of a wide range of DNAs from various sources in the extracts obtained from airborne spore traps, only two and one PCR products could be reliably sequenced on the COI and HSP90 loci, respectively. The BLASTn searches using the sequences obtained against NCBInr. confirmed, for these two samples, the presence of *Pi*. The COI and HSP90 loci have been detected in other *Phytophthora*.

Genotyping of *Pi* assayed from 16 spore trap DNA samples revealed the presence of genotypes EU_36_A2 and EU_37_A2 at Gembloux and Libramont in January and February of 2021 and 2020, respectively (Table 3). Of the seven samples tested in Gembloux, only one, collected in July 2021, was successfully identified as EU_36_A2 and two other samples, collected in January and February 2021, were identified as EU_37_A2 and EU_36_A2. These two genotypes were also identified in the Libramont in January or February 2020. Another unidentified lineage (referred to as "Other") was detected in September 2021 at Libramont. Genotyping was successful in samples containing 86–1448 equivalent sporangia but failed in samples containing 12–1456 sporangia. Genotype

Table 3 *Pi* genotype identification in DNA extracts from Melinex tape samples exposed to the air in fields in Belgium with Burkard traps

Sample Name	Date	Location	Number of sporangia	Genotype
BG20-07–08	08–07-20	Gembloux	12.1	INA[a]
BG20-07–11	11–07-20	Gembloux	41.6	INA
BG20-12–30	30–12-20	Gembloux	14.1	INA
BG21-01–27	27–01-21	Gembloux	86.6	EU_36_A2
BG21-02–01	01–02-21	Gembloux	168.9	EU_37_A2
BG21-07–12	12–07-21	Gembloux	400.9	EU_36_A2
BG21-08–21	21–08-21	Gembloux	202.4	INA
BL20-21_r2	21–01-20	Libramont	12.4	INA
BL20-01–29	29–01-20	Libramont	1448.5	EU_36_A2
BL20-33_r2	02–02-20	Libramont	217.2	EU_37_A2
BL20-07–26	26–04-20	Libramont	42.8	INA
BL20-12–20	20–12-20	Libramont	44.0	INA
BL21-01–09	09–01-21	Libramont	1456.6	INA
BL21-07–25	25–07-21	Libramont	200.7	INA
BL21-07–29	29–07-21	Libramont	10.7	INA
BL21-09–21	21–09-21	Libramont	706.0	Other

[a]Identification Not Achievable

identification from several samples failed, possibly because of a mixture of DNA from different origins.

Utility of aerial inoculum data to improve forecast systems

Relationship between airborne inoculum with the infection events predicted using the GD model and the onset of PLB symptoms

The metric used to evaluate the performance of the GD model was the number of predicted infection events over the entire season. All predicted infection events during the season were considered false alarms (predicted infection events but not observed) for the data sets without infection during the season. A predicted infection event was considered true (= true alarm) when disease symptoms appeared in the field within 1–3 days of the event. The analyses were only carried out with data from the pre-infection period, as the aim was to reduce the number of false alarms before the first infection. Without infection, this period corresponded to the entire season (Table 4).

The GD model triggered 14, 19, 9 and 19 infection events in Ath, Gembloux Louvain-la-Neuve and Libramont, respectively, in 2019. Most (5, 13, 9 and 11 cases; Table 4) were not followed by the development of symptoms in the field and were thus considered 'false alarms.' The presence of an airborne inoculum in the prediction model enabled the detection of two false alarms each in Louvain-la-Neuve and Libramont. Airborne inoculum monitoring in 2020 resulted in three false alarms in Ath but none in the other locations because all predicted infection periods were concomitant with the airborne inoculum. Depending on the location, 31–37 infection events were predicted by the GD

Table 4 The number of predicted infection events from the Guntz–Divoux model over a season, the number of false alarms (predicted infection event but not observed), the number of false alarms when the presence of airborne inoculum the day before is included in the model, the maximum severity and date of first symptoms

Year	Location	GD predicted infections over-season	Number of GD predicted infections but not observed[a]	False alarm in the presence of AI[a]	Performance of the GD model (%)	Performance of the model with AI (%)	Maximum LB severity at the end of the season (%)	Date of disease onset
2019	Ath	14	5	5	64.3	64.3	10	24–06
	Gembloux	19	13	13	31.6	31.6	80	15–07
	Louvain-la-Neuve	9	9	7	0.00	22.2	0	
	Libramont	19	11	9	42.1	52.6	100	06–08
2020	Ath	32	21	18	34.3	43.7	1	20–07
	Gembloux	21	12	12	42.9	42.9	0.3	23–07
	Louvain-la-Neuve	13	13	13	0.00	0.00	0	
	Libramont	19	19	19	0.00	0.00	0	
2021	Gembloux	31	10	6	67.7	80.6	100	20–07
	Louvain-la-Neuve	42	13	8	69.0	80.9	100	21–07
	Libramont	37	14	9	62.2	75.7	80	20–07
2022	Gembloux	9	9	6	0.00	33.3	0	
	Louvain-la-Neuve	9	9	3	0.00	66.7	0	
	Libramont	11	11	11	0.00	0.00	0	

GD: Guntz-Divoux

AI: Airborne inoculum

LB: Late blight

[a]Before LB onset

model in 2021 because of the favourable climatic conditions for late blight development. However, 10–14 were not followed by symptoms, but 4–5 false alarms were identified by considering the airborne inoculum. Despite the unfavourable conditions for PLB and the absence of disease in the three experimental fields in 2022, 9–11 infection events were predicted by the GD model, depending on the location. Three of these false alarms were detected by the airborne inoculum data in Gembloux and six in Louvain-la-Neuve.

The daily airborne inoculum trapped during the growing season, along with the date of onset of late blight symptoms in the crop for the four tested locations from 2019 to 2022, is displayed in Fig. 3. Viable inoculum was detected at all four locations a few days before the onset of potato late blight (represented by a dashed line), with the exception of Gembloux in 2019. Viable inocula without any disease outbreak were detected in Gembloux and Libramont in July 2022 and Louvain-la-Neuve in 2019 and 2020.

Impact of airborne inoculum data in improving the prediction of true infection periods by Vigimap DSS in fields

The late blight pressure was high in Libramont in 2021. Based on the Vigimap warnings alone, 13 treatments were applied during the season starting on June 16 (Fig. 4). When combined with airborne inoculum data (spore trap programme), the first fungicide application was delayed until July 5 and 10 treatments were applied. The first symptoms were observed in untreated plots on July 18 for the cultivars Bintje and Agria and on August 3 for Louisa. The disease progression followed a typical S-curve. The first symptoms appeared on August 10 and 17 in Bintje, when the spore traps and Vigimap programmes were applied, respectively. Less than 2% of the foliage was

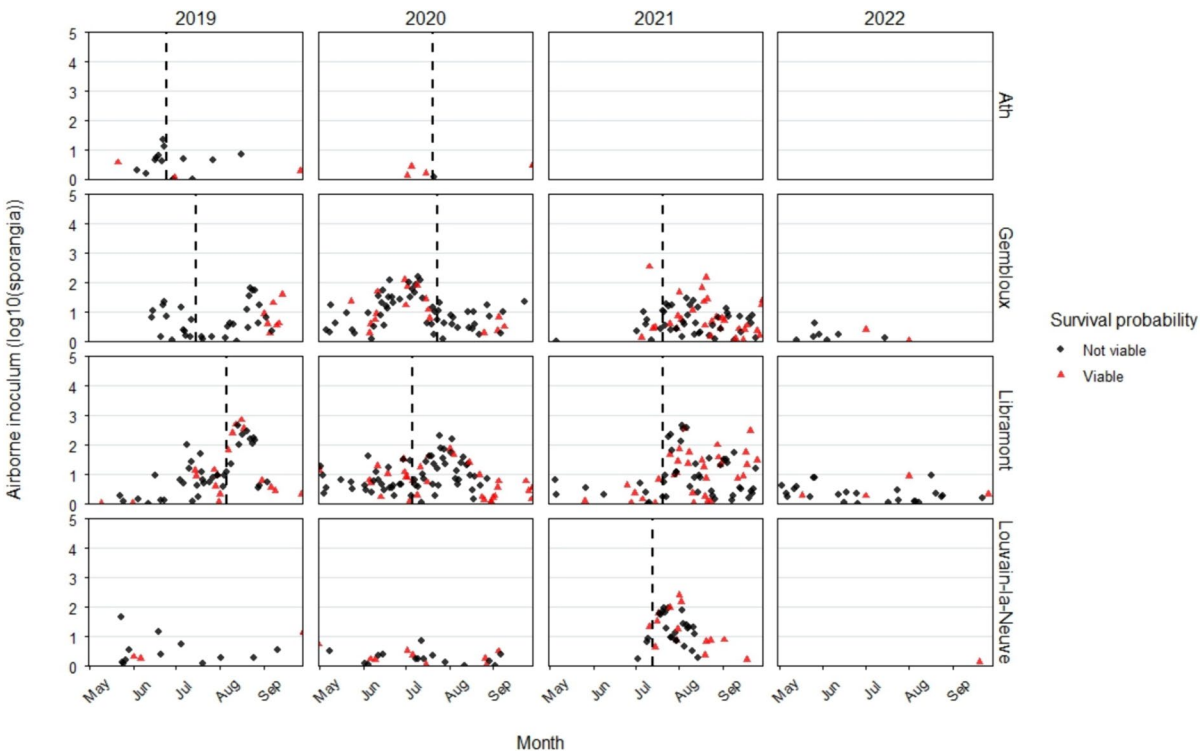

Fig. 3 Quantities of airborne inoculum trapped in potato fields from 2019 to 2022 in Ath, Gembloux, Libramont and Louvain-la-Neuve during the cropping seasons (May–September). Airborne inoculum data are not available for Ath in 2021 and 2022 due to technical issues. Detected airborne inoculum with a probability of survival greater than 0.6 is represented with red triangles and airborne inoculum represented with black dots have a probability lower than 0.6. The dashed lines represent the dates of onset of potato late blight

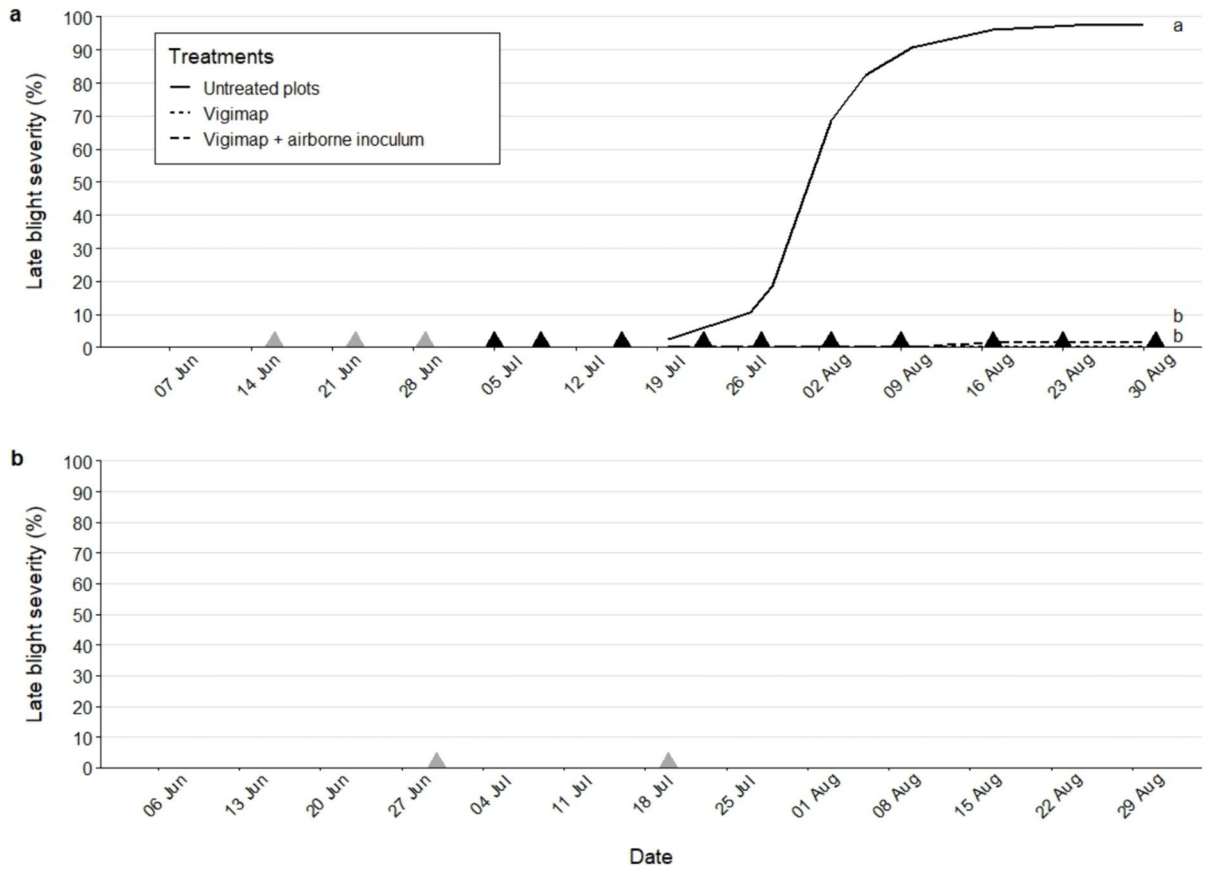

Fig. 4 Number of fungicide treatments and potato late blight progress (% of leaf surface covered by the disease) in field trials in Libramont with the susceptible Bintje cultivar according to the fungicide treatment programme in 2021 (**a**) and in 2022 (**b**). Gray triangles (▲) represent fungicide treatments done following Vigimap and black triangles (▲) following Vigimap and the detection of airborne inoculum. Disease progress curves with different letters are significantly different (Tukey's test, p-value < 0.05)

affected by August 30 and no significant difference in severity was observed between the two programmes, indicating that the level of protection was similar. The symptoms visible on the treated Agria were limited to a few leaves, whereas no symptoms were observed in the treated plots with the Louisa variety.

No symptoms were observed in any of the plots, including the untreated plots, throughout the season in 2022. Two treatments were recommended based on Vigimap DSS.

Discussion

The data on *Pi* airborne inoculum patterns in four Belgian regions over the growing seasons in this study revealed that the presence of airborne inoculum varied depending on both location and time. In total, a greater amount of the inoculum was captured at Libramont and Gembloux than at the other two locations. This variation could not be linked to the spore trap itself, as the spore traps were placed and calibrated in a standardized manner and the quantities captured by the two samplers placed 15 m apart in Libramont during a three-month period were comparable. The high level of airborne inoculum in Libramont was unexpected considering that, unlike the other three locations, potatoes are not cultivated as a major crop in this region, which is more focused on pasture and livestock. No large-scale potato cultivation is present in Libramont, but lower temperatures were usually recorded in this higher-altitude

region (approximately 500 m) and greater precipitation, with an average of 1150 mm of rainfall, compared to an altitude of 800 m in Louvain-la-Neuve. These climatic conditions may be more favourable for the sporulation of *Pi.* In contrast, Gembloux and Louvain-la-Neuve are very close and located in the centre of the country. The higher global quantities of airborne particles captured in Gembloux than in Louvain-la-Neuve are more surprising and could be related to a local situation tied to crop rotation or the landscape (for example, vegetation and density of potato fields) around the plots. Further research on the spatial variability of *Pi* airborne inoculum, related to topography and its impact on long-distance spore dispersion is needed.

The profiles of airborne inoculum in three or four experimental fields for four growing seasons showed that this inoculum was not consistently present early in the season but was detected in all fields before the appearance of the disease. Furthermore, the GD model and Vigimap DSS predicted the periods of infection in the field when no inoculum was in the air.

Our results on the heterogeneous distribution of *Pi* airborne inoculum are in line with the results obtained by Fall et al. (2015b) in Canada, suggesting that developing a spore sampling network may be usefull approach for presymptomatic disease monitoring. Such spore-trapping networks have already been developed for other diseases, such as onion leaf blight in Canada, where the use of one or a few samplers per field has allowed successful monitoring and disease management (Carisse et al., 2012). Similar results have been reported for *Puccinia striiformis* f. sp. *tritici* and *Puccinia triticina* in Belgium (Dedeurwaerder et al., 2011; Duvivier et al., 2016) and *Plasmopara viticola* in France (Douillet et al., 2022). The intermittent detection of airborne inoculum throughout the season has already been reported for several fungal pathogens such as *Pyrenopeziza brassicae,* the causal agent of light leaf blight on oilseed rape (Karolewski et al., 2012), *Hymenoscyphus falxinus* and *H. albinus* (Dvorak et al., 2015), *H. pseudoalbinus* (Chandelier et al., 2014), the causal agents of ash dieback, *Zymoseptoria tritici*, responsible for septoria tritici blotch on wheat (Duvivier et al., 2014), *Sclerotinia sclerotinium*, responsible of sclerotinia stem rot on oilseed rape (Almquist & Wallenhammar, 2015; Rogers et al., 2009) and *Botrytis cinerea*, responsible for grey mould on vine (Leyronas & Nicot, 2013). In our study, *Pi* was systematically detected within four days before the appearance of the first symptoms of late blight in the field. These results reveal that the detection threshold of the technology that integrates the use of volumetric spore traps and a quantitative real-time PCR assay is sufficient. Our results also highlighted that the presence of an airborne inoculum is not systematically followed by visible plant infections. The presence of an airborne inoculum that does not lead to infection has already been reported by Escuredo et al. (2019) and Meno et al. (2023), and can easily be explained by unfavourable climatic conditions for infection, such as low relative humidity. Although high relative humidity (RH > 90%) is required for late blight development and sporulation (Iglesias et al., 2009), the release and dispersion of sporangia require a period of time with lower values of RH (RH < 90%) (Skelsey, 2008). Insolation (direct solar radiation) is another infection-limiting factor affecting sporangia survival (Skelsey, 2008). This factor is considered in certain forecasting models, such as Blite-SVR in Korea (Gu et al., 2016) or Nærstad in Norway (Hjelkrem et al., 2021), to reduce the risk of infection. As illustrated in Fig. 4, sunny days with low levels of humidity could explain the cases of airborne inoculum detection not followed by the onset of infection in our results. These findings emphasize the importance of integrating airborne inoculum monitoring and insolation to improve late blight prediction.

Since inoculum dispersal is the key to the epidemic development of *Pi* (Aylor et al., 2001; Skelsey et al., 2009) and to confirm the interest in monitoring airborne inoculum, which has already been identified as being of major importance for late blight management (Fall et al., 2015a, 2015b; Meno et al., 2023), we conducted experiments in 2021 and 2022 by integrating airborne inoculum measurements with the disease support system VIGIMAP to predict fungicide application programmes. This successfully delayed the first fungicide application in comparison with treatment programmes based on the Vigimap warning system alone in 2021; thus, three treatments were saved. However, late blight symptoms appeared in the untreated controls on July 19, whereas the first fungicide treatment was applied on July 5 with the spore trap programme. This indicated that it may have been possible to save two additional treatments by adjusting the threshold of the airborne inoculum and better integrating the disease epidemiology.

These results were consistent with those of a similar study conducted by Thiessen et al. (2016) on grape powdery mildew (*Erysiphe necator*), where 3.3 fungicide applications were avoided when using the airborne inoculum in the decision system compared to that with the standard treatment programme. These results are encouraging; however, more trials should be conducted to test soil and climatic conditions, potato varieties and *Pi* genotypes further. The integration of spore traps in disease monitoring has already been conducted for other diseases, such as *Venturia inaequalis* in apple orchards (Torfs et al., 2019).

Variable quantities of *Pi* airborne inoculum were captured during winter at all locations. This finding is surprising as there is no potato cultivation in Belgium during winter. However, the genotype identification of several samples failed, possibly due to a mixture of DNA from distinct clonal lineages. This DNA mixture, combined with low quantities of DNA, made the identification of several samples impossible. The identification process can be improved using metagenomic techniques, which require smaller DNA quantities and provide better resolution. To our knowledge, no airborne inoculum of *Pi* has been reported in winter in temperate climates; however, studies on airborne inocula have usually focused on the cropping season (Aylor et al., 2001; Fall et al., 2015a, 2015b; Iglesias et al., 2009; Lees et al., 2019; Nielsen et al., 2007). Conversely, the presence of *Pi* airborne sporangia throughout the year in Brazil, even in months less favourable to the disease, was highlighted by Lima et al. (2009). However, their study was conducted in a tropical/subtropical area where winters are less severe, several crop seasons occur in succession during the year and alternative hosts may be present. In a study on light leaf spot disease of winter oilseeds in Poland and in the UK, low quantities of airborne inoculum of *Pyrenopezeza brassicae* were detected in November in the United Kingdom (Karolewski et al., 2012). Airborne inoculum of *Rynchosporium secalis* (barley leaf blotch) was also detected in January and February in the UK, as barley is grown in winter and spring; however, the quantities trapped were negligible, except on three dates (Fountaine et al., 2010). The detection of airborne inoculum during the winter has not been widely studied and studies are lacking on *Pi* as potatoes are grown during the summer. Growth tests were conducted at low temperatures using three *Pi* strains (13_A2, 36_A2 and 6_A1) to investigate the overwinter detection of *Pi* airborne inoculum. All strains were able to grow and sporulate *in vitro* (with media V8 and Rye) at 5 °C. The sporangia produced were viable and infectious, as reinoculation of the detached leaves was successful. These results agree with those of Gigot et al. (2009), who investigated the survival of *Pi* at low temperatures. The authors were also able to demonstrate the sporulation of this oomycete on potato tubers under temperatures as low as 4 °C. *In vivo* tests should be conducted to confirm these results and investigate *Pi* potential sporulation under lower temperatures (2–3 °C). These results also raise the question of the origin of this winter inoculum, as the average winter temperature where the spore traps were located is between 2.5 and 6.9 °C. The genotypes identified in this study are European. The first source of inoculum could be cull piles or volunteer tubers surviving winter and their management should be considered (Kirk, 2003). This inoculum could also originate from fields in neighboring regions, where the climate might be more favourable. *Pi* inoculum covers large distances on the wind (up to 20 km in three hours) (Aylor et al., 2001). Fall et al. (2015b) suggested that, in the early season, most sporangia originate from long-distance flights. However, long-distance flights result in small quantities of airborne inoculum being trapped because they are diluted by distance. This hypothesis contradicts the quantities trapped, as they equal or exceed the airborne inoculum detected during the growing period. The involvement of a secondary host in the winter may also be an option. A list of non-cultivated and cultivated plant species recognized as hosts for *Pi* was established by Erwin and Ribeiro (1996). Therefore, investigation of potential winter hosts might be of interest. Many wild *Solanum* species, such as *S. nigrum* (Deahl et al., 2004) and *S. sarrachoides* (Vartanian & Endo, 1985), are alternate hosts for *Pi* but they are unlikely to act as overwinter reservoirs as these species are annual and lack frost resistance. *S. dulcamara* acts as an overwinter reservoir for *Pi* as it is present in the host rhizosphere (Vetukuri et al., 2020). As Belgium is included in the distribution area of *S. dulcamara*, its presence near the experimental fields could explain the airborne inoculum trapped in winter. Solar radiation is a major determinant of sporangial survival (Aylor et al., 2001; Mizubuti et al., 2000). As the days are particularly dark during winter in Belgium (3.456 MJ.

m^{-2} per day on average), it is reasonable to suggest that the airborne inoculum trapped in winter may be viable. Potato refuse piles were described as one of the most important sources of primary airborne inoculum (Evenhuis et al., 2007; Montarry et al., 2007) as *Pi* can survive several months in the middle of the cull pile under mild temperatures (around 5 °C) (Kirk, 2003). Recent tests under controlled conditions demonstrated that *Pi* is able to sporulate on potato tubers at 4 °C (personal data). The highest quantities of airborne inocula detected in winter were in Libramont, where potato cultivation was not conducted on an industrial scale. The presence of refuse piles is unlikely to occur in this area and, therefore, does not explain the quantities of aerial inoculum collected.

Conclusion

This study provides new insights into the epidemiology of PLB caused by *P. infestans* by examining the spatiotemporal dynamics of its airborne inoculum across Belgium from 2019 to 2022. The key findings highlight significant variability in the presence of airborne inoculum both spatially and temporally. This variability underscores the need for localized monitoring to improve the accuracy of disease predictions. We established a clear correlation between the detection of airborne inoculum and the subsequent appearance of potato late blight symptoms in untreated fields. Notably, inoculum presence was detected a few days before the onset of visible disease, although not all inoculum presence led to infection, indicating the critical role of favourable climatic conditions in disease development. Our comparative analysis of existing DSSs revealed that fungicide treatments are often recommended when no airborne inoculum is present. By integrating inoculum data into these systems, it is possible to reduce unnecessary fungicide applications. Field trials conducted in 2021 and 2022 demonstrated that using airborne inoculum data allowed for delaying the first fungicide treatment and reducing the total number of treatments without compromising disease control. An unexpected but significant finding was the detection of airborne inoculum during winter, suggesting that *Pi* can survive and disperse even outside the typical growing season. This raises new questions about the *Pi* survival mechanisms and the potential for long-distance dispersal. Overall, the results underscore the importance of incorporating airborne inoculum monitoring into existing decision support systems to enhance the precision of potato late blight management. This approach not only optimizes fungicide use but also contributes to sustainable agricultural practices by reducing chemical inputs. Future research should focus on understanding the environmental factors influencing inoculum distribution and exploring the potential for year-round monitoring to further refine PLB management strategies.

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Declarations

Conflict of interests The authors state that there are no conflicts of interest.

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